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RESEARCH ARTICLE

Effect of CATA PRO® on Hemato-biochemical Parameters, Fecal Shedding of *Escherichia coli* and Frequency of Diarrhea in Neonatal Buffalo Calves

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Abstract

In Europe from 2006 and nowadays all over the world, there are restrictions about the addition of antibiotics to calves' food in order to minimize the rates of bacterial resistance to antibiotics. Thus, this study was conducted to determine the anti-diarrheal effect of probiotic supplementation in neonatal buffalo calves at a dose of 10 g for each animal once daily for three weeks comparing with non-supplemented control group that fed only on milk replacer. Fecal score, selective hematological and biochemical studies were analyzed to evaluate the effect of probiotics on the frequency of diarrhea caused by *Escherichia coli* (*E. coli*) and the Probability of having any side effects on blood picture and liver function. Results revealed that the probiotics mixture used at this study had no significant changes at the values of red and white blood cells, liver enzymes, glucose and electrolytes. however, a significant decrease in the count of fecal *E. coli* wihout diarrhea was observed all over the experimental periods compared to the non-supplemented animals. It could be concluded that the addition of probiotics to calves food at the period before weaning is safe and achieving antidiarreal and antimicrobial benefits.

Keywords: Probiotic, Electrolytes, Calf Scour, Erythrocyte, Leukocyte

Introduction

One of the major concerns in dairy farms is the mortality of young calves, and it is considered that diarrhea is the most common disease among newly born calves that also increases the risk of their mortality. Young calves throughout the pre-weaning period are predisposed to several infectious pathogens that cause primary damage to the intestinal villi [1].

Calf scour diseases lead to impairment in the absorption of fluids from the intestine causing life-threatening electrolyte disturbances and death, which usually results from dehydration, acidosis, and electrolytes disturbances [2]. One mode of actions of the probiotic is the competitive exclusion; in which normal microflora protection occur against the harmful pathogens. It competes with harmful pathogens in its adhesion sites and competes for nutrients. As it block the receptor sites and avoid the attachment of harmful pathogens, by this way the probiotic can exclude pathogenic bacteria and prevent the infection. [3].

Antibiotics have been greatly used in milk replacer (MR) in the USA and Japan to increase body weight and decrease scours in dairy calves [4]. At times of stress; such as weaning, digestive upsets become very common among calves. In these conditions, it is preferred to include probiotics in foods rather than the usage of antibiotics; as it not only destroys the harmful bacteria but also the desirable species. Probiotics are known as healthy bacteria that help to promote certain strains of bacteria that are beneficial to the immune status. Animal's diet does not have all the requirements and nutrients needed by the gut flora, probiotics act mainly by improving or restoring the gut flora by providing these nutrients needed [5].

Probiotics are very beneficial live microorganisms (bacteria, yeasts, fungi) that can be added to food or water either single or mixed cultures to add value to it. In newborn animals where milk is their main food, their stomach tissue structure is still underdeveloped so probiotics alter the flora in

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the intestine and prevent cases of diarrhea in calves. They act by increasing the speed of development of flora and fauna in the intestine and consequently decreasing mortality rates. In addition, they reduce the cost of feed per unit gain and thus accelerate weaning process [6].

Probiotics can modify bacterial flora of the gastrointestinal tract efficiently and result in potentially decreasing the incidence of disease and improving the outcome. These beneficial effects include prevention of harmful bacterial growth, activation of the mucosal barrier function and alteration of the process of immune regulation Usage of antibiotics revealed that they destroy both beneficial and the harmful species, in contrast to probiotics. Probiotics are used to activate many bacteria in the gut and decrease the effect of the unfavorable ones. The nutritional requirements of the useful bacteria may not be supplied properly by the diet of the animal. Consequently, supplying the animal with these essential nutrients will help to encourage the growth of the useful bacteria [7].

The purpose of the current study was to assess the effect of probiotics on the frequency of diarrhea and fecal *E. coli* count with assessing the hemato-biochemical adverse effects.

Materials and Methods

Study design, Feeding and housing

The present study was carried on 65 newly born buffalo calves of both sexes, their age range from 3 days to 1 month and were fed on milk replacer in a private farm in Sharkia Governorate to determine the effect of probiotic on blood parameters , fecal *E. coli* count and the prophylaxis of calf diarrhea in them. They were examined and divided into the following groups:

Group (1): included 30 apparently healthy calves were fed on milk replacer without addition of probiotic (non-supplemented control group).

Group (2): included 35 apparently healthy calves were fed on milk replacer with addition of probiotic in which animals received 10 g probiotic orally for each animal every day for 4 weeks (probiotic supplemented group).

The usual diet in the first ten days was 3 L/d of milk replacer. Then in the rest of the month, it was increased to 4 L/d of milk replacer. Hot water was used during the preparation of the diet to dissolve the fat particles. Then temperature was adjusted to 38°C by adding cool water. Food was applied twice daily using a plastic bucket at 8 AM and 5 PM. At each feeding, a bucket containing milk replacer was fitted into the stand and removed after feeding. The composition of diet was listed in Table (1). Water was supplied unlimited and changed twice every day. Feeding neonatal buffalo calves with the probiotic at a dose of 10 g for each animal every day with the morning milk during the study period. The probiotic used in the experiment was called CATA PRO ® (Catalysis,S.L. Macarena, 14.28016 Madrid, SPAIN), which is a mixture of *Lactobacillus acidophilus* (0.32 x10 CFU), *Lactobacillus blantrum* (0.21 x10 CFU), *Enterococcus facium* (0.22 x10 CFU), *Eifidobacterium bifidum* (0.05 x10 CFU) and *Bacillus subtilus* extract (0.25 x10 CFU).

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 [29].
**ND = Not determined.

Sampling and measurements

Fecal samples were taken at the 7^{th}, 14^{th}, 21^{th}, and 28^{th} after start of the experiment directly from the rectum using sterile rubber gloves in a clean container. The fecal samples were stored at 4°C for a maximum period of 2 hours. One-gram fecal sample was diluted in 9 ml of 0.1% sterile peptone water and complete set of tenfold serial dilutions were done.

The dilutions of 10^{-5}, 10^{-6} and 10^{-7} were done by using pour plate technique for colony counting. Plates were incubated at temperature 37°C for 2 days. Colonies grown on EMB (Eosin Methylene Blue) agar (Lab Supply Company, Heliopolis, Cairo, Egypt) plates were counted at 1 day and 2 days of incubation. The number of colonies showing green metallic sheen was counted selecting a colony count of 30 to 300. The average of colonies of three plates was taken as *E. coli* count. Log colony-forming units per gram of feces were the expression of number of colony-forming units [8].

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 Ethylene diaminetetraacetic acid (EDTA) as anti-coagulant and were examined within an hour of taking the blood sample. This sample was used for evaluation of blood parameters including red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb) and packed cell volume (PCV%). Blood parameters were done automatically by CBC device (complete blood counter) and its model was (Mindray Bc-2800 CBC). The second sample (coagulated) blood and centrifugation at 3000 rpm for 15 minutes to remove residual red cells, then stored in the deep freezer - 20^0C and kept for determination of total protein, albumin and globulin, which were determined spectrophotometrically using kits produced by Diamond according to the method described by Grant *et. al.*[9]. Also serum enzymes (Aspartate Aminotransferase "AST" and Alanine Aminotransferase "ALT") were analyzed by colorimetric method using test kits according to Tietz [10], and electrolytes (Na: sodium; Cl: chloride; K: potassium) determined by using flame photometer according to the method applied by Bhattacharya *et al.* [11] using test kits produced by Spinreat company.

Fecal scoring

The procedure of Larson *et al.* [12] was used for estimation of fecal fluidity and it was conducted daily in the morning. Scoring was represented as for fecal fluidity, one = normal, two = soft, three = runny or four = watery. A scour day was recorded if fecal fluidity = three or four. The data was averaged per week.

Statistical Analysis

Statistical analysis was performed using SAS Institue [13]. Differences in fecal bacterial count between experimental groups were evaluated using t-test. Difference of fecal scoring was evaluated by repeated measurements ANOVA.

Results

Hemato-biochemical changes

Mean values of hematological and biochemical parameters in probiotic treated calves and their control are shown in Table 2. Blood hematological profile (total erythrocytic count, Hb, total leukocytic count and PCV %) and albumin, globulin, enzymes (AST and ALT), glucose and electrolytes (Na, Cl, and K) were analyzed and the values were all in normal values in probiotic group compared with the control. Probiotic supplementation revealed no significant changes on either the hematological or biochemical traits measured ($P>0.05$).

Table 2: Effects of probiotic supplementation on calves' blood parameters (Mean ± SE):

Parameters (Mean ± SE)	non-supplemented group (control) n= (30)	Probiotic group n = (35)	Sig (2-tailed)
RBCs (10^6/µI)	8.32±0.04	8.29±0.03	0.641
WBCs (10^3/µl)	9.58±0.03	9.52±0.04	0.310
PCV (%)	30.26±0.03	30.29±0.03	0.604
Hb (g/dl)	10.71±0.03	10.70±0.04	0.904
ALT (IU/L)	64.58±0.02	64.52±0.03	0.170
AST (IU/L)	85.71±0.04	85.65±0.03	0.223
Glucose (mg/dl)	77.69±0.04	77.68±0.03	0.855
Total protein (gm/dl)	7.41±0.02	7.37±0.01	0.240
Albumin (gm/dl)	4.20±0.05	4.18±0.01	0.85
Globulin (gm/dl)	3.21±04	3.18±0.01	0.521
Na (mmol/l)	136.71±0.01	136.74±0.02	0.375
Cl (mmol/l)	93.86±02	93.84±0.02	0.696
K (mmol/l)	4.25±0.02	4.28±0.02	0.271

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.

Effect of probiotic on fecal E. coli count

The effect of probiotic on fecal *E. coli* count for the experimental period is shown in Table 3. The results revealed that calves fecal *E. coli* count 023+ the 2nd week of the experiment, the control and the probiotic group's fecal *E. coli* count were 7.48±0.12 and 7.02±0.22 CFU/g, respectively. While after the 3rd week of the experiment, the fecal *E. coli* count in the control and probiotic groups were 7.32±0.15 and 6.85±0.40 CFU/g, respectively. Finally, at the 4th week of the experiment, the fecal *E. coli* count in the control group and probiotic group were 7.44±0.12 and 6.74±0.15 CFU/g, respectively.

Table 3: Effect of probiotic on mean *Escherichia coli* count (CFU/g ± SE) in feces of neonatal buffalo calves

Day	non-supplemented gr591oup (control)(n=30)	Probiotic group (n = 35)	Sig (2-tailed)
7	7.40±0.45 [a]	7.12 ±0.22 [b]	0.213
14	7.48±0.12 [a]	7.02±0.22 [b]	0.213
21	7.32±0.15 [a]	6.85±0.40 [b]	0.213
28	7.44±0.12 [a]	6.74±0.15 [b]	0.213

Effect of probiotic on diarrhea frequency

The fecal score was used for assessment of calf diarrhea during one month before weaning. Diarrhea occurred in the control group during the study period. Nevertheless, those fed on the probiotics; diarrhea was not present beyond the second week (Figure 1).

There was significant difference in the fecal score between the probiotic treated group and the control group after two weeks of the experiment; subsequently, fecal score became constant in the probiotic treated group and never exceeded the normal value (Figure 1).

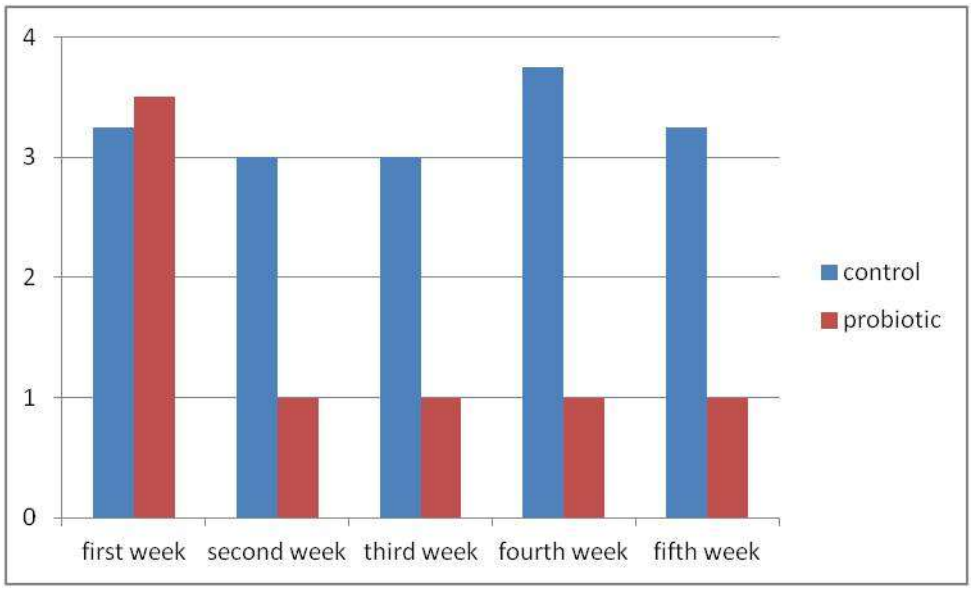

Figure 1: Fecal score of neonatal buffalo calves supplemented with or without probiotics. Calve diarrhea was evaluated using the fecal score and recorded according to Larson *et al.* [11] recommendations. For fecal fluidity, scoring was done as follows: 1 = normal, 2 = soft, 3 = runny and 4 = watery during the experimental period.

Discussion

The current study was designed to assess the effect of probiotics on the frequency of diarrhea and fecal *E. coli* count with assessing the hemato-biochemical parameters in neonatal buffalo calves.

Probiotic feeding had no significant changes on both the hematological and biochemical parameters. This was in agreement with the findings of Adams *et al* [14, Moslemipur *et al.* [15] and Riddell *et al.* [16] who reported no significant differences regarding hematological and biochemical parameters when comparing probiotic treated calves and their control. These results attributed to that probiotic has no side effect on the body organs and blood components probiotics are live normal flora of the intestine. On the other hand, these results disagree with Abe *et al.* [6] who reported an increase in WBCs in probiotic treated calves and this might attributed to the effect of probiotic in the immune response and improving the gut immunity against harmful pathogens.

The results of fecal *E. coli* count indicated that there was significant decrease in the count of E. coli in probiotic treated calves on 7^{th}, 14^{th} and 28^{th} day. The results showed that probiotic treatment was effective in decreasing the fecal *E.coli* count up to 28th day of supplementation, which can be explained by better balance in intestinal bacteria. These results are in agreement with the findings of Roodposhti and Dabiri [17] who reported a significant reduction in fecal *E.coli* count in calves supplemented with probiotic compared to control group.

In addition, Shim [18] reported significantly lower *E.coli* number in the intestine of piglets given probiotic than the control group. The study of Elam *et al.* [19] reported that probiotics can reduce undesired bacteria such as *E. coli* by production of inhibitory materials like organic acids and H_2O_2 having antimicrobial-like action and by competitive inhabitation with bacterial adhesion on intestinal surfaces. By the same manner, *Lactobacillus acidophilus* was found to decreases the shedding of *E. coli* in the feces.

However, Abdel- Raheem *et al.* [20] reported no significant change in fecal coliform and *E.coli* count among probiotic supplemented group and control group. The reason for the ineffectiveness of supplementation of probiotic post 30 days may

be due to the development of immunity in growing calves.

Regarding fecal score, there was significant difference in the fecal score between the probiotic treated group and the control after two weeks of the study. Then fecal score became constant in the probiotic treated group and never exceeded the normal value. The results indicated that there was a significant reduction in the incidence of diarrhea after two weeks of application. The reduced incidence of diarrhea may be as a result of an improvement in the intestinal bacterial flora in calves supplemented with probiotics.

This outcome agrees with the results of Dezfouli *et al.* [21] and Khuntia. and Chaudhary,. [22]. While in the study of Cruywagen *et al*. [23] no reduction of the occurrence of diarrhea was observed in the probiotics group.

The studies of Kawakami *et al.* [24] and Gorgulu *et al*. [25] reported that with respect to diarrhea and fecal scoring calves fed probiotics were superior to control group and explained that by that lactic acid bacteria can enhance the development of the immune action against harmful pathogens.

Probiotics was found to decrease the incidence of diarrhea in calves fed milk replacer as the probiotic has bactericidal activity as some types of bacteria produce lactic acid such as *Lactobacilli spp* in which they decrease the pH level and damage the harmful pathogens. In addition, probiotics produce different agents that can reduce the number of pathogenic organisms. These agents are antioxidants, organic acids, H_2O_2 and bacteriocins. However, these agents can also affect the microbial metabolism and the production of toxins. Bacteriocins have been found to be able to permeate the outside membrane of certain type of bacteria and thus inactivate these types of bacteria. Probiotics are useful for the action of immunity [3].

Enhancing of the immune system by stimulation of the production of antibodies and enhancing phagocytic activity can be achieved by probiotic bacteria and subsequently increase the animaldefense against harmful ones in the gastrointestinal. Harmful pathogenic bacteria are eliminated when the immune system is involved following exposure to probiotic bacteria and hostile ones as reported by Frizzo *et al*. [26].

Furthermore, Gorgulu *et al*. [25] also reported 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*. [27] for piglets and calves. Their finding is in agreement with this present study.

However, Cruywagen *et al*. [23] did not find any encouraging effect of the introduction of probiotic in milk replacer on diarrheal incidence.

Probiotics help in improving gastrointestinal health of the calves when experiencing challenges. Travelling of the animals to a far distance can induce harmful changes and affect their gut flora and this cause diarrhea and adding of the probiotics to their diet might help in decreasing the occurrence of diarrhea through improving their gut flora Timmerman *et al*. [28].

Conclusion

Therefore, it can be concluded that adding probiotic to milk replacer significantly reduced the incidence of diarrhea and reduced fecal *E. coli* count.

It is recommended that probiotics should be used in animal production so that we can reduce the use of antibiotics in animal industry, which has harmful effect on the consumers' health. More studies should be carried out using high number of animals to assess the probiotic benefits on animal growth performance and health condition of neonatal calves.

Conflict of interest

The authors declare that they have no competing interests.

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الملخص العربي

تأثير البروبيوتيك (الكتا برو) على قياسات صورة الدم البيوكيميائية وافراز بكتريا الأ شريشيا كولاى فى البراز وتكرار الاسهال فى عجول الجاموس حديثة الولادة

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في أوروبا منذ عام ٢٠٠٦ وفي الوقت الحاضر في جميع أنحاء العالم، هناك قيود على الإضافات الغذائية للمضادات الحيوية إلى العجول من أجل تقليل معدلات المقاومة البكتيرية للمضادات الحيوية. وبالتالي، أجريت هذه الدراسة لتحديد تأثير مضاد للإسهال من مكملات البروبيوتيك في العجول الجاموس حديثه الولادة بجرعة ١٠ جرام لكل حيوان كل يوم مقارنة مع المجموعه الضابطه التي تتغذى فقط على بديل اللبن. تم إجراء دراسات على البراز،و ايضا دراسات انتقائية في الدم والكيمياء الحيوية لتقييم تأثير البروبيوتيك على تواتر الإسهال الناجم عن الإشريكية القولونية (الاى كولاي) وإمكانية حدوث أي آثار جانبية على الدم والكبد. أظهرت النتائج أن خليط البروبيوتيك المستخدم في هذه الدراسة لم يحدث أي تغيرات معنوية في قيم خلايا الدم الحمراء والبيضاء، وأنزيمات الكبد، والجلوكوز بينما سجل انخفاض ملحوظ في عدد بكتريا الايشرشيا كولاى ببراز العجول المعالجة مع عدم حدوث إسهال طوال الفترات التجريبية مقارنة بالمجموعة الضابطه

نستنتج من ذلك أن اعطاء البروبيوتيك للعجول مع الغذاء في فترة ما قبل الفطام آمنة وتحقيق فوائد مضادة للإسهال ومضادة للميكروبات.