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Effect of Oral Administration of *Lactobacillus acidophilus* on the Intestinal Mucosal Immune Cells in Young Bactrian Camels (*Camelus bactrianus*)

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Abstract: The intention of this study was to examine the effects of oral administration of *L. acidophilus* L3 on the number of intestinal mucosal immune cells of young bactrian camels. Young camels were fed daily with *L. acidophilus* L3 (a concentration of 2×10^9 CFU kg^{-1} feed) and their intestinal immune cells were assessed on day 28 by the histology, histochemistry and Cell Counting Methods. The number of Intraepithelial Lymphocytes (IELs), Goblet Cells (GCs), plasma cells and mast cells were counted, recorded and compared with the control group. Statistical analysis showed that the number of those intestinal mucosal immune cells were all increased in the probiotic group, compared with the control group and the difference was statistically significant ($p<0.05$). The distribution tendency of those cells in small intestine was that the number of intraepithelial lymphocytes, goblet cells and mast cells was gradually reduced from duodenum to ileum in two groups whereas the number of plasma cells was gradually increased from duodenum to ileum. The results indicated that *L. acidophilus* L3 has intense influence on the number of mucosal immune cells in small intestine of young camels, supplementation of the diet with *L. acidophilus* L3 is able to enhance the intestinal mucosal immunity of young camels.

Key words: Young camels, *Lactobacillus acidophilus*, intestinal, immune cells, groups

INTRODUCTION

The intestinal microflora play a crucial role in host defense as demonstrated by their ability to modulate both innate and acquired immunity at the local as well as systemic levels (Isolauri *et al*., 2001; Macfarlane and Cummings, 2002). Due to these immunological properties, specific strains of Lactic Acid Bacteria (LAB) defined as probiotics have raised considerable interest in recent years. When ingested as a feed supplement in suficient numbers, probiotics are live microorganisms that beneficially affect the gastrointestinal balance going far beyond the conventional nutritional effect (Penner *et al*., 2005). Many studies report positive effects of probiotic supplementation on the performance and health of animals. Despite the fact that several studies have shown disease prevention or immune enhancement resulting from oral administration of probiotics (Billoo *et al*., 2006; Cong *et al*., 2003; Galdeano and Perdigon, 2006) few studies are available on their specific effects on the gut defense mechanisms, the mechanisms underlying the immune modulating properties of probiotics are not fully understood.

Alashan Bactrian camels inhabit the desert area of China they were praised boat in the desert in China. With the worsen of the survival environment of the camel, the gastrointestinal disease is becoming more and more, mortality of young camel is increased. It has been established in laboratory rodents that lactic acid bacteria given orally can signicantly affect both the systemic and mucosa-associated immune responses (Perdigon and Alvarez, 1992). Despite the fact that several studies have shown disease prevention or immune enhancement resulting from oral administration of probiotics, few studies are available on their specic effects on the gut defense mechanisms in camels. The present research was conducted to help characterize some of these actions, the specific objectives were to determine the effects of *L. acidophilus* L3 on the number of intestinal mucosal immune cells (intraepithelial lymphocytes, goblet cells, plasma cells and mast cells) of young camels. If *L. acidophilus* L3 has obviously positive effect on camel and enhance the camel's defense system, growth performance will be improved, diarrhea, mortality and morbidity will also be decreased.

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MATERIALS AND METHODS

Study animals: All experimental procedures were approved by the welfare authority of Mingqing County of Gansu Province.

A group of 16 healthy young camels (1-1.5 years) was randomly divided into two groups, one of which (probiotic group) was supplemented with *L. acidophilus* L3 which was procured from China General Microbiological Culture Collection Center (CGMCC) while the other group of 8 camels remained untreated (control group). *L. acidophilus* L3 was provided to the probiotic group as a feed supplement at a concentration of 2×10^9 CFU kg^{-1} feed. The probiotic group received the food supplemented with *L. acidophilus* L3 for 28 days, then the animals were anesthetized with sodium pentobarbital and were then exsanguinated. Researchers investigated the small intestine of the probiotic group and the control group, the abdomen was incised and the small intestine was taken out. Tissues were taken for histology from duodenum, distal jejunum and terminal ileum and they were fixed in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 18 h at 4°C and processed routinely for wax histology, paraffin sections were stained by the following methods: hematoxylin and eosin, Periodic Acid-Schiff, Unna-Pappenheim methyl green-pyronin and toluidine blue those Staining Methods were used to show Intraepithelial Lymphocytes (IELs), goblet, plasma and mast cells.

Cell Count Method: IELs and goblet cells were counted using the same standard microscope. The total number of IELs and goblet cells per 100 epithelial cells were counted at 400x magnification on haematoxylin/eosin stained slides and Periodic Acid-Schiff stained slides and the mean number was recorded for each case. Results were expressed as IELs/100 villus epithelial cells and GCs/100 villous epithelium cells. The number of plasma cells and mast cells per 5 fields at a magnification of 400x was counted under light microscopy. These results were assessed semi quantitatively by two researchers and the average count was used as the final score.

Statistical analysis: All the date were analysed using Independent t-test and one way ANOVA (SPSS for Windows Version 11.5). The statistical analysis was based on the comparison between the groups (using independent t-test) and comparison within the groups (using one way ANOVA). Differences were considered statistically significant where $p<0.05$.

RESULTS

The comparison of the number of IELs in the probiotic group and the control group: As shown in Table 1, the number of IELs in small intestine of young camel in the probiotic group was more than control group. The difference between two groups was statistically significant ($p<0.05$). The number of IELs in duodenum, jejunum and ileum increased by 59.97, 62.54 and 54.52% in the probiotic group compared with the control group. The present investigation indicated that the number of IELs was gradually reduced from duodenum to jejunum in two groups (Fig. 1-8).

The comparison of the number of goblet cells in the probiotic group and the control group: As shown in Table 2, the number of epithelial goblet cells in small intestine of camel in the probiotic group was more than the control group. The difference between two groups was statistically significant ($p<0.05$). The number of epithelial goblet cells in duodenum, jejunum and ileum increased by 38.11, 45.17 and 53.80% in the probiotic group compared with the control group. From duodenum to jejunum, the number of epithelial goblet cells was gradually reduced in two groups and the difference was statistically significant ($p<0.05$) (Fig. 1-8).

The comparison of the number of plasma cells in the probiotic group and the control group: As illustrated in

Table 1: Number of intraepithelial lymphocytes in small intestinal of young camel in the control and probiotic groups ($\bar{x}\pm S$)

	(IELs/100 villous epithelium cells)			
Groups	Duodenum	Jejunum	Ileum	Small intestinal
Control	20.01 ± 1.99^{Aa}	16.23 ± 1.73^{Ba}	13.06 ± 1.25^{Ca}	16.37 ± 2.31^{a}
Probiotic	32.01 ± 3.04^{Ab}	26.38 ± 3.17^{Bb}	20.18 ± 2.03^{Cb}	26.19 ± 3.95^{b}

The data with different capital letter within the same row or column differ significantly ($p<0.05$). The capital letter (A, B, C) represent different small intestine segment in the same group, lowercase (a, b) represent the same small intestine segment in different group

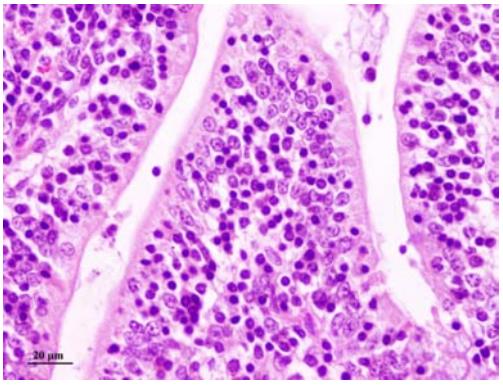

Fig. 1: Light photomicrograph of the IELs in small intestine of young camel in the control group (H&E, bar = 20 μm). The arrow points to the IEL

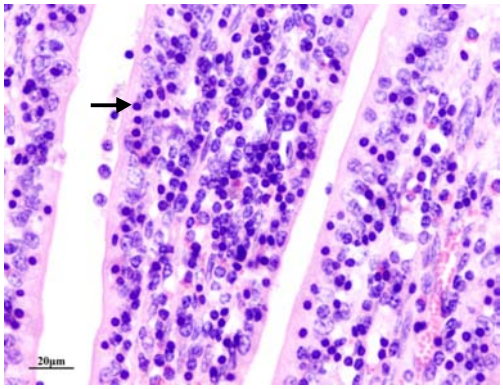

Fig. 2: Light photomicrograph of the IELs in small intestine of young camel in the probiotic group (H&E, bar = 20 μm). The arrow points to the IEL

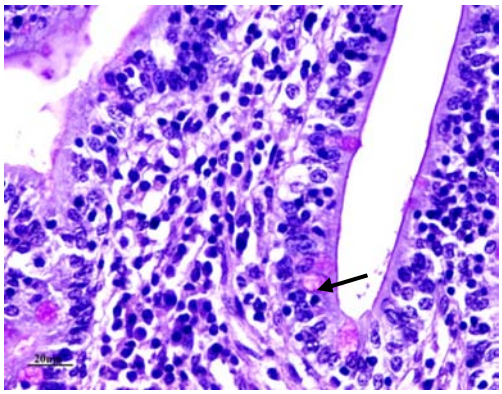

Fig. 3: Light photomicrograph of the goblet cells in small intestinal epithelium of young camel in the control group (Periodic Acid-Schiff stain, bar = 20 μm). The arrow points to the goblet cell

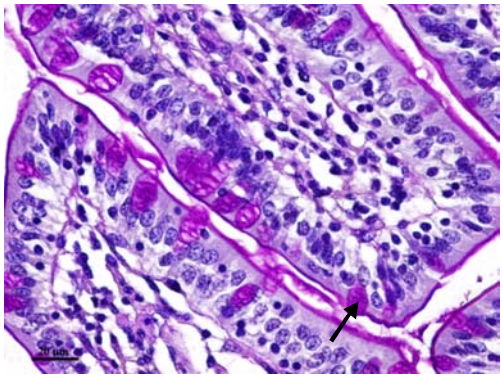

Fig. 4: Light photomicrograph of the goblet cells in small intestinal epithelium of young camel in the probiotic group (Periodic Acid-Schiff stain, bar = 20 μm). The arrow points to the goblet cell

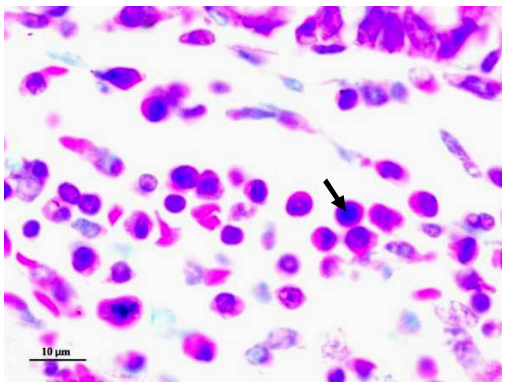

Fig. 5: Light photomicrograph of the plasma cells in small intestine of young camel in the control group (Unna-Pappenheim methyl green-pyronin stain, bar = 10 μm). The arrow points to the plasma cell

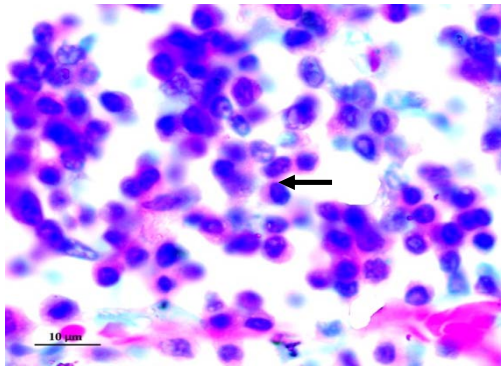

Fig. 6: Light photomicrograph of the plasma cells in small intestine of young camel in the probiotic group (Unna-Pappenheim methyl green-pyronin stain, bar = 10 μm). The arrow points to the plasma cell

Table 2: Number of goblet cells in small intestinal of young camel in the control and probiotic groups ($\bar{x}$±S)

	GCs/100 villous epithelium cells			
Groups	Duodenum	Jejunum	Ileum	Small intestinal
Control	18.13 ± 2.07^{Aa}	15.12 ± 1.91^{Ba}	13.16 ± 1.55^{Ca}	15.47 ± 2.20^{a}
Probiotic	25.04 ± 3.11^{Ab}	22.03 ± 2.34^{Bb}	20.24 ± 2.54^{Cb}	22.44 ± 3.08^{b}

The data with different capital letter within the same row or column differ significantly ($p<0.05$). The capital letter (A, B, C) represent different small intestine segment in the same group, lowercase (a, b) represent the same small intestine segment in different group

Table 3 the number of plasma cells in small intestine of camel in the probiotic group was more than the control group and the difference between two groups was statistically significant ($p<0.05$). At the same time, the number of plasma cells in duodenum, jejunum and ileum increased by 63.49, 52.11 and 48.52% in the probiotic

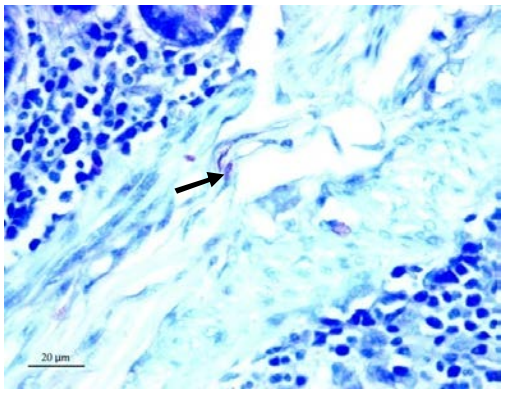

Fig. 7: Light photomicrograph of the mast cells in small intestine of young camel in the control group (toluidine blue stain, bar = 20 μm). The arrow points to the mast cell

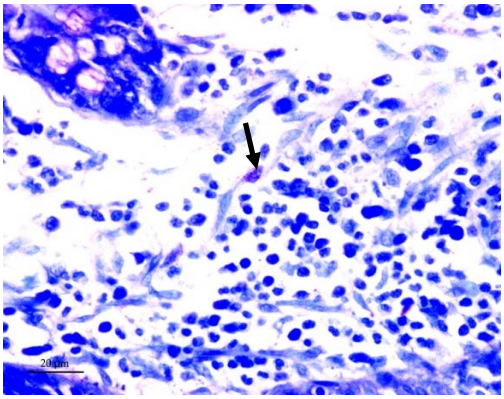

Fig. 8: Light photomicrograph of the mast cells in small intestine of young camel in the probiotic group (toluidine blue stain, bar = 20 μm). The arrow points to the mast cell

group compared with the control group. The number of plasma cells was gradually increased from duodenum to jejunum in two groups and the number of plasma cells in duodenum and jejunum was significantly different ($p<0.01$) whereas there was no difference in the number of plasma cells in jejunum and ileum ($p>0.05$). Histological observation found that plasma cells were mainly distributed in the lamina propria of small intestine there were few plasma cells in the solitary lymphoid nodule and aggregated lymphoid nodules.

The comparison of the number of mast cells in the probiotic group and the control group: The number of mast cells in the infected and normal group is shown in Table 4, researchers found that the number of mast cells in the probiotic group was higher than the control group

Table 3: Number of plasma cells in small intestinal of young camel in the control and probiotic groups ($\bar{x}\pm S$)

	Plasma cells/HPF			
Groups	Duodenum	Jejunum	Ileum	Small intestinal
Control	11.23 ± 1.28^{Ba}	21.13 ± 2.18^{Aa}	22.34 ± 2.76^{Aa}	18.23 ± 1.39^{a}
Probiotic	18.36 ± 2.04^{Bb}	32.14 ± 3.87^{Ab}	33.18 ± 3.45^{Ab}	27.89 ± 3.01^{b}

Table 4: Number of mast cells in small intestinal of young camel in the control and probiotic groups ($\bar{x}\pm S$)

	Mast cells/HPF			
Groups	Duodenum	Jejunum	Ileum	Small intestinal
Control	29.13 ± 3.04^{Aa}	23.06 ± 2.87^{Ba}	17.89 ± 1.96^{Ca}	23.36 ± 2.25^{a}
Probiotic	39.15 ± 4.12^{Ab}	32.01 ± 3.98^{Bb}	26.85 ± 2.94^{Cb}	32.67 ± 3.23^{b}

The data with different capital letter within the same row or column differ significantly ($p<0.05$). The capital letter (A, B, C) represent different small intestine segment in the same group, lowercase (a, b) represent the same small intestine segment in different group

and the difference between two groups was statistically significant ($p<0.05$). The number of mast cells in duodenum, jejunum and ileum increased by 34.40, 38.81 and 50.08% in the probiotic group compared with the control group. The number of mast cells was gradually decreased from duodenum to jejunum in two groups and the difference was statistically significant ($p<0.01$). Histological observation demonstrated that mast cells were mainly distributed around the intestinal gland, blood vessels and lymphatic vessels (Fig. 6-8).

DISCUSSION

This is the first report of the effects of oral administration of *L. acidophilus* L3 on the number of intestinal mucosal immune cells of young camels, the results of this study showed that all the immune cells (intraepithelial lymphocytes, goblet cells, plasma cells and mast cells) in small intestine of camel were increased in the probiotic roup.

The intestinal tract is the crossroad between the needs of nutrient absorption and host defense (Macdonald and Monteleone, 2005). It has been suggested that the gut has the most important role in the maintenance of homeostasis of the body (MacDonald and Monteleone, 2005). As a complicated immune system tissue, the intestinal tract plays a critical role in the first line of defense against ingested pathogens. The main site of the mucosal immune system in the intestine is referred to as gut-associated lymphoid tissue and immune associated cells including IELs, goblet cells, plasma cells and mast cells are involved in many processes to prevent pathogen invasion (Oswald, 2006). The collaboration of those immunocompetent cells and probiotic help the animals to compete against all kinds of infectious pathogens (Blum and Schiffrin, 2003).

The gastrointestinal tract plays the key role in uptake of fluids and nutrients and at the same time it forms the main protective barrier between the sterile environment of the body and the outside world (Artis, 2008). The intestinal Intraepithelial Lymphocytes (IELs) form the first line of the host immune defence system and play an essential role against infections caused by certain microorganisms or parasite (Guk *et al*., 2003; Inagaki-Ohara *et al*., 2006). This study found that the number of of IELs in small intestine of camel in the probiotic group was more than normal group at the same time, numerous investigators have demonstrated that raised IELs are often seen after feeding a probiotic. Dalloul *et al*. (2003) examined the effects of feeding a Lactobacillus-based probiotic on the intestinal IEL subpopulations and any subsequent enhancement of intestinal immunity against coccidiosis they found that the number of IELs in the probiotic group was more than the control group they also found that IELs sustain the epithelial barrier function against coccidiosis infection during coccidiosis infection, IEL increased production of gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) and decreased transforming growth factor beta (TGF-β) production. These results suggest that IEL play important multifunctional roles in protection of the epithelium against infections. The increased number of IELs in small intestine of young camels in this research indicate that *L. acidophilus* L3 has the function of immune stimulation, oral administration of *L. acidophilus* L3 can enhance the intestinal mucosal immunity.

The study find that the number of epithelial goblet cells in small intestine of camel in the probiotic group was more than the control group. Researchers know that goblet cell can secrete mucins, mucins are the major protein components of the protective mucus barrier that cover epithelial surfaces in the gastrointestinal tract. This barrier is considered a first line of defense against colonization by gut pathogens (Harrison *et al*., 1999). Mahdavi *et al*. (2005) found using different levels of probiotic caused highly significant increase ($p<0.01$) in goblet cell numbers they provide the probiotic feed containing *Bacillus subtilis* (CH201) and Bacillus licheniformis (CH200) to hens for 12 weeks, the number of intestinal goblet cells markedly increased in the probiotic group. Another research also found that the dietary probiotic significantly increase the number of goblet cells and mucins throughout the small intestine compared with the other groups in chickens (Smirnov *et al*., 2005). Goblet cell mucins play a key role in mucosal defence, it seem as the selective barrier for the intestinal pathogens thus the increase in the number of goblet cells seem to be an unspecific defensive mechanism. The hyperplasia of epithelial goblet cells in small intestine of camels show that *L. acidophilus* L3 has the function of immune stimulation, oral administration of *L. acidophilus* L3 can reinforcement of the intestinal mucosal barrier against infection.

The study found that the number of plasma cells in each part of the small intestine in the probiotic group was more than that of the control group and the difference between two groups was statistically significant. The main founction of plasma cells is to produce Immunoglobulin A (IgA) most of the IgA in the gut is generated by B cells in the PP germinal centers (McGhee, 2005; Mestecky and Elson, 2008). On epithelial surfaces, the main specific immune defense of the host is the protection afforded by secretory IgA antibodies. In a study of Jain *et al*. (2008), the protective effect of probiotic dahi supplemented with *Lactobacillus acidophilus* and *L. casei* against *Salmonella enteritidis* infection in mice is investigated. The 7 days pre-feeding with probiotic dahi significantly increased anti-*S. enteritidis* secretary IgA antibodies and lymphocyte proliferation in *S. enteritidis* infected mice. The mucosal immune system forms the largest part of the entire immune system, containing about three quarters of all lymphocytes and producing grams of secretory IgA daily to protect the mucosal surface from pathogens (Macpherson, 2006). A great deal of IgA secreted by plasma cells can prevent the pathogens inhabiting.

The probiotic group showed apparently higher number of mast cells in each part of the small intestine than that of the control group. The increase of mast cell in the intestinal mucosa is known to play an important role in host defense against infections (Caldwell *et al*., 2004; Zareie *et al*., 2006). Some studies indicate that intestinal mucosal mast cells play an important role in the local mucosal immune response (Caldwell *et al*., 2004; Morris *et al*., 2004). Mast cells are important immunocompetent cells in the intestinal mucosal immune response that exert multifunctional roles by releasing prestored and de novo synthesized mediators such as histamine, proteases, serotonin and others (Metcalfe *et al*., 1997).

The present research supported that the specific effects on the gut defense mechanisms was that *L. acidophilus* L3 has intense influence on the number of mucosal immune cells, the increased intestinal mucosal immune cells can enhance the defense system of the body so the diarrhea, mortality and morbidity will be decreased.

CONCLUSION

The present study suggests that *L. acidophilus* L3 has intense influence on the number of mucosal immune

cells (IELs, goblet cells, plasma cells and mast cells) in small intestine of young camels, the hyperplasia of those cells can strengthen the anti-infections ability of camels. The main action of *L. acidophilus* L3 can be summarized as a reinforcement of the intestinal mucosal barrier and increase the number of intestinal mucosal immune cells.

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