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Effect of fermented liquid feed on feed efficiency, gut health and immunity of Large White Yorkshire (LWY) fattener pigs

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ABSTRACT

The study assessed effect of fermented liquid feed (FLF) on feed efficiency, gut health and immunity of LWY fattener pigs. Weaned piglets (24) were assigned four dietary treatments (T_1, T_2, T_3, T_4) in two phases in a randomised block design for 180 days. T_1 was fed standard ration (SR), T_2 was fed SR mixed with water (1:2, w/w), T_3 and T_4 were fed FLF fermented by *Lactobacillus acidophilus* (T_3) and *Enterococcus faecium* (T_4) respectively. Feed efficiency was calculated fortnightly; diarrhoea score and incidence up to day 14; faecal pH, NH_3-N, lactic acid, LAB, *Escherichia coli* and *Salmonella* on day 1, 60, 120 and 180; immune responses and histomorphology of small intestine at 42nd day. Final body weight and gain, feed efficiency was significantly higher in T_3 and T_4. The DS and DI reduced significantly in T_3 and T_4. Increased faecal pH and reduced NH_3-N recorded in T_3 and T_4 on 180th and 120th day respectively. Faecal LAB was significantly higher on 120th day and increasing trends on 60th and 108th day recorded in T_3 and T_4. Faecal *Escherichia coli* and *Salmonella* was significantly reduced in T_3 and T_4. Increased VH, decreased CD and higher AVSA with infiltration of mononuclear and lymphoid follicles observed in T_3 and T_4. Antibody titre (HI $\log_{10}$/ml) on 7th and 21st day post-inoculation was recorded significantly higher in T_3 and T_4. Based on findings of the study, feeding of FLF by *Lactobacillus acidophilus* or *Enterococcus faecium* was recommended to LWY fattener pigs.

Keywords: Faecal metabolites, Feed efficiency, Fermented liquid feed, Gut health, Immunity, Pig

Pig farming generates valuable animal protein accounting to 30% of the global meat production annually (FAO 2023). According to 20th Indian Livestock census (2019), pig population is 9.06 million, which is 1.69% of the total livestock population of India. Piggery provides livelihood resource to poor rural communities/farmers and source of supplementary and cash income for the seasonally employed rural farmers, unemployed youth, women and agri-entrepreneurs.

Optimum feeding is essential for economically sustainable piggery. In the recent years, feeding cost has increased manifold due to increased feed ingredient prices because of the raw material crisis in all the countries across the globe (Anonymous 2022). Searching ways to improve feed efficiency has become inevitable in today's scenario as feeding cost accounts nearly 2/3rd of total production cost in piggery. Feed delivery methods have significant impact on feed utilization by pigs. During recent decades, feeding of fermented liquid feed (FLF) has gained momentum for advantages over other feed delivery methods and has emerged as one of the promising alternatives to convention feeding methods. FLF favourably modifies intestinal microbiota, improves feed consumption and digestibility and growth performance. It can also be considered as cost-effective and bio-safe alternatives to antibiotics growth promoters in pig rations (Xu *et al*. 2020, Xin *et al*. 2021, Cullen *et al*. 2021).

Positive correlation exists between feed efficiency and gut health of pigs. Optimum gut health favours effective digestion and absorption, stable intestinal microbiota, effective immunity and wellbeing. FLF can favourably affect gut health by reducing digestive problems, inhibiting pathogens, preventing atrophy of intestinal villi, positively affecting integrity of intestinal barrier function and stimulating immune system (van Winsen *et al*. 2000, Wang *et al*. 2023, Sun *et al*. 2024). Dry or liquid feed delivery methods result in considerable wastage of feed, may affect digestion by changing physiological processes of GIT, contribute respiratory problems for dustiness, and have chances of toxicity for shorter shelf life and spoilage. Considering potentialities of FLF, present study attempted to standardise a method of preparing FLF with *Lactobacillus acidophilus* and *Enterococcus faecium* and to study effects on feed efficiency, gut health and immunity of LWY fattener pigs under intensive system of management.

MATERIALS AND METHODS

Location of study and experimental design: The

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experiment was carried out during 2018-19 at the experimental animal unit of Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl, Mizoram. The study was approved by the Institutional Animal Ethics Committee (IAEC) (Letter no. 770/ac/CPCSEA/F.V.Sc./AAU/IAEC/17-18/481 dated 09.08.2017).

Twenty-four weaned LWY piglets (11.45±2.42 to 11.46±2.37 kg) were selected and randomly allotted to four dietary treatments (T_1, T_2, T_3 and T_4) in a randomised block design (CRD) considering each animal as one replicate. T_1 was offered standard ration (SR) in dry form, T_2 was fed non-fermented liquid feed (SR: water 1:2, w/w), T_3 and T_4 were offered FLF prepared by fermenting SR (SR: water 1:2, w/w) with *Lactobacillus acidophilus* and *Enterococcus faecium,* respectively. The experiment was conducted for 180 days in two phases - grower phase (6^{th} to 18^{th} week of age) and finisher phase (19^{th} to 30^{th} week of age).

Preparation of fermented liquid feed (FLF): Freeze-dried cultures of *Lactobacillus acidophilus* (ATCC 11975) and *Enterococcus faecium* (ATCC 19434) were procured from National Collection of Dairy Cultures, Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal (Haryana), India-132001 and revived through standard procedure viz. a loop full of culture was transferred aseptically to 100 ml of De Man, Rogosa and Sharpe (MRS) broth for *Lactobacillus acidophilus* and to 100 ml Brain Heart Infusion (BHI) broth for *Enterococcus faecium* and incubated for 24 hours at 37°C in an automatic incubator shaker. This 100 ml 24 hours old culture was then used for fermenting 1 kg ground maize moistened with water (1:1, w/w) at 37°C for 24 hours. This fermented maize was further utilized for fermenting SR after mixing with water (1:2, w/w) at 1:5 (w/w) and fermented for 48 hours at room temperature. After 48 hours, 50% of the FLF was utilized for feeding to pigs of T_3 and T_4 and the remaining 50% FLF was utilized for back-slopping for another 7 days.

Feeding and housing management of experimental pigs: The pigs were housed individually in cemented floor pens equipped with feeding and water troughs. Feed was offered twice daily (morning 8.00-9.00 AM; evening 2.00-3.00 PM) to allow *ad libitum* feeding with the provision for free access for drinking water. Vaccination and deworming were done as per schedules for growing and finishing pigs.

Calculation of feed conversion efficiency (FCR) of pigs: The feed conversion ratio (FCR) was calculated as the ratio of total feed consumed (kg) and total body weight gain (kg) at fortnightly interval throughout the feeding trial.

Calculation of diarrhoea score (DS) and incidence (DI), estimation of faecal metabolites: The DS was recorded during 1^{st} to 14^{th} day of experimental period. Faecal consistency was evaluated following classification scale developed by Pedersen and Toft (2011) for growing pigs. The DS and DI of T_1, T_2, T_3 and T_4 were calculated as per the formula given by Giang *et al*. (2010).

$$\text{DS} = \frac{\text{(Sum of the diarrhoea score over the period)}}{\text{(experiment days)}}$$

$$\text{DI (\%)} = \frac{\text{(Numbers of pigs with diarrhoea of each group} \times \text{diarrhoea days} \times 100)}{\text{(Total pigs in group} \times \text{experiment days)}}$$

Faecal samples were collected directly from rectum through grab technique on 1^{st}, 60^{th}, 120^{th} and 180^{th} day for estimation of faecal pH, NH_3-N and lactic acid. The pH was measured by digital pH meter. Faecal NH_3-N was estimated by calorimetric method (Weatherburn 1967), and lactic acid by method of Baker and Summerson (1941).

Counting of faecal bacteria: Faeces collected directly from rectum on 1^{st}, 60^{th}, 120^{th} and 180^{th} day of feeding trial were enumerated for total lactic acid bacteria (LAB), *E. coli* and *Salmonella* count by pour plate method. The colonies were counted after 24 hours of incubation at 37^0 C as colony forming unit (cfu/g faeces) and expressed as $\log_{10}$ cfu/g of faeces.

Immune status of pigs: The immunological parameters, namely cell-mediated immune response (CMI) and humoral immune response (HI), were measured during the last month of feeding trial. The CMI was assessed through *in vivo* sub-cutaneous delayed-type hypersensitivity (DTH) reaction against phytohaemagglutin-p (PHA-p) following method of Kim *et al*. (2000) and HI was measured by micro-hemagglutination assay as described by Wegmann and Smithies (1966).

Histo-morphology of small intestine of pigs: Duodenal, jejunal and ileal parts of small intestine were collected for estimation of villi height (VH), crypt depth (CD) and apparent villi surface area (AVSA) following methods of Culling *et al*. (1985), Zhang *et al*. (2009) and Iji *et al*. (2001).

Analysis for nutritional composition: The nutritional compositions of the SRs and faeces were analysed following methods of AOAC (2012).

Statistical analysis: The findings of the study were analysed using SPSS version 16.0 by one-way ANOVA and means were compared between the groups as per Duncan's multiple range test at 1% and/or 5% level of significance (Duncan 1955).

RESULTS AND DISCUSSION

Nutritional composition of standard rations and fermented feeds: Ingredient and nutritional composition of SRs are presented in supplementary table 1 and 2. DM% of LF and FLF ranged from 26.48 to 29.26, and 24.02 to 28.93 in T_3, and 24.27 to 28.08 in T_4 respectively. The LAB counts of FLFs of T_3 and T_4 were between 10^8 to 10^9 cfu/g throughout the experiment.

Growth and feed efficiency: The FLFs had significant effects (P<0.01) on growth rate and feed efficiency of LWY grower-finisher pigs (Table 1). Growth rate at 18^{th} and 30^{th} week of age were significantly (P<0.01) higher in T_3 and T_4. Similarly, total body weight gain and FCR at 30^{th} week of age were also significantly (P<0.01) higher in FLF fed

Table 1. Growth rate and feed efficiency of LWY pigs

Parameter	T_1	T_2	T_3	T_4	SEM/p-value
IBW (kg)	11.46±0.96	11.45±0.98	11.46±1.05	11.45±1.13	0.48/1.00
GBW (kg)	44.08^{a}±1.04	46.00^{ab}±2.29	51.38^{b}±2.18	48.53^{ab}±1.78	1.05/0.07*
GBWG (kg)	32.63^{a}±1.31	34.54^{ab}±1.40	39.92^{b}±2.41	37.08^{ab}±1.12	0.95/0.03*
FBW (kg)	91.55^{a}±1.15	96.53^{a}±3.04	105.78^{b}±1.91	105.73^{b}±1.85	1.61/<0.01**
FBWG (kg)	47.47^{a}±0.79	50.53^{ab}±0.81	54.40^{bc}±0.80	57.20^{c}±0.88	0.86/<0.01**
TBWG (kg)	80.09^{a}±0.94	85.08^{a}±2.12	94.32^{b}±1.95	94.28^{b}±1.24	1.49/<0.01**
TFI (kg)	305.83±2.16	310.22±11.33	316.78±3.86	326.18±7.41	3.69/0.23
FCR	3.82^{a}±0.07	3.64^{b}±0.05	3.36^{c}±0.04	3.46^{c}±0.07	0.05/<0.01**

Means bearing different superscripts (a, b, c) in a row differ significantly (*P<0.05, **P<0.01). IBW: BW at 6th week of age; GBW: BW at 18th week of age; GBWG: BW gain at 18th week of age; FBW: BW at 30th week of age; FBWG: BW gain from 19th to 30th week; TBWG: BW gain from 6th to 30th week of age; TFI: total feed intake; FCR: feed conversion ratio; BW: body weight.

pigs. During 6th to 18th week, 22.34% and 13.64% higher body weight gain were recorded for T_3 and T_4, respectively compared to T_1; and 15.58% and 6.85% respectively than T_2. Similar changes were also recorded between 19th to 30th week. In the finishing phase, average body weight gain was 14.59% and 20.49% higher in T_3 and T_4 than T_1; while it was 7.66% and 13.20% higher than T_2 respectively. Overall improvement of 17.76% for T_3 and 17.71% for T_4 and 10.86% for T_3 and 9.76% for T_4 were recorded than T_1 and T_2, respectively in growth and feed efficiency.

The findings indicated efficient utilization of FLFs by pigs of T_3 and T_4, which might be due to optimum gut health that might had positively influenced the digestion, absorption and utilization of nutrients. FLF also helps in enhancing feed consumption in early post-weaning period improving acceptability by eliminating dustiness. Efficient utilization of feeds in T_3 and T_4 might also be for improved enzymatic activity of the gut, breakdown of anti-nutritional factors during fermentation, reduced gastrointestinal pathogens and improved intestinal health (Missotten *et al*. 2015, Tiwari *et al*. 2019, Lee *et al*. 2023).

Effect of FLF on post-weaning diarrhoea (PWD) of piglets: Post weaning diarrhoea (PWD) is a serious problem in piggery, associated with proliferation of enterotoxigenic *Escherichia coli* in the intestine (Rhouma *et al*. 2017), negatively affecting feed intake, nutrient digestibility and growth rate of piglets. In present experiment, average DS were 1.80±0.09, 1.50±0.18, 1.36±0.12 and 1.42±0.08, respectively for T_1, T_2, T_3 and T_4 and DI (%) were 23.81, 16.67, 5.95 and 4.76 respectively**.** Results indicated significantly (P<0.05) reduced DS and DI (%) for piglets of T_3 and T_4. LAB of FLF could favourably modulate intestinal microbiota by inhibiting the growth of invasive pathogens like *Escherichia coli* (Huang *et al*. 2020). LAB produce antimicrobials like bacteriocins which helps in inhibiting the production of bacterial toxins or adhesion of pathogens to the intestinal mucosa. As reported by Cernauskiene *et al*. (2011), *Enterococcus faecium* could produce lactic acid reducing the intestinal pH and preventing the growth of invasive pathogens. Similar favourable effect of FLF was also reported by Dowarah *et al*. (2017) in Landrace × Local pigs. Sayan *et al*. (2018) also reported reduced diarrhoea incidence of 9.16% in growing pigs fed *Lactobacillus salivarius* compared to 13.26% of control group fed basal ration.

Effect of FLF on faecal pH: Alkaline faecal pH is desirable in growing-finishing pigs. Faecal pH, estimated on 180th day of feeding trial was significantly (P<0.05) higher in T_3 and T_4, while on 60th and 120th day, it was insignificant among the treatments, but an increasing trend was recorded in T_3 and T_4 (Table 2). Reduced substrates in the large intestine of FLF fed pigs might resulted in less microbial growth and therefore reduced VFA production in the lower part of gastrointestinal tract increasing the pH (Urlings *et al*. 1993). Increased pH might also be result of increased secretion of pancreatic juice in pigs fed FLF.

Effect of FLF on Faecal Ammonia nitrogen (NH_3-N): Ammonia is a potent environment pollutant and affects both pigs and human beings. In pigs, approximately 70% of nitrogen intake is excreted either as faecal nitrogen (about 20%) or as urinary nitrogen (about 50%). About 80% of faecal nitrogen is in the form of organic nitrogen, whereas 97% of urinary nitrogen is in the form of urea. Urea is easily converted to NH_3 by faecal enzyme urease (Dai and Karring 2014). Thus, transferring nitrogen from the urea and then converting faecal nitrogen into bacterial protein would be an effective way to reduce nitrogen excretion and thereby NH_3 emission in piggery. Significantly (P<0.05) reduced faecal NH_3-N level in T_3 and T_4 was recorded on 120th day of feeding (Table 2). FLF might have favourable effect in reducing the ammonia emission. It is feasible that more nitrogen being excreted via the faeces in the form of bacterial protein and less from the urine as urea could reduce NH_3 emissions (Cheng *et al*. 2017). There might be higher transfer of nitrogen or urea into the gastrointestinal tract in T_3 and T_4 and it might lead to an increased fixation of nitrogen within microbial protein.

Effect of FLF on faecal lactic acid: No significant changes of faecal lactate level were recorded during the feeding trial. However, a decreasing trend was noticed in FLF fed groups which might be indications of efficient absorption or conversion of lactic acid in FLF fed-pigs (Table 2) leading to decreased lactate along the GIT with very low concentrations in the caecum, colon and rectum contents, thereby less faecal lactic acid content.

Effect of FLF on faecal LAB, Escherichia coli and

Table 2. Faecal pH, NH_3-N (mg/g) and lactic acid (µg/g) of pigs fed FLF

Day	T_1	T_2	T_3	T_4	SEM/p-value
			Faecal pH		
1	6.43±0.66	6.66±0.04	6.39±0.20	7.20±0.43	0.14/0.14
60	6.23±0.32	6.08±0.20	6.35±0.21	5.76±0.33	0.09/0.12
120	5.73±0.45	5.65±0.44	6.37±0.17	6.30±0.14	0.13/0.06
180	5.81^a±0.27	6.01^{ab}±0.22	6.21^b±0.13	6.34^b±0.06	0.08/0.04*
			Faecal NH_3-N level (mg/g)		
1	8.683±1.13	9.685±0.55	6.915±2.59	8.635±3.10	0.60/0.49
60	8.465±1.25	9.736±0.49	6.542±3.36	6.948±3.54	0.73/0.44
120	5.653^b±0.91	4.842^b±0.65	2.484^a±1.20	2.902^a±0.82	0.46/0.01*
180	2.581±2.36	2.580±1.03	2.483±1.02	1.752±3.54	1.42/0.09
			Faecal lactic acid level (µg/g)		
1	2.37±0.73	3.19±1.04	2.75±1.22	2.72±0.63	0.41/0.94
60	5.40±0.55	4.77±0.95	3.31±0.63	2.65±0.36	0.43/0.06
120	5.87±0.77	5.15±0.78	3.27±0.13	3.22±0.76	0.45/0.05
180	4.66±0.75	3.43±0.93	2.68±0.26	2.85±0.83	0.39/0.30

Means bearing different superscripts (a, b) in a row differ significantly (*P<0.05, **P<0.01)

Salmonella counts: An increasing trend of faecal LAB count was recorded in T_3 and T_4 on 60th and 120th day of feeding experiment, while LAB count was significantly (P<0.01) higher in T_3 and T_4 on 180th day of experiment (Table 3). It might be due to favourable effects of FLF on gut microbiota. Gut microbial balance is negatively influenced by weaning stress in piglets and results in growth retardation, diarrhoea and other digestive problems. Feeding FLF is known to alleviate digestive problems in weaned piglets re-establishing gut microflora in favourable way. As reported by Zhao *et al.* (2018), faecal *Lactobacillus* concentration increased in weanling pigs supplemented with 0.1% *Lactobacillus reuteri* and *Lactobacillus plantarum* (10^6 cfu/g) complex diet. Mallo *et al*. (2010) also observed that supplementation of *Enterococcus faecium* (10^6 cfu/g) increased faecal *Lactobacilli* population, but did not influence *E. coli* counts on 56th day in weanling pigs. Increased faecal *Lactobacilli* and decreased *E. coli* counts in weanling pigs fed high energy diets with *Enterococcus faecium* (10^6 cfu/g) on 14th and 28th day of age was also reported by Zhang *et al*. (2014).

Escherichia coli is associated with wide range of diseases in pigs, including PWD (Fairbrother and Gyles 2012). Enteric colibacillosis causes significant economic losses due to increased mortality, decreased weight gain, and increased cost for treatments, vaccinations and supplemental feedings. Feeding probiotics or feed fermented with probiotic bacteria reduces the incidence of PWD for their bacteriostatic and bactericidal effects. Faecal *E. coli* count was significantly (P<0.01) reduced in T_3 and T_4 on 120th and 180th day indicating that FLF might have positive effects in reducing the *E. coli* population in the gut. The prevalence of *E. coli* associated with pathogenic groups was lower with the use of probiotics in pigs (Figueiredo-Silva *et al*. 2010). Hu *et al*. (2014) also reported that probiotic supplementation reduced *E.*

Table 3. Faecal microbial profile (log_{10} cfu/g) of experimental pigs

Day	T_1	T_2	T_3	T_4	SEM/p-value
			LAB		
Day 1	7.735±1.15	8.766±1.16	8.879±0.52	9.107±0.59	0.27/0.32
60 days	7.159±0.77	8.403±1.30	8.994±0.82	8.568±0.90	0.32/0.20
120 days	7.835^{ab}±0.80	7.315^a±0.62	10.133^c±0.51	8.755^b±0.37	0.36/<0.01**
180 days	8.646±0.42	8.366±0.58	9.339±0.39	9.017±0.65	0.17/0.19
			E. coli		
Day 1	7.016±0.28	6.619±0.77	6.860±0.59	6.465±0.82	0.17/0.74
60 days	7.683±1.09	8.255±1.74	6.434±1.11	7.434±0.51	0.36/0.37
120 days	7.273^a±0.76	7.497^a±0.92	4.958^b±0.54	5.085^b±0.65	0.40/<0.01**
180 days	6.359^a±0.51	5.805^a±0.96	3.670^b±0.47	3.958^b±0.54	0.38/<0.01**
			Salmonella		
Day 1	5.059±1.08	4.867±0.51	5.085±1.13	5.851±0.56	0.24/0.56
60 days	5.705±1.46	6.444±0.06	5.774±1.81	5.085±1.88	0.40/0.75
120 days	6.064±0.68	5.780±0.66	4.767±0.40	5.100±0.85	0.23/0.14
180 days	6.114^a±0.71	6.693^a±0.53	3.460^b±0.56	3.781^b±0.42	0.45/<0.01**

Means bearing different superscripts (a, b) in a row differ significantly (*P<0.05, **P<0.01) *coli* counts in weaned piglets. Lee *et al*. (2018) observed comparatively lesser faecal count of *E. coli* and higher faecal count of *Lactobacillus* in crossbred finishing pigs fed fermented wheat and barley with *Lactobacillus* spp. It was also reported that a decrease of *E. coli* in probiotic-fed piglets led to improved LAB/*E coli* ratio, which is an indicator of good gut health (Dong *et al*. 2013).

A decreasing trend was noticed for faecal *Salmonella* counts in FLF-fed pigs. On 180th day, the *Salmonella* count was significantly (P<0.01) reduced in T_3 and T_4. This might be the pathogen inhibitory effects of *Lactobacillus acidophilus* and *Enterococcus faecium* of FLF in T_3 and T_4 hindering the adhesion of pathogenic bacteria in the gastro-intestinal tract, for the production of an acidic environment, and bacteriocins inhibiting the growth or adhesion of harmful bacteria. By reducing the pH in the stomach of pigs, feeding fermented liquid feed might have prevented the proliferation of pathogens such as coliforms and *Salmonella* from developing in the gastrointestinal tract (Missotten *et al*. 2015).

Effect of FLF on histo-morphology of small intestine: Functional status of small intestine is one of the main indicators of gut health and characterized in part by villus height (VH) and crypt depth (CD) (Laudadio *et al*. 2012). Increased VH and a decreased CD are positively correlated with nutrient digestion, absorption and assimilation (Munyaka *et al*. 2012, Shang 2014). The significantly (P<0.05) higher intestinal VH and AVSA in T_3 and T_4 might be the indication of positive effects of FLF on nutrient digestibility of LWY pigs (Supplementary Table 3). FLF can reduce viscosity of the intestinal content and dry matter content of digesta influencing favourably the ecophysiology of pig's GIT and thereby contributing to the improvement of absorptive ability of small intestine (Sayan *et al*. 2018).

The intestinal villi showed hyper-cellularity with more mononuclear cells in T_3. The widths of the villi were more with more glandular epithelial cells and crypts between the villi contained more mononuclear epithelial cells. Lymphoid follicles were also observed above the lamina propria and about 5-7 numbers of lymphoid follicles were seen per section in entire circumference of duodenum (Fig. 1).

Large numbers of mononuclear cells and lymphoid follicles were visible in the duodenal villi of T_4, but were less in numbers than T_3. About 2-3 lymphoid follicles were visible per section in the entire circumference of the duodenum. Mild infiltration of mononuclear epithelial cells was visible with 4-8 lymphoid follicles per section of entire circumference in the jejunum. Bigger lymphoid follicles occupying most of the spaces of *lamina propria* were visible both in T_3 and T_4. The *lamina propria* lymphocytes and/or intraepithelial lymphocytes are not only involved in immune surveillance, but their presence is also indispensable for normal mucosal regeneration (Sipos and Muzes 2011). Majority of these cells are IgA-secreting B-cells which are transported through the epithelial cells into the lumen where it interferes with adhesion and invasion of bacteria.

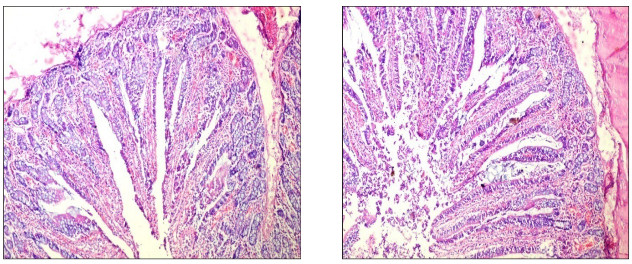

Fig. 1. Small intestinal section showing hypercellularity with infiltration of mononuclear cells.

Effect of FLF on cell-mediated and humoral immune responses: The intradermal reaction after injection of phytohaemagglutinin-p (PHA) is a marked acute delayed-type hypersensitivity like reaction resulting in swelling, erythema and induration of the skin (Fritz *et al*. 1990). In the present study, skin-fold thickness of FLF-fed pigs was significantly (P<0.01) higher than pigs of T_1 and T_2 (Supplementary Table 4). The skin reaction showed an increasing trend up to 24 hours after which it was slowly subsided. Joling *et al.* (1993) also reported maximum skin reaction at 24 hours although the reaction was pronounced in 6 hrs and 48 hrs. It might be for colonization of *Lactobacilli* in the GIT epithelium forming a protective membrane against pathogenic microbes modulating immunity by stimulating epithelial lymphocytes (Yu *et al*. 2008).

Antibody titre (HI $\log_{10}$/ml) on 7th and 21st day post-inoculation was significantly (P<0.05) higher in T_3 and T_4 (Supplementary Table 5). These observations indicated a positive immune response of FLF-fed pigs compared to pigs fed dry and liquid feeds. Kirjavainen *et al*. (1999) reported that oral administration of 10^8 cfu *Lactobacillus* strains could modulate cellular immunity or (and) humoral immunity response.

Significant improvements in growth rate, feed efficiency, gut health and immunity of LWY pigs were recorded for feeding FLF fermented by *Lactobacillus acidophilus* or *Enterococcus faecium* than the pigs fed liquid feed and dry feed. Therefore, FLF fermented either by *Lactobacillus acidophilus* or *Enterococcus faecium* was recommended to LWY fattener pigs for better performance and profit under intensive system of management.

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