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Full Research Article

Effect of Live *Lactobacillus acidophilus* NCDC 15 and CURD as probiotics on Blood Biochemical Profile of Early Weaned Piglets

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Abstract

The present experiment was done to ascertain the effect of *Lactobacillus acidophilus* NCDC 15 and curd as probiotics on blood biochemical profile of early weaned piglets. A total of 36 crossbred (Landrace×Desi) piglets weaned at 28 day were assigned to three different treatments (Control group, *L. acidophilus* NCDC 15 fed group, Curd fed group; n=12) each containing 4 replicates of 3 animals following completely randomized block design. Piglets of control group (first) were fed basal diet without any probiotics, while second and third groups were fed basal diet fermented with live *L. acidophilus* NCDC 15 (200 g day^{-1} $piglet^{-1}$; 1.7×10^9 cfu g^{-1}) and Curd (200 g day^{-1} $piglet^{-1}$; 2.0×10^9 cfu g^{-1}), respectively for a period of 120 days. The blood was collected on 0, 60 and 120 days of experimental period. The mean glucose level was significantly ($p<0.01$) higher in curd fed group as compared to *L. acidophilus* NCDC 15 fed group followed by control group. Total cholesterol level was significantly ($p<0.05$) lower in both the *L. acidophilus* NCDC 15 and curd fed group as compared to control group. The period-wise comparison of mean values of serum albumin was similar among the groups; however, period-wise comparison of total protein and globulin was significant. The results indicated that supplementation of live *Lactobacillus acidophilus* NCDC 15 and curd had higher glucose but low cholesterol level with similar serum protein value, which indicated good utilization of feed as well as health status of early weaned piglets.

1. Introduction

Weaning of piglets is a crucial factor since it is a very stressful experience for young piglets because of changes from milk to a solid feed. When piglets are weaned, the intestinal microflora of piglets is altered (Jensen, 1998). It has been suggested that it may take 4 to 6 weeks to establish a stable flora (Mul and Perry, 1994). The gut represents a dynamic microbial ecosystem in which microorganisms in the intestine (microflora) have important and specific metabolic and protective functions (Massi et al., 2004). A gut imbalance frequently occurs in post weaning piglets due to increase population of *E. coli* and decrease population of *Lactobacillus* (Thu et al., 2011). After weaning, piglets are commonly exposed to multi-stressors such as nutritional, psychological, microbiological, environmental and immunological. To ameliorate the weaning stress by maintaining GIT microflora through probiotics can be one of the strategies. Maintaining intestinal microflora balance in animals is important to prevent diseases by controlling the overgrowth of potentially pathogenic bacteria as well. Earlier antibiotics were used for growth promotion and prophylactics against gastrointestinal diseases but the injudicious use of antibiotics could lead to bacterial resistance and certain amount of residues in animal products. Therefore, maintenance of intestinal microflora balance through a non-antibiotic approach is urgently needed and for that probiotics is now preferred over antibiotics. With the growing popularity of yoghurt/curd as probiotic carrier food, yoghurt is emerging as a new therapeutic leading edge with different diseases. Therefore, an attempt was made to study the effect of feeding *Lactobacillus acidophilus* NCDC 15 and curd (mixed culture) on the health of the growing piglets using blood indices as indicator for the health status of the animals.

2. Materials and Methods

2.1. Ethical approval

Prior approval for experiments was taken from Institutional Animal Ethics Committee as per CPCSEA (Govt. of India) norms.

2.2. Animals and diets

Experiment was conducted on a total of 36 [18 Male (M) and 18 Female (F)] crossbred (Landrace×Desi) piglets weaned at day 28 selected from Swine production farm, Indian Veterinary Research Institute, Izatnagar from November, 2013 to April, 2014. Piglets were assigned to three different groups (Control group, *L. acidophilus* NCDC 15 fed group, Curd fed group) containing 4 replicates of 3 animals in each following completely randomized block design. First group was fed basal diet without probiotic *i.e.* control group while second and third groups were fed basal diet fermented with live *L. acidophilus* NCDC 15 and Curd, respectively. Basal diet was formulated with maize, soyabean meal, fish meal, wheat bran, salt, mineral mixture and vitamin supplements as per NRC (1998). Physical composition of the diet was as given in (Table 1). Feed was fermented with *Lactobacillus acidophilus* NCDC 15 (1.7×10^9 cfu g^{-1} feed) and curd (2.0×10^9 cfu g^{-1} feed) as described below and fed @ 200 g (on fresh basis) per piglet per day to second and third groups, respectively. The study was continued for 120 days.

2.3. Biochemical parameters of serum samples

Blood was collected at 0, 60 and 120 days of experimental trial and serum was collected and then stored at -20 °C until analysis. Finally, after thawing, serum was analyzed for biochemical profiles (glucose, total protein, Albumin, globulin, total cholesterol and Triglycerides) by standard protocol as per kit (Span Diagnostics Ltd., India) using spectrophotometer.

2.4. Fermented feed preparation from L. acidophilus NCDC 15

Lactobacillus acidophilus NCDC 15 was grown in Man Rogosa and Sharpe (MRS) broth for 24 h at 37 °C. Basal feed (2 kg) was mixed with 2000 ml of tap water, inoculated with 200 ml of 24 h old *Lactobacillus* culture (10% inoculum) and incubated for 24 h at 37 °C. The fermented material was fed as 200 g^{-1} day^{-1} animal and the same fermented material was used as inoculum (20%) for preparation of next day's fermented feed. After 15 days, fresh culture was taken and as described above and used consecutively for next 15 days.

Table 1: Physical composition of diets for piglets

Ingredients (%)	Body weights (kg)			
	5-10	10-20	20-50	50-80
Crushed maize	46	54	62	71
Deoiled soybean meal	30	22	15	10
Wheat bran	16	16	15	13
Fish meal	06	06	06	04
Mineral mixture	1.5	1.5	1.5	1.5
Common salt	0.5	0.5	0.5	0.5
CP (%)	23.7	20.8	18.4	15.5
DE (kcal kg^{-1})	3400	3399	3399	3390

2.5. Preparation of fermented feed from curd

The 200 ml of curd was directly used as inoculums (10% inoculum) and mixed with feed which were incubated for 24 hours at 37 °C. The fermented material was fed as 200 g day^{-1} $piglet^{-1}$ and the same fermented material was used as inoculum (20% of concentrate mixture) for preparation of next day's fermented feed. A similar process was followed as described above.

2.6. Evaluation of probiotic product

The counting of lactic acid bacteria was done by using pour plate method in MRS agar (HiMedia Lab) at pH 6.8. 90 ml normal saline (0.85%) were prepared in 250 ml Erlenmeyer flasks and distributed into test tubes (9 ml). MRS agar, test tubes having normal saline were autoclaved at 15 lbs pressure for 20 minutes. Fermented feed (1 g) was mixed with 9 ml of sterile normal saline. The content was mixed for 4-5 minutes in vortex mixture and the supernatant (1 ml) was used for the colony counting. The sample was diluted up-to 10 folds (10^{10}) with normal saline (0.85%). After serial dilution, from appropriate dilution, 1 ml culture was poured in petri plates and 15-20 ml of MRS agar was poured and mixed gently by circular motion. The inoculated plates were incubated at 37 °C for 48 hours. Individual colonies were counted after 48 hours of incubation and the average of duplicate plates was taken. The average number of colonies was multiplied by the dilution factor to get the number of colony forming units (CFU) per gram of sample.

The count of *Lactobacillus* sp. was done at 1st, 4th, 7th, 9th, 12th and 15th of each 15 days batch and mean was taken to observe the CFU of *Lactobacillus* g^{-1} of sample actually fed to the animal. The fermented feed was used as probiotic and this fermented feed was evaluated periodically to see the status of the probiotics cells. It was observed that the number of cells went down gradually, hence after 15 days a fresh culture was used to prepare fermented probiotic product, and *Lactobacillus* spp. (feed fermented with curd) was presented in (Table 2).

Table 2: Microbial profile of probiotics at different periods

Mean of Each 15 days batch	*Lactobacillus acidophilus* NCDC 15 (cfu g^{-1})	*Lactobacillus* spp. (cfu g^{-1}) (feed fermented with curd)
1st d	3.1×10^9	3.3×10^9
4th d	2.8×10^9	3.2×10^9
7th d	2.2×10^9	2.9×10^9
9th d	2.0×10^9	2.3×10^9
12th d	1.8×10^8	2.2×10^8
15th d	1.6×10^8	1.7×10^8
Average	1.7×10^9	2.0×10^9
pH	3.9-4.6	4-4.8

2.7. Statistical analysis

The experimental data generated were analyzed using statistical package SPSS 20.0 and means were compared using Duncan's Multiple Range Test (Snedecor and Cochran, 1994).

3. Results and Discussion

Serum profiles are affected by age, nutrition, heredity, and various diseases. The amount of serum glucose, cholesterol, total protein, triglycerides, albumin and globulin under different treatment groups has been presented in (Table 3).

In the present study, the mean glucose level was significantly (p<0.01) higher in curd supplemented group as compared to *Lactobacillus acidophilus* NCDC 15 supplemented group followed by control. It might be due to the fact that higher glucose level required during the growing period. The present findings were in agreement with the observation of Kumar et

Table 3: Effect of probiotic feeding on blood biochemical parameters in piglets

Treatment	Period (Days)			Treatment mean±SE	P value		
	0	60	120		T	P	T*P
Glucose (mg dl^{-1})							
Control	98.16±0.76	107.90±0.83	108.09±0.73	104.71±0.90^{a}	0.000	0.000	0.000
L. acidophilus	97.69±0.88	115.33±0.85	118.55±0.91	110.52±1.63^{b}			
Curd	98.08±0.77	118.69±0.91	121.97±0.93	112.91±1.85^{c}			
Mean±SE	97.98±0.45^{a}	113.97±0.90^{b}	116.20±1.11^{c}				
Cholesterol (mg dl^{-1})							
Control	52.03±3.74	50.24±3.55	53.46±3.06	51.91±1.95^{b}	0.023	0.010	0.132
L. acidophilus	51.79±3.66	44.67±3.22	40.20±2.90	45.55±2.01^{a}			
Curd	52.28±3.43	46.77±2.24	38.88±1.45	45.98±1.69^{a}			
Mean±SE	52.03±2.03^{b}	47.23±1.76^{ab}	44.18±1.82^{a}				
Triglyceride (mg dl^{-1})							
Control	30.99±0.88	34.43±0.98	35.12±1.00	33.51±0.61	0.393	0.000	0.580
L. acidophilus	31.50±0.89	32.71±0.93	34.25±0.97	32.82±0.55			
Curd	30.23±1.05	34.02±0.68	32.83±1.65	32.36±0.72			
Mean±SE	30.91±0.54^{a}	33.72±0.50^{b}	34.07±0.72^{b}				
Total protein (g dl^{-1})							
Control	7.70±0.22	8.27±0.24	8.02±0.24	8.00±0.14	0.536	0.008	0.594
L. acidophilus	7.65±0.28	8.48±0.25	8.50±0.25	8.21±0.16			
Curd	7.91±0.26	8.13±0.25	8.50±0.24	8.18±0.15			
Mean±SE	7.75±0.14^{a}	8.29±0.14^{b}	8.34±0.14^{b}				
Albumin (g dl^{-1})							
Control	3.63±0.30	3.59±0.30	3.71±0.30	3.64±0.17	0.965	0.841	0.978
L. acidophilus	3.74±0.31	3.45±0.29	3.55±0.30	3.58±0.17			
Curd	3.70±0.30	3.62±0.23	3.52±0.29	3.61±0.16			
Mean±SE	3.69±0.17	3.55±0.15	3.59±0.17				
Globulin (g dl^{-1})							
Control	4.07±0.36	4.68±0.37	4.31±0.37	4.35±0.21	0.654	0.047	0.667
L. acidophilus	3.91±0.43	5.03±0.37	4.95±0.37	4.63±0.24			
Curd	4.21±0.39	4.51±0.34	4.98±0.42	4.57±0.22			
Mean±SE	4.06±0.22^{a}	4.74±0.21^{b}	4.75±0.22^{b}				
A:G							
Control	1.01±0.13	0.86±0.11	0.97±0.13	0.95±0.07	0.855	0.165	0.717
L. acidophilus	1.14±0.16	0.76±0.09	0.79±0.10	0.89±0.07			
Curd	1.00±0.13	0.88±0.10	0.83±0.14	0.90±0.07			
Mean±SE	1.05±0.18	0.83±0.06	0.87±0.27				

^{abc}Means bearing different superscripts in a row differ significantly: (p<0.05) (p<0.01) al. (2012). However, level of glucose in all the groups was within the normal physiological range (Kaneko et al., 2008). Similarly, significant increased in serum glucose levels in probiotic treated buffalo calves was observed by Bakr et al. (2009). The serum protein concentration at any given time is in turn a function of hormonal balance, nutritional status, water balance and other factors affecting the state of health. The serum protein level indicates the balance between anabolism and catabolism protein in the body. In the present study, serum total proteins, albumin and globulin remained within normal range (Kaneko et al., 2008) and did not differ significantly among different dietary treatments. The period-wise comparison of mean values of total protein and serum globulin were significantly (p<0.05) lower at day 0 than 60 and 120. This may be due to improvement in animal appetite and feed utilization by the animals Kumar et al. (2012). The finding was also in harmony with that recorded by Bakr et al. (2009) who reported no significant difference in the levels of serum globulin in probiotic fed calves; however, they observed a significant increase in the blood serum levels of total proteins. Cholesterol and triglyceride levels in piglet blood are affected by age, heredity, nutrition and various diseases. The mean serum triglyceride level was comparable among the treatments; however, mean serum triglyceride level was significantly higher at day 60 and 120 as compared to day 0. A similar result was found by Kumar et al. (2012) who also observed no significant difference between probiotic fed piglets and control during 120 days of study. The serum cholesterol level was significantly (p<0.05) lower in probiotic fed groups as compared to control group. As period-wise comparison, the mean value at day 120 showed significant decrease (p<0.01) in cholesterol level than day 0, whereas at day 60 it was statistically similar with 0 and 120 day In the agreement with the result of cholesterol in this experiment, it is reported that supplementation of crushed maize fermented with live *Saccharomyces cerevisiae* (2×10^6 cfu g^{-1} feed) and fed @ 200 g $piglet^{-1}$ significantly reduces serum cholesterol as compared to control (Kumar et al., 2012). Similarly, it is reported that the use @ 100 mg kg^{-1} of the probiotic supplement (*L. acidophilus, Bifidobacterium* and *Aspergillus oryzae*) significantly decreases the serum cholesterol level of chickens (Panda et al., 2001). Similarly Zacconi et al. (1992); Taranto et al. (1998) have also reported that the serum cholesterol was lower in mice receiving diet containing probiotics. This may be due to the probiotic exerts their action by the activity of lactic acid bacteria, production of enzymes, disintegrating bile salt and de-conjugating them, as well as decreased intestinal pH and simultaneously, they are absorbed less from the intestine and are excreted more in feces (Klaver and Van der Meer, 1993). Consequently, the liver converts more cholesterol concentration into the tissues and therefore their level in the blood is reduced (Ros, 2000).

Probiotic bacteria with active bile salt hydrolase or products containing them have been suggested to lower cholesterol levels through interaction with host bile salt metabolism (De Smet et al., 1998). Several studies (Taranto et al., 1998; Grunewald, 1982; Xiao et al., 2003) have shown that probiotic has the ability to reduce cholesterol in blood. In agreement with these findings we found a significantly decreased cholesterol level over the period-wise might be due to the fact that cholesterol is required during growing period for development of muscle and tissue. Age of piglets also played a part in the level of serum cholesterol; the older the piglets were the lower the serum cholesterol level.

4. Conclusion

Supplementation of live *Lactobacillus acidophilus* NCDC 15 and curd had higher glucose but low cholesterol level with similar serum protein value, which indicated good utilization of feed as well as health status of early weaned piglets. Hence, curd can be utilised as an alternative to probiotics for the feeding in early weaned piglets.

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