

SUCKLING CALVES PERFORMANCE AND IMMUNE STATUS AS AFFECTED BY LACTOCOCCUS LACTIS AS A PROBIOTIC SOURCE

U.A. Nayel, G.A. Baraghit, B.M. Ahmed and M.A. Elmeshtawy
Department of Animal Production, Faculty of Agriculture, Menoufia University,
Menoufia, Egypt.

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ABSTRACT: *This study sought to investigate the effects of Probiotic, Lactococcus lactis bacteria (LC.) on growth performance, nutrients digestibilities, diarrhea incidence and immune status of buffalo calves. Thirteen suckling buffalo calves were divided into two groups according to their body weight, age and sex (T1- Control (No LC. supplementation, T2- 4 g LC. Per calf in breast milk). The Calves were fed 10% of their body weight fresh milk 2 times daily and provided calf starter and clover hay to meet their requirements of DM according to Kearn (1982). Calves body weight was recorded at the start of the experiment and thereafter regularly at 10 days interval. Diarrhea occurrence also faecal scores have been detected. After completion of the feeding trial, three animals per treatment were used in a metabolism trial. We obtained blood samples at the end of the study from each calf through jugular vein puncture. Obtained results showed significant ($P < 0.5$) increase in roughage, concentrate and total DM intakes with calves fed LC-supplemented than the control (T1) group during the periods of P3, P4 and P5. Supplementation of LC. L to calves in T2 group had showed an increase in body weight than T1- group; the increase in total gain and average daily gain (ADG) was significant ($P < 0.05$), CP, CF, EE and NFE digestion coefficients keep on the same trend. Diarrhea occurred on T1 calves were more than T2 calves, and the duration of diarrhea at the same time was longer in control calves than treated calves. The number of E.coli in feces was more in control calves than treated calves. Immunological parameters (IgA, IgG and interleukin 2) have been increased in treated calves than control with no significant difference ($P > 0.05$).*

Key words: *Probiotic, Lactococcus lactis, Performance, immune, calves.*

INTRODUCTION

The young calf encounters potentially stressful situations in its first few months of life including transportation, dehorning, castration, weaning, and commingling. The risk of disease and the immune system repression can be increased by stress in the presence of a pathogen (Salak-Johnson and McGlone, 2014). Average daily gain and leukocyte function have been decreased in young calves which lives in group housing system, the risk of bovine respiratory disease increased in these calves (Hulbert and Ballou, 2012).

Consequently, research specifically involving methods to aid calves in maintaining health and growth through stressful situations, such as commingling, may be beneficial.

Recently, probiotic expression of lactic acid bacteria has been emphasized. But, few studies on the probiotic state of Lactococcus strains since they are traditionally not reasoned to be elemental inhabitants of the human gastrointestinal tract (Teuber et al. 1992). Still, various activity showed the possibility of the proximity of Lactococcus strains in the

human flora or animal gastrointestinal tract (Gruzza et al. 1992; Grahn et al. 1994; Klijn et al. 1995b). The present work was carried out in order to investigate the effect of *Lactococcus lactis* as a probiotic source on the growth performance, digestibility, immunity state, diarrhea incidences and GIT pathogenic microorganism of young calves.

MATERIALS AND METHODS

This study was conducted at the Experimental Station and Nutrition Laboratory, Animal Production Department, Faculty of Agriculture, Menoufia University (Shebin El-Kom) in order to investigate the effect of lactococcus lactis as a probiotic source on the performance of young calves. Thirteen suckling buffalo calves were divided into two groups according to their live body weight, seven calves in treated group and six calves in control group. Daily feed allowance was changed quantitatively according to the change in body weight. Feed was offered twice daily at 8:00 and 16.00 hr. Fresh water was getable at all times. Calves were fed 10% of their body weight fresh milk 2 times daily and provided calf starter and clover hay to meet their requirements of DM according to (Kearl (1982). Two experimental rations were prepared: Ration 1 (R1): Control (No probiotic supplementation). Ration 2(R2): Control +

4g probiotic (*Lactococcus lactis*)/calf. (According to manufacturer's instructions)

Specification and analysis of probiotic:

** Culture identify is confirmed at the genus/species level based on 16s rRNA sequencing

Product name: Lactococcus Lactis

Type: LLL-G25-110

Batch No.: 20170207

Shelf life: 24 months

| Items | Specification | Results |
|--------------------------|------------------------------------|-----------|
| Appearance | fluffy light yellow powder | complies |
| | no off-flavor, no visible impurity | |
| Water | ≤ 8% | 1.4% |
| Content of activity | ≥1.0x10 ¹⁰ cfu/g | |
| | 1.5x10 ¹⁰ cfu/g | |
| Non lactic acid bacteria | | <10cfu/g |
| Coliforms | ≤10cfu/g | <10cfu/g |
| Mold & Yeast | ≤10cfu/g | <10cfu/g |
| Pathogenic bacteria | | negative |
| Lead(Pb) | ≤1.0mg/kg | <1.0mg/kg |
| Total Arsenic(As) | ≤0.5mg/kg | <0.5mg/kg |

The complete chemical composition of the experimental rations is shown in Table (1).

Table 1: Chemical composition (%) of the experimental rations.

| Item | DM | OM | CP | EE | NFE | CF | Ash |
|-------------------|------|------|------|-----|------|----|-----|
| Concentrate (CS) | 91.5 | 95 | 19.2 | 7.1 | 64.7 | 4 | 5 |
| Forage Clover hay | 88.6 | 91.7 | 10.5 | 4 | 48.2 | 29 | 8.3 |

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NFE, nitrogen-free extract; CF, crude fiber. Calf Starter (CS): corn 65%, wheat bran 6%, soya bean meal 26%, caco₃ 1.6%, sodium chloride 1%, minerals & vitamins 4kg/ton

Body weights of calves in a 50-day trial were recorded at the beginning of the experiment and every ten days through the experimental period. Weighing was done at early morning before the calves were allowed to access feed and water. After completion of the feeding trial, three animals per treatment were used in a metabolism trial. After ten days as an adjustment period feces were collected from the experimental animals. Feces voided by each calf was weighed and recorded every day, mixed and 20% representative samples were taken and frozen at -20°C, also similar procedure was done for the provided feed and refusal samples collected. Offered feed samples, refusal and feces were integrated well and oven dried at 70°C for 48 h. The dried samples of feeds and feces were ground through 1 mm strainer then stored for analysis. Nutrient digestibility (%) was calculated as a difference between nutrient intake and nutrient voided in the feces divided by nutrient intake and the quotient multiplied by 100.

Each day before feeding, calves were observed in their pens and fecal scores recorded according to the method of Larson *et al.* (1977). For fecal fluidity, scoring was done as follows: 1 = normal, 2 = soft, 3 = runny and 4 = watery.

Rectum collected fecal samples were placed in sterile plastic tubes with lid, then stored at -20°C for determine of *E. coli* enumeration. *E. coli* count procedure was done by using dilution plate count method. Three plates were inoculated with 1 ml of the suitable dilution and poured on Eosin methylene blue (EMB) medium. The plates were incubated at 35-37°C for 24 hours for counting total coliform bacteria (Gue *et al.*, 2006). Immunoglobulin A, immunoglobulin G and Interleukne 2 concentrations in

serum were determined using single radio-immunodiffusion methods derived primarily from the works of Fahey and McKelvey (1965) and Mancini *et al.* (1965). These methods are specific for the quantitative determination of individual protein groups in biological fluids.

The obtained results were statistically analyzed using SPSS (Statistical Package for Social Sciences) program version 19, (2010). The following statistical model was applied:

Model:- $Y_{ij} = \mu + T_i + e_j$

Where:

Y_{ij} = the parameters under analysis.

μ = the overall mean.

T_i = the treatment effect, (i = 1 and 2).

e_j = the random error.

RESULTS AND DISCUSSION

Effect of *Lc. Lactis* (LC) on growth performance

Many efforts have been focused around the therapeutic action of probiotics. Probiotics were first documented by Metchnikoff (1907) after his observance on the longevity of Bulgarian farmers who consumed great quantities of yogurt. He thought that hurtful organisms, detrimental to humans, were inhibited by beneficial bacteria existing in yogurt. Lilley and Stillwell (1965) described probiotics as materials secreted by one organism that encourages the growth of another. recently, probiotics have been described as microorganisms that have beneficial effects on the host animal by supplying intestinal microbial balance (Fuller, 1989).

In the present study, it was found that supplementing calves' diet with LC significantly ($P < 0.5$) increased intakes of roughage, concentrate and total DM during the periods of P3, P4 and P5 (Table 2 and Fig. 1)..

Table 2: Feed intakes of calves as affected by LC supplementation

| Period | Treatments* (kg/d) | | Sig |
|-----------------|--------------------|---------------|------|
| | LC | Co | |
| P1 | 2.44 ± .076 | 1.94 ± .236 | NS |
| P2 | 2.64 ± .093 | 2.08 ± .281 | NS |
| P3 | 3.02 ± .091 | 2.21 ± .305 | 0.05 |
| P4 | 3.14 ± .093 | 2.37 ± .343 | 0.05 |
| P5 | 3.59 ± .141 | 2.63 ± .411 | 0.05 |
| Roughage | 9.49 ± .212 | 7.40 ± .981 | 0.05 |
| Concentrates | 5.35 ± .330 | 3.85 ± .611 | 0.05 |
| Total DM Intake | 14.85 ± .478 | 11.25 ± 1.576 | 0.05 |

*Co, Control (un-supplemented); LC, treated ration (supplemented with *LC. Lactis*)
P1 (period: 10 days after experimental beginning. P2,P3,P4,P5 :10 days interval)

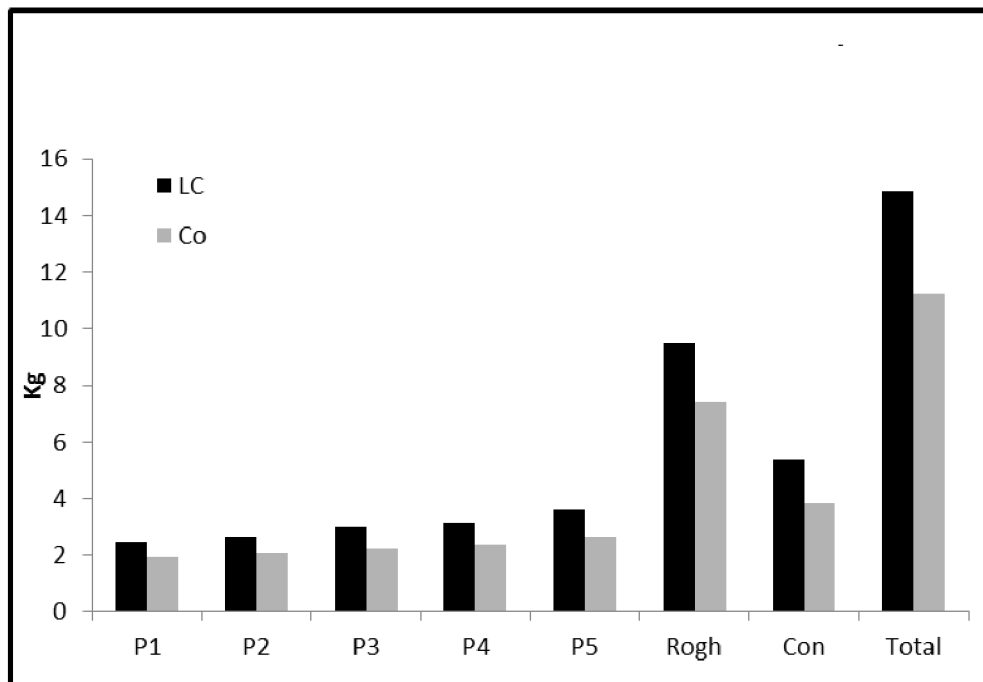


Fig. 1: Feed intake of calves as affected by LC.

Table (3) showed an increase in body weight in LC-group than Co- group; the increase in total gain and ADG was

significant ($P < 0.05$). The results were illuminated in Figs 2, 3 and 4.

Suckling calves performance and immune status as affected by

Table 3: Change in body weight of calves fed the experimental rations Mean \pm SE

| Period | Treatments* (kg) | | Sig |
|------------|-------------------|------------------|------|
| | LC | Co | |
| IBW | 74.42 \pm 6.27 | 73.16 \pm 4.99 | NS |
| P1 | 80.14 \pm 6.85 | 77.16 \pm 6.00 | NS |
| P2 | 87.00 \pm 7.90 | 80.83 \pm 6.72 | NS |
| P3 | 91.00 \pm 8.07 | 85.16 \pm 7.55 | NS |
| P4 | 99.71 \pm 8.87 | 90.66 \pm 9.24 | NS |
| P5 | 107.85 \pm 9.30 | 95.66 \pm 9.86 | NS |
| Total gain | 33.42 \pm 3.20 | 22.50 \pm 4.99 | 0.05 |
| ADG | 0.64 \pm 0.061 | 0.43 \pm 0.096 | 0.05 |

*Co, Control (un-supplemented); LC, treated ration (supplemented with *LC. Lactis*)
 IBW, initial body weight; P (period: 10d); ADG, average daily gain
 P1 (period: 10 days after experimental beginning. P2,P3,P4,P5 :10 days interval)

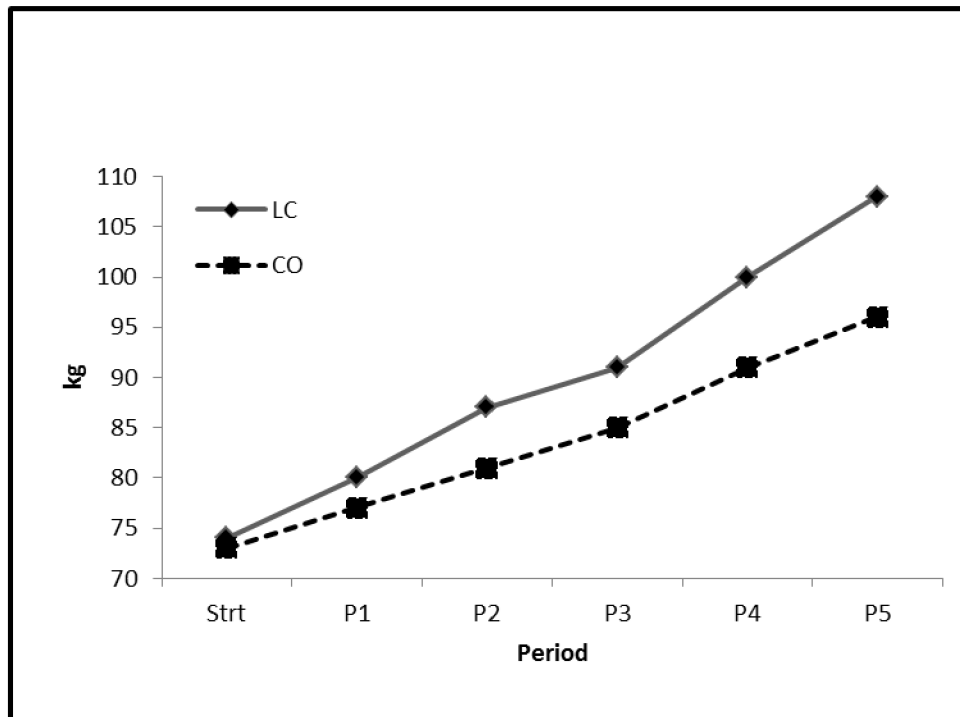


Fig. 2: Change in body weight of calves as affected by LC.

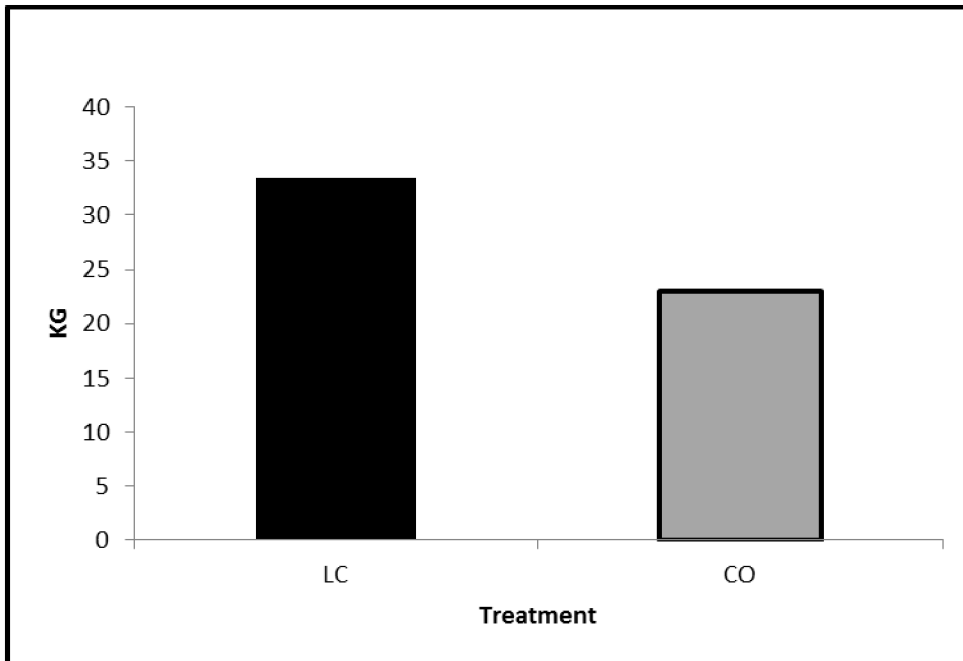


Fig. 3: Total gain of calves as affected by LC.

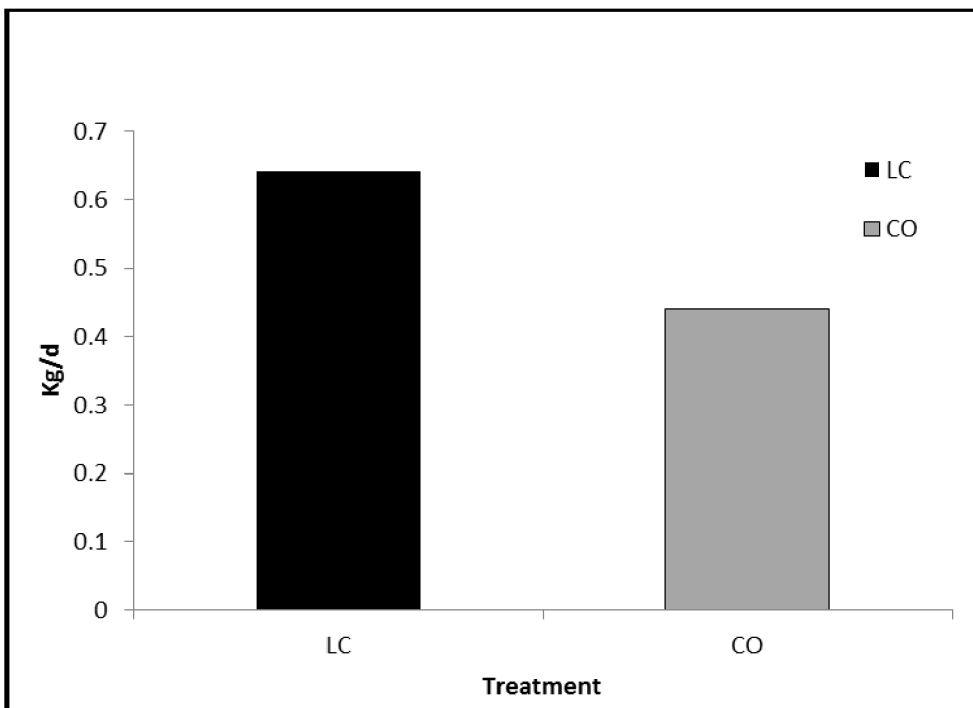


Fig. 4: ADG of calves as affected by LC.

Numerous investigators reported a positive effect of probiotic supplementation on nutrient intake, bodyweight gain and feed conversion rate in small ruminants (Antunovic *et al.*, 2006 and Whitley *et al.*, 2009).

It has been stated that influence of probiotics in animals performance may differ, as supplementation can enhance feed intake (Abd El-Ghani, 2004; Antunovic *et al.*, 2005; Desnoyers *et al.*, 2009), feed conversion ratio (Khalid *et al.*, 2011) or bodyweight gain (Jang *et al.*, 2009; Hussein, 2014). Haddad and Goussous (2005) reported increase in body weight of Awassi lambs which supplemented with yeast culture compared to controls (266 *versus* 212 g daily). The administration of probiotic to dairy calves (Le *et al.*, 2016) guide to an development in feed intake, ADG and feed conversion ratio emulate with the control calves. The management of milk fermented with lactic acid bacteria to calves was evaluated by Maldonado *et al.* (2018); they found that calves fed with probiotic evidenced an improvement in nutritional parameters, body condition and weight gain.

Various lactic acid bacteria strains are oftentimes employed in farm animals as feed additives because they bearing probiotic features, so they can raise animal performance, enhance health and reduce the gravity of mucosal infection (Sandes *et al.*, 2017).

Effect of *Lc. lactis* on digestibility:

Feeding LC-supplemented diet significantly increased digestibility of CP, CF, EE and NFE than the control group; the results were illustrated in Table (4) and Fig (5).

Probiotics was reported to improve nutrient digestibility (Krehbiel *et al.*, 2003; Abd El-Ghani, 2004), fibers degradation

(El-Waziry and Ibrahim, 2007) and rumen digestion (Kamel *et al.*, 2004).

Haddad and Goussous (2005) have reported that supplementation with yeast culture (Diamond VRYC) in the diets of Awassi lambs had resulted in increased digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF). Whitley *et al.* (2009) have reported improved apparent digestibility of DM, CP, NDF and ADF in meat-goat given a diet with probiotics.

The addition of *Bacillus licheniformis* to the diet of lactating cows increased NDF digestibility (Qiao *et al.*, 2010). Similarly, Boyd *et al.* (2011) found that lactating cows fed diets supplemented with *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* had higher NDF and protein digestibility. Khalid *et al.* (2011) found that supplementing the diet of growing lambs with a commercial probiotic increased NDF and ADF digestibility. Similarly, a mixture of *Lactobacillus* probiotics increased digestibility of protein and fiber as well as increased microbial synthesis in growing goats (Galina *et al.*, 2009).

Effect of *Lc. lactis* on diarrhea.

The effect of *Lactococcus lactis* on diarrhea presence and diarrhea period is illustrated in Fig (6). It was observed that diarrhea occurred on control calves more than calves that treated with *Lactococcus lactis*, and the duration of diarrhea at the same time was longer in control calves than with treated calves.

Le *et al.* (2016) presented that the administration of probiotic to young calves and they reported that diarrhea occurrence duration was 2 days less for the calves than the control.

Effect of *Lc. lactis* on the number of *E. coli* in calves feces:

By microbiological examination for each calf's feces (7 calves in treated group and six calves in control group), it was observed as shown at Table (5) that the number of *E.coli* was more in control calves than in treated calves. This explains the incidence of diarrhea and increased duration of diarrhea in control calves than treated calves.

Lactic acid bacteria (LAB) are natural components of the normal intestinal microbiota in both humans and animals

(Schneider *et al.*, 2004) and have been used to inhibit the effects of pathogens such as *Salmonella* spp. (Gill *et al.*, 2001) and *Escherichia coli* (Shu and Gill, 2002). These two pathogens are the most frequent bacterial etiologic agents in calf scours during the first week of life (Barrington *et al.*, 2002; Millemann, 2009).

Jozala *et al.* (2011) illustrate that, *Lactococcus lactis* can produce extracellular peptide, this peptide has been applied as a natural additive once it presents broad antibacterial activity.

Table (4): Nutrient digestibility (%) as affected by dietary treatments Mean ± SE

| Item | Treatment* | | P< | Sig. |
|------|--------------|--------------|------|------|
| | LC | CO | | |
| CP | 69.35 ± 1.23 | 61.52 ± .66 | .005 | *** |
| CF | 54.10 ± .79 | 49.48 ± .72 | .013 | ** |
| EE | 74.07 ± .51 | 51.77 ± 1.27 | .005 | *** |
| NFE | 66.46 ± 1.45 | 50.69 ± 1.89 | .003 | *** |

*Co, Control (un-supplemented); LC, treated ration (supplemented with *LC. Lactis*)

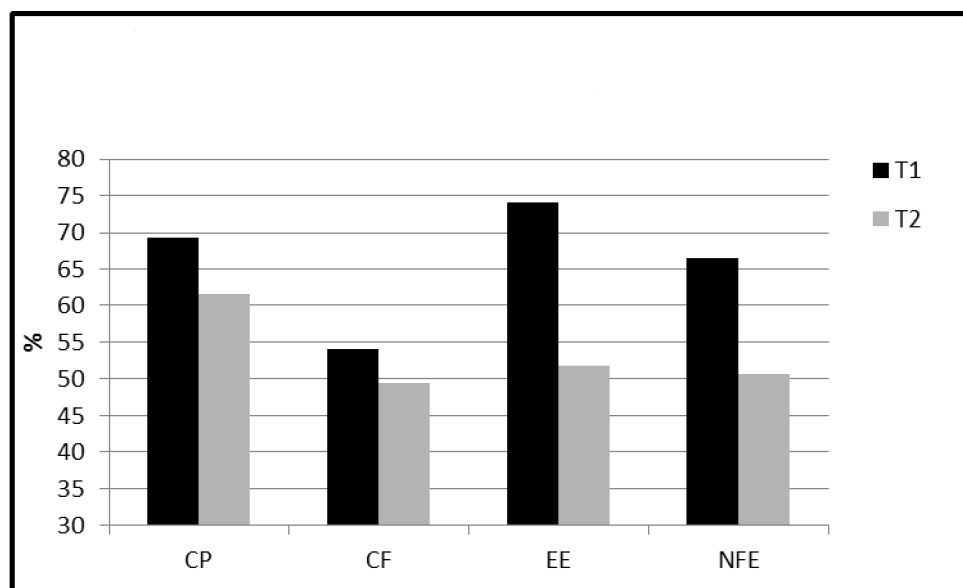


Fig. 5: Nutrient digestibility as affected by dietary treatments.

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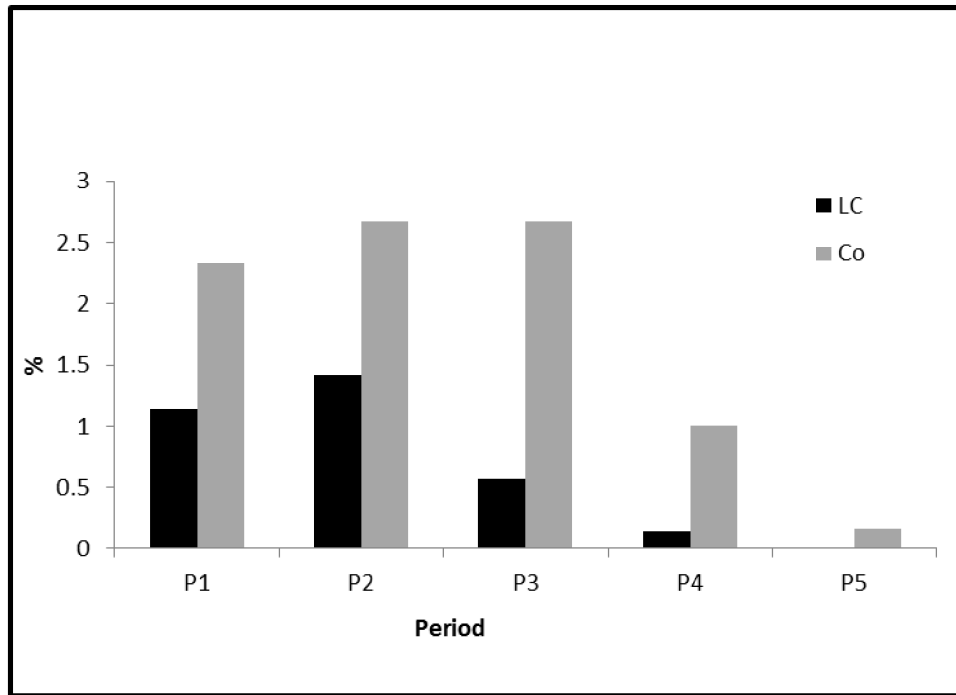


Fig. 6: Diarrhea incidence of calves as affected by LC.

Table 5: Effect of Lc.lactis on E. coli number in feces

| Treatment | E.coli number |
|------------|----------------------|
| Lc. Lactis | 2x10 ³ |
| | 1.1 x10 ³ |
| | 2.2 x10 ³ |
| | 2x10 ³ |
| | 2.3x10 ⁴ |
| | 2.1 x10 ³ |
| | 2.4x10 ³ |
| Control | 2x10 ⁵ |
| | 2.8x10 ⁵ |
| | 5.6x10 ⁴ |
| | 4.6 x10 ⁵ |
| | 2.3x10 ⁵ |
| | 8.4x10 ⁴ |

Effect of *Lc. lactis* on immunological parameters:

As shown in Table (6), it was observed that, the immunological parameters increased in treated calves than control calves, meaning that *Lc. lactis* promoted gut health via stimulation of the innate immune response, so treated calves immunity system produce larger amount of immunological parameters than control calves that have lower hygiene than treated calves. However, differences did not reach a significant level due to the less animal number used; also differences between animals within groups was higher than between treatments (the large standard error masked the difference between groups).

Immuno-modulatory effects of several strains of *Lactococcus lactis* have also been studied extensively under both *in vitro* and *in vivo* trials (Kitazawa et al., 1992; Kimoto et al., 2004). Kimoto et al. (2004) studied both *in vitro* as well as *in vivo* induction of cytokine production by strains of *Lc. lactis*. They achieved *in vitro* and *in vivo* trial on murine macrophage cell line J774.1 (as an APC) and spleen cells of experimental mice, respectively. On the basis of their study, they reported an increased level of cytokine production by various lactococcal strains tested. Among all the Lactococcal strains *Lactococcus lactis* subsp. *Lactis* G50 was found to induce the highest cytokine production.

Kitazawa et al. (1992) studied the effect of *Lactococcus lactis* subsp.

cremoris KVS20 strain on mitogenic activity of B-cells. An increased proliferation of B-cells because of slime (phospho-polysaccharide consisting of rhamnose, glucose, galactose and phosphorus) produced by KVS20 strain was observed.

Probiotics may improve the immune system (Table 6) by stimulating antibody production, increasing epithelial barrier integrity, and up-regulating immune cells. Qadis et al. (2014b) found that supplementing calf feed with a probiotic mixture of *Lactobacillus plantarum*, *Enterococcus faecium* and *Clostridium butyricum* increased the number of leukocytes in both diarrheic and healthy calves and in the diarrheic calves the severity of diarrhea was also reduced. Probiotics can indirectly affect the ruminant's immune system by changing the microbe community. Kawakami et al. (2010) showed that *Lactobacillus plantarum* supplementation to Holstein Friesian calves increased the number of LAB which resulted in GIT-associated lymphoid and epithelial cells activating local immune responses in the intestine. Sun et al. (2010a) found that supplements of *Bacillus subtilis* fed to pre-weaned calves increased the levels of serum immunoglobulin G (IgG) and T helper cell 1 (Th1) cytokines such as interferon gamma (IFN-γ). In addition, *Bacillus* added to the diet of dairy calves with diarrhea promoted the activation and maturation of regulatory T cells (Novak et al., 2012).

Table (6): Effect of *Lc.lactis* on serum concentrations of IgA, IgG and Interleukne 2.

| Item | Control | <i>Lc.lactis</i> | Sig |
|-----------------------|--------------|------------------|-----|
| IgA (mg/dl) | 107.6±8.68 | 183±55.15 | NS |
| IgG (mg/dl) | 1007.3±109.7 | 1106.6±159.6 | NS |
| Interleukne 2 (Pg/ml) | 61.6±13.04 | 72.8±9.5 | NS |

Probiotics offered to humans enhanced the immune response, reduced serum cholesterol levels and colon cancer, improved calcium absorption, vitamin synthesis, and lactose tolerance (Isolauri *et al.*, 2001), as well as reduced diarrhea in children (Van Niel *et al.*, 2002).

Various pathogens, alone or most often in combining with another pathogens, are etiologic agents of diarrhea in new born calves. Utmost of these agents are mainly transferred by the fecal oral path from the infected animals feces to the mouths of susceptible animals (Barrington *et al.*, 2002). In young pre-weaned ruminants, where the rumen is not yet fully functional, the conventional target of probiotic application is to stabilize the GIT micro-biome to decrease the risk of pathogen colonization in the intestine and to facilitate a successful weaning. Further, Lee *et al.* (2005) found that probiotics enhance immune responses by proliferation of T-cell and B-cell, cytokine, immunoglobulin (IgA, IgE) production etc., which directly and indirectly help in the prevention of certain diseases like atopic eczema, AIDS, allergy (a hypersensitive reaction), etc. (Kimoto *et al.*, 2007). Immunomodulatory effects of several strains of *Lactococcus lactis* have also been studied extensively under both *in vitro* and *in vivo* trials (Kimoto *et al.*, 2004; Kitazawa *et al.*, 1992). Kimoto *et al.* (2004) studied both *in vitro* as well as *in vivo* induction of cytokine production by strains of *Lc. lactis*.

Conclusion

Probiotic (*Lactococcus lactis*) supplementation generally improved average daily gain, nutrients digestibility, reduces the incidence of diarrhea, improve immunity status.

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تأثير بكتريا اللاكتوكوكاس لاكتيس كمصدر للبروبيوتك على الأداء والحالة المناعية للعجول الرضيعة

أسامة ابوالعز محمد نايل ، جمال أحمد براغيت ، بركات محمد أحمد ، محمود أحمد المشطاوى
قسم الانتاج الحيوانى - كلية الزراعة - جامعة المنوفية

الملخص العربى

أجريت هذه الدراسة على العجول الرضيعة بمزرعة الجاموس ومعمل تغذية الحيوان بقسم الانتاج الحيوانى بكلية الزراعة جامعة المنوفية بهدف معرفة تأثير بكتريا اللاكتوكوكاس لاكتيس كمصدر للبروبيوتك على الاداء الإنتاجي والحالة المناعية والصحية وزيادة قدرة العجول الرضيعة على تخطى الفترات الحرجة من عمرها خاصة فى مرحلة ما قبل الفطام وتم اجراء التجربة على ثلاثة عشر من العجول والعجلات الجاموس الرضيعة والتي قسمت الى مجموعتين مجموعة المقارنة وفيها تم تقديم لبن الرضاعة للحيوانات دون اى اضافات والمجموعة الثانية مجموعة المعاملة وفيها تم اضافة بكتريا اللاكتوكوكاس لاكتيس على لبن الرضاعة لدراسة تأثير هذه البكتريا على العوامل السابق ذكرها , مع مراعاة ثبات باقى العوامل التى يتعرض لها الحيوانات فى كلا المجموعتين من عليقة البادئ والدريس ومياه الشرب أشارت النتائج الى وجود زيادة معنوية فى معدل المأكول اليومي وكذلك اوزان ومعدلات نمو عجول المجموعة الثانية (مجموعة المعاملة مقارنة بالمجموعة المقارنة, تحسنت معاملات الهضم البروتين والالياف والكربوهيدرات والدهن بصورة معنوية نتيجة المعاملة أظهر الفحص الميكروسكوبى للروث تسجيل مجموعة المقارنة حالات اسهال أكثر واستمرار مدة الاسهال لفترات اطول وأعداد أكبر من بكتريا الايشيريشيا كولاى (E.coli) مقارنة بالمجموعة المعاملة زادت نسبة معلمات المناعة I2 او I1 او I2 فى مجموعة المعاملة عن مجموعة المقارنة دون فروق معنوية وذلك يوضح دور بكتريا اللاكتوكوكاس لاكتيس فى تحسين نظام المناعة فى المجموعة المعاملة من خلال تحفيزها للجهاز المناعى بها وافراره لكميات اكبر من هذه المعلمات المناعية.

أسماء السادة المحكمون:

- ١- أ.د. سعيد أحمد محمود كلية الزراعة - جامعة كفر الشيخ
- ٢- أ.د. كمال محمد عبدالرحمن كلية الزراعة - جامعة المنوفية