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R Buragohain

Department of Animal
Nutrition, College of Veterinary
Sciences and Animal Husbandry,
Central Agricultural University
Imphal Selesih, Aizawl,
Mizoram, India

BN Saikia

Department of Animal
Nutrition, College of Veterinary
Sciences, Assam Agricultural
University, Guwahati, Assam,
India

AK Samanta

Department of Animal
Nutrition, College of Veterinary
Sciences and Animal Husbandry,
Central Agricultural University
Imphal Selesih, Aizawl,
Mizoram, India

TK Rajkhowa

Department of Veterinary
Pathology, College of Veterinary
Sciences & A.H., CAU Imphal
Selesih, Aizawl, Mizoram, India

Effect of fermented liquid feed on gut morphology of Large White Yorkshire (LWY) grower-finisher pigs

R Buragohain, BN Saikia, AK Samanta and TK Rajkhowa

Abstract

The study was for comparative assessment of gut morphology of grower-finisher LWY pigs fed dry feed, non-fermented liquid feed and fermented liquid feed prepared with *Lactobacillus acidophilus* and *Enterococcus faecium*. After 180 days of feeding trial, 3 pigs from each group were slaughtered and duodenal, jejunal and ileal samples were collected and processed following standard procedures for histo-morphological study. Significant ($P < 0.05$) improvements of villi height, crypt depth and apparent villi surface area were recorded in all the sections of small intestine of pigs fed fermented liquid feed as compared to pigs fed dry and non-fermented liquid feed. Histo-morphology enumerated in all the three segments of the small intestine revealed that the villi and epithelial cellular structures were normal indicating that feeding of fermented and non-fermented liquid feeds did not have any adverse effect. The tips of the villi showed hyper-cellularity with more mononuclear cells and crypts between the villi were also observed to have more mononuclear epithelial cells in pigs fed fermented liquid feed. Except in pigs fed dry feed and non-fermented liquid feed, numerous lymphoid follicles were also observed above the lamina propria of the duodenal sections of pigs fed fermented liquid feed. No significant difference was observed between pigs fed fermented liquid feeds with different inoculums. The findings of the study revealed that feeding of liquid feed/fermented liquid feed had favourable effect on the development of the intestinal epithelium and infiltration of mononuclear cells with large number of glandular epithelium and lymphoid follicles in the intestinal sections of pigs fed fermented liquid feed might be the indication of better digestive efficiency and immune status of pigs.

Keywords: Fermented liquid feed, gut morphology, Large White Yorkshire pigs

Introduction

The gastrointestinal tract (GIT) is the main digestive and absorptive organ in animals. The GIT permits the uptakes of dietary nutrients into systemic circulation excluding the toxic compounds simultaneously [1]. Temporary starvation immediately after weaning may result villous atrophy and reduces crypt depth [2] in piglets resulting in reduced digestive activity [3]. Transition from sow's milk to plant-based solid feed [4], underdeveloped GIT at weaning also cause disruption of intestinal mucosal integrity [5] contributing to significant alterations in size and structure of the gut, its functional capacity, digestion processes and nutrient absorption [6]. Feeding rations in dry form with the provision of *ad libitum* drinking water is the common feeding practice of pigs. However, in recent decades, feeding of rations in liquid form (i.e. mixing with drinking water before feeding to the animals) is gaining popularity among the pig farmers for its manifold advantages [7]. Weaned piglets adapt more easily to liquid feed and liquid feeding also reduces feed wastage as dust, increases acidity of the diet and availability of phosphorous, improves accessibility to substrates by the digestive enzymes, reduces viscosity of gut [8, 9]. It is also known that when liquid feed is fermented with lactic acid bacteria (LAB), it further enhances the usefulness of feeds for the animals. LAB are known to modify the intestinal microbiota in favourable way excluding the enteropathogens [10] which has positive effect on gut health and nutrient utilization. Liquid feeding is known to prevent atrophy of the intestinal villi in the post-weaning period [11, 12] and helps in the development of healthier and intact villi-structure of the small intestine of pigs. Feeding of fermented liquid feed can positively affect the integrity of the intestinal barrier against colonization by pathogens [13] through establishment of a balanced microbiota stimulating the immune system in an anti-inflammatory manner [14].

Corresponding Author:**R Buragohain**

Department of Animal
Nutrition, College of Veterinary
Sciences and Animal Husbandry,
Central Agricultural University
Imphal Selesih, Aizawl,
Mizoram, India

Keeping in view the importance of a healthy gut for efficient utilization of feeds, in the present study, an attempt was made for comparative assessment of gut morphology of pigs fed dry feed, liquid feed and fermented liquid feed prepared with *Lactobacillus acidophilus* and *Enterococcus faecium* in grower-finisher LWY pigs.

Materials and Methods

Approval of Animal Ethics Committee

The study was approved by the Institutional Animal Ethics Committee via approval letter no. 770/ac/CPCSEA/FVSc/AAU/IAEC/17-18/481 dated 09.08.2017.

Location of the study and experimental animals

The study was carried out at the experimental unit of Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl, Mizoram. Twenty four weaned piglets (42-days of age, average body weight 11.46 ± 2.37 kg) were selected from the Piggery Unit of

Instructional Livestock Farm Complex (ILFC), College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl, Mizoram and randomly distributed into 4 homogenous groups (3 males and 3 females in each group). The experimental groups were designated as follows:

T₁: Pigs fed dry feed.

T₂: Pigs fed non-fermented liquid feed.

T₃: Pigs fed fermented liquid feed prepared with *Lactobacillus acidophilus* ($1-2 \times 10^9$ cfu/g).

T₄: Pigs fed fermented liquid feed prepared with *Enterococcus faecium* ($1-2 \times 10^9$ cfu/g).

Formulation of experimental rations and preparation of non-fermented and fermented liquid feed

Four experimental rations were formulated as per NRC [15] recommendations for grower-finisher pigs (Table 1) i.e. Ration I (11-25 kg body weight), Ration II (25-50 kg body weight), Ration III (50-75 kg body weight) and Ration IV (75-100 kg body weight).

Table 1: Ingredient composition (%) of basal experimental ration

Name of Ingredient	Experimental Ration			
	Ration I (11-25 kg)	Ration II (25-50 kg)	Ration III (50-75 kg)	Ration IV (75-100 kg)
Yellow Maize	52.45	59.06	62.00	66.00
Wheat Bran	0.00	0.00	6.00	8.00
Soya bean meal	27.00	21.00	15.00	11.00
De-oiled ground nut cake (SE)	8.00	8.00	7.00	5.60
Fish Meal	5.00	5.00	4.00	3.00
L-Lysine	0.50	0.40	0.30	0.30
DL- Methionine	0.05	0.04	0.00	0.00
Vegetable oil	4.00	3.50	3.20	3.60
Common Salt	0.50	0.50	0.50	0.50
Commercial mineral mixture	2.50	2.50	2.00	2.00
Total	100	100	100	100

The non-fermented liquid feed was prepared by mixing basal ration with drinking water at 1:2 ratio (w/w) immediately before feeding to the experimental pigs.

For preparation of fermented liquid feed, freeze-dried cultures of *Lactobacillus acidophilus* and *Enterococcus faecium* were procured from National collection of Dairy Cultures, Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal (Haryana), India-132001. The experimental ration was mixed with drinking water (1:2, w/w) and inoculated with *Lactobacillus acidophilus* culture and fermented for 48 hours in sealed plastic container under environmental temperature. Back-slopping was done for preparation fermented liquid feed with *Lactobacillus acidophilus* for the next 7 days after which the process was started again from the beginning. Fermented liquid feed with *Enterococcus faecium* was also prepared following the same procedure as that of *Lactobacillus acidophilus* fermented liquid feed.

Feeding and management of experimental animals

The experimental animals were housed in individual pen with separate feeding and watering provisions. The feeding was done twice daily (morning 8.00-9.00 AM; evening 2.00-3.00 PM) to allow *ad libitum* feeding with provision for *ad libitum* drinking water. The feeding trial was continued for 180 days.

Collection of samples and protocol for Histo-morphology

At the end of the feeding experiment, 3 animals per treatment (the lightest, heaviest, and the middle by weight) were

slaughtered. The GIT was removed and samples of intestinal segments (i.e. duodenum, jejunum and ileum) were collected. Each of the intestinal segments (approximately 2 cm in length) were excised and flushed with 0.9% saline to remove the contents. Gut segments were fixed in 10% neutral-buffered formalin for histology. Samples were dehydrated, cleared and paraffin embedded, stained with hematoxylin and eosin staining (H&E). Intestinal segments from 3 pigs per dietary treatment were sectioned at a 6- μ m thickness, placed on glass slides and processed for examination by light microscopy according to Culling *et al.* [16]. The morphometric indices evaluated were villus height (i.e. from the tip of the villus to the crypt), crypt depth (i.e. from the base of the villi to the sub-mucosa), and the villus height to crypt depth ratio [17]. The apparent villus surface area was calculated by the following formula according to Iji *et al.* [18].

Apparent villus surface area:

[(Villus width at one-third + villus width at two-thirds of the height of the villus) $\times 2^{-1} \times$ villus height].

Statistical analysis

For interpretation of results, data were analysed using SPSS version 16.0 according to one-way ANOVA. The means were compared between the groups as per Duncan's multiple range test at 1% and 5% level of significance [19].

Results and Discussion

Intestinal morphology is the main indicator of gut health which reflects the development of the digestive tract and the response of intestine to certain feed substances [20]. The functional status of small intestine is characterized in part by villus height (VH) and crypt depth (CD) [21]. Intestinal development can be evaluated through measurement of the crypt, a region in which new intestinal cells are formed, as well as VH and surface area, to determine the area available for digestion and absorption [22, 23]. It was also reported is commonly believed that an increased VH and a decreased crypts depth CD were positively correlated to the digestive and absorptive functions of in the GIT of animals accounting for an enlarged absorptive area and a reduced tissue turnover rate [24,25,26].

Villus height, crypt depth and VH: CD ratio and apparent villus surface area (AVSA)

In the present study, in the duodenum, the VH in the duodenum was significantly higher ($P < 0.05$) in fermented liquid feed fed-pigs (T_3 and T_4) than the T_1 and T_2 , and without any significant ($P > 0.05$) difference between T_3 and

T_4 (Table 2). VH was significantly higher in T_2 than the T_1 . CD and AVSA were also significantly ($P < 0.05$) more in T_4 than the other groups. In the jejunum, VH was significantly ($P < 0.05$) higher in T_3 and T_4 , but no significant ($P > 0.05$) difference was seen for CD among the groups. The AVSA was significantly ($P < 0.05$) more in T_3 and was numerically more in T_4 than the T_2 and T_1 . In the ileum also, the VH, CD and AVSA was significantly higher in T_3 and T_4 than T_1 and T_2 . There was no significant difference in VH: CD ratio among the groups. The findings, thus, indicated that there was significant increase of VH, CD and AVSA in fermented liquid feed fed-pigs. The feeding of fermented liquid feed might resulted in proper development of intestinal epithelium for efficient utilization of nutrients compared to T_1 and T_2 . Improvement in villus architecture of fermented liquid feed fed-pigs might be for improvement in feed intake [27, 28, 29] as liquid diet is known to reduce the transition gap from milk to the weaner diet [30, 31]. Improvements seen in T_2 for VH and CD than the T_1 might also indicated the usefulness of feeding liquid feed to weaned pigs compared to traditional dry feeding practice.

Table 2: VH (mm), CD (mm) and VH: CD of experimental pigs under different feeding regimes

Area	Para.	Experimental group				p-value	SEM
		T ₁	T ₂	T ₃	T ₄		
Duodenum	VH (μm)	350.00 ^a ±28.86	450.00 ^{ab} ±28.86	483.33 ^b ±60.09	550.00 ^b ±28.86	0.039	27.41
	CD (μm)	356.67 ^a ±23.33	470.00 ^b ±35.11	516.67 ^{bc} ±16.67	566.67 ^c ±33.33	0.004	26.32
	VH:CD	0.99±0.08	0.98±0.14	0.93±0.09	0.98±0.08	0.977	0.04
	AVSA (μm ²)	31.50 ^a ±3.01	35.67 ^{ab} ±5.48	58.50 ^{bc} ±9.76	64.50 ^c ±10.85	0.045	5.45
Jejunum	VH (μm)	316.67 ^a ±16.67	350.00 ^a ±28.87	450.00 ^b ±28.87	383.33 ^{ab} ±16.67	0.021	17.94
	CD (μm)	350.00±28.87	356.67±23.33	366.67±16.67	400.00±28.87	0.532	12.12
	VH:CD	0.92±0.12	0.99±0.13	1.24±0.13	0.97±0.09	0.304	0.06
	AVSA (mm ²)	28.17 ^a ±7.07	30.50 ^a ±1.80	77.08 ^c ±7.98	34.42 ^a ±0.82	0.001	6.48
Ileum	VH (μm)	250.00 ^a ±28.86	316.67 ^{ab} ±16.67	383.33 ^b ±16.67	366.67 ^b ±33.33	0.022	18.92
	CD (μm)	266.67 ^a ±16.67	366.67 ^{ab} ±33.33	483.33 ^b ±60.09	433.33 ^b ±33.33	0.021	29.59
	VH:CD	0.94±0.13	0.88±0.07	0.82±0.12	0.85±0.08	0.860	0.05
	AVSA (mm ²)	20.67 ^a ±1.83	43.50 ^b ±6.87	47.25 ^b ±5.37	47.67 ^b ±7.36	0.031	4.16

Means bearing different superscripts (a, b, c) in a row differ significantly.

As reduced feed intake immediately after weaning could lead to adverse morphological and functional changes in the intestine [32], it might be the reason for significantly reduced VH, CD and VH: CD ratio in T_1 compared to T_2 , T_3 and T_4 . Besides, fermentation of feed is known to reduce viscosity [33, 34] and dry matter content of digesta [35] which contributes to changes of ecophysiology of pig's GIT in favourable way. LAB produces short chain fatty acids which stimulate epithelial cells and enterocytes [36] and this might be the reason for significantly more VH, CD and VH: CD ratio in T_3 and T_4 compared to T_2 .

Histo-morphology of small intestine

The fastest growing tissue in piglets is the epithelial lining of the small intestine. Adequate feed/nutrient intakes in the initial post-weaning period ensures optimum development of intestinal epithelium. As reported by Scholten *et al.* [37], liquid feed, either fresh or fermented, was observed to improve feed intake and Pluske *et al.* [38] and Vente-Spreuwenberg and Beynen [39] stated that it was mainly due to higher nutrient intake which helped to maintain the villus architecture.

In the present study, histo-morphological studies of the small intestine revealed that the villi and epithelial cellular structures were normal in all the experimental groups indicating that feeding of non-fermented and fermented liquid

feed might not have any adverse effects. However, in T_3 , tips of the villi showed hyper-cellularity with more mononuclear cells. The widths of the villi were more with more glandular epithelial cells. Crypts between the villi contained more mononuclear epithelial cells. Lymphoid follicles were also observed above the lamina propria. About 5-7 numbers of lymphoid follicles were observed per section in entire circumference of duodenum in T_3 . In T_4 also, large numbers of mononuclear cells and lymphoid follicles were visible in the duodenal villi, but were less in numbers compared to T_3 . About 2-3 lymphoid follicles were visible per section in entire circumference of the duodenum. The villi were seen all and wider.

In the jejunum of T_4 , the villi heights were shorter than T_3 , but are wider than T_3 . Mild infiltration of mononuclear epithelial cells were visible with 4-8 lymphoid follicles per section in entire circumference. In T_1 , however, no mononuclear epithelial cells and lymphoid follicle were visible. In T_2 , the villi were wider with more glandular epithelium compared to T_1 , but no mononuclear cells were visible. In the fermented liquid feed fed-groups, bigger lymphoid follicles occupying most of the spaces of lamina propria were seen. The histo-morphological changes of the small intestine of pigs fed fermented liquid feed compared to pigs fed dry and non-fermented liquid feed might be the indication of enhancement

of intestinal mucosal immunity and maintenance of intestinal barrier, also reported by many workers [40, 41, 42, 43]. Sipos and Muzes [44] also reported that 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. 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.

Conclusion

The findings of the study revealed that feeding of fermented liquid feed might have favourable effect on the development of the intestinal epithelium significantly improving the VH, CD and AVSA compared to pigs fed non-fermented and dry feed. Compared to dry feed fed-pigs, non-fermented liquid feeding also resulted improvement in the development of gut epithelium of LWY pigs. The intestinal villi of duodenum and jejunum of fermented liquid feed fed-groups showed infiltration of mononuclear cells with large number of glandular epithelium and lymphoid follicles in the entire circumference. These might be the indication of better digestive efficiency and immune status of pigs fed fermented liquid feed compared to dry feed and non-fermented liquid feed.

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