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Effect of fermentation on pH, microbial profile and proximate composition of compound feed of growing-finishing pig

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Abstract
The study was to evaluate the effect of fermentation of compound pig feed on pH, microbial profile and proximate composition. A standard grower-finisher ration was prepared following NRC (2012) [14] standard incorporating conventional ingredients. Two probiotic cultures, namely *Lactobacillus acidophilus* and *Enterococcus facium,* were used separately as inoculum for fermentation process. After 48 hours of fermentation of 1st batch of liquid compound feed (Feed: water: 1:2), half of the amount was replaced with new liquid compound feed for fermentation again for 48 hrs. This back-slopping process was continued for seven times. Samples of fermented feeds were collected after completion of each cycle of fermentation. Fermented feed samples were analysed for pH and microbial profile [Lactic acid bacteria, *E. coli* and *Salmonella*] and proximate principles (AOAC 2012) [3]. The pH of the fermented feed was variable from 1st to 7th cycles of back slopping, but without any significant difference (p>0.05) and varied from 4.28-4.45 and 4.31-4.43 respectively for fermentation with *Lactobacillus acidophilus* and *Enterococcus facium*. The dry matter contents of fermented feeds ranged from 20.66-28.93% and 22.72-28.08%, respectively. Comparatively increasing trend was observed for crude protein (%) from 1st to 7th cycle for both *Lactobacillus acidophilus* and *Enterococcus facium* inoculation, and reverse was recorded for ether extract (%). Other proximate principles were variable without any significant difference (p>0.05). The numbers of Lactic acid bacteria in fermented feed with *Lactobacillus acidophilus* and *Enterococcus facium* decreased from 1st to 7th cycle (average 8.69 to 7.30 CFU $\log_{10}$/ml and 8.78 to 6.47 CFU $\log_{10}$/ml, respectively). The *E. coli* count decreased as the fermentation cycle progressed. *Salmonella* was not detected in any samples of fermented feeds. The baseline information of the present study may be utilised while deciding feeding strategy for pigs comparing with convention ways of feeding of dry and liquid feed for better performance and profit.

Keywords: pH, fermentation, compound pig feed, microbial profile, proximate principle

1. Introduction

Optimal metabolic utilization of dietary nutrients ensures better feed efficiency and profit from piggery as feeding cost accounts for more than two-thirds of the total expenditure (Liao and Nyachoti 2017) [11]. Better digestibility of feed depends not only on its nutritional composition and proportion, but also on its form in which it is offered. Conventionally, compound feed is provided to pigs in '*dry form*' along with drinking water. However, this method of feeding results wastage of feeds and digestibility of nutrients is low negatively affecting profitability. Thus, there has been growing interest shifting to offering feed in '*liquid form*' i.e. mixing with water for better advantages than the dry feeding method, Brooks 1994 [5], Geary *et al*. 1996 [10], Pluske *et al*. 1996 [16], Brooks *et al*. 2001 [8], Brooks *et al.*, 2003a [6], Canibe and Jensen 2012 [9]. Weaned piglets adapt more easily to liquid feed, feed wastage reduces, improves accessibility to substrates for digestive enzymes enhancing digestibility of nutrients, reduces viscosity of guts. In the recent years, however, it has been further realised that improvements of palatability, digestibility of nutrients and better metabolic utilization of nutrients are possible by feeding fermented feed to pigs, Piepera *et al*. 2010 [15], Yan and Kim 2013 [20], Ahmed *et al*. 2014 [1], Balasubramanian *et al*. 2016 [4], Saliu *et al*. 2024 [17].

Probiotics microbes of fermented feed favourably modify the gut microbiome eliminating or inhibiting the growth of pathogens and thereby improving gut health and performance of the animals. In recent years, there has also been growing interest for alternatives to antibiotic growth promoters for negative impacts relating to emergence and spread of antibiotic resistance bacteria through residues in meat, unapparent carriage of antimicrobial drug-resistant bacteria and exchange of plasmids from antimicrobial resistant bacteria of pigs to human pathogens. For the action of probiotics bacteria, fermented feed may also be considered as alternative to antibiotics growth promoters.

Fermentation is one of the oldest, safest and most natural methods of feed preservation (Brooks *et al*. 2003b) [7]. Feeding of fermented feed may be considered as a cost-effective and bio-safe feeding strategy to replace antibiotic growth promoters in pig rations (Niba 2008) [13]. Fermentation also significantly changes of the physical and chemical properties of feed in favourable ways. In the present study, therefore, an attempt was made to standardise a method of fermentation of compound feed of pigs by back-slopping and to study the effect of fermentation on pH, microbial profile and proximate composition of compound feed of growing-finishing pig.

2. Materials and Methods

2.1 Place of study

The study was carried out at the Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Aizawl, Mizoram, India.

2.2 Formulation of compound feed for fermentation

A standard compound feed for growing-finishing pigs (25-50 kg body weight) was formulated following NRC (2012) [14] standards. Conventional feed ingredients namely, yellow maize grain, soya bean meal, de-oiled ground nut cake, fish meal, L-Lysine DL-Methionine, vegetable oil, commercial mineral mixture and iodised salt were used for ration formulation. The composition of the compound feed was yellow maize grain 59.06%, soyabean meal 21%, de-oiled ground nut cake 8%, fish meal 5%, L-Lysine 0.4%, DL-Methionine 0.04%, vegetable oil 3.5%, iodised salt 0.5% and mineral mixture 2.5%.

2.3 Fermentation methods

The freeze-dried ATCC cultures of *Lactobacillus acidophilus* and *Enterococcus faecium* were procured from National collection of Dairy Cultures, ICAR-National Dairy Research Institute, Karnal, India, revived, and sub-cultured in De Man, Rogosa and Sharpe (MRS) and Brain Heart Infusion (BHI) broth, respectively following standard methods. For fermentation of compound feed, ground maize grain was fermented first with the probiotics cultures. One kg ground maize was mixed with 1 litre of drinking water and then mixed thoroughly with 100 ml of probiotics culture. It was fermented at 37ºC for 24 hours. The fermented ground maize was utilized for fermentation of 5 kg of compound feed. Before inoculated the fermented maize, the compound feed was mixed with drinking water (1:2 w/w) and then fermented for 48 hours at room temperature in plastic container sealing the cover tightly with adhesive tape. Minimum gap was maintained between feed and the container cover while fermenting. After 48 hrs. of fermentation, approximately 50% of the fermented feed was removed from the container (ready for feeding to pigs) and similar quantity of compound feed with water (1:2 w/w) was mixed with the remaining fermented feed and fermented again for 48 hrs. At room temperature sealing the cover as mentioned above. This process of back-slopping was continued for 7 consecutive cycles.

2.4 Collection of samples and analytical methods

Samples of fermented feed was collected after completion of each cycle of fermentation for 7 consecutive cycles. The samples were aseptically collected and immediately analysed for pH using digital pH meter. For analysis of proximate principles, samples were collected separately following standard procedures and analysed for proximate principles by AOAC (2012) [3] methods.

The fermented feed was enumerated for *Lactic acid bacteria* (LAB), *E coli, and Salmonella* by pour plate method. 10 g sample were mixed with normal saline (1:10 w/v) immediately after collection and vortexed for 3-4 min and the supernatant was collected for microbial counting. It was serially diluted to 10^{10} with normal saline and inoculated in MRS, EMB and SS agar (HiMedia) respectively for LAB, *E. coli, and Salmonella* counts. The agar plates were incubated at 37ºC for 24 hrs and colonies were counted and expressed as $\log_{10}$ cfu/g of sample.

2.5 Statistical analysis

For interpretation of results, data were analysed following standard methods of Snedecor and Cochran (1994) [18].

3. Results and Discussion

The nutritional composition of unfermented compound feed was dry matter 89.23%, crude protein 19.49%, ether extract 3.28%, crude fibre 4.02%, total ash 8.81%, nitrogen free extract 64.40%, organic matter 91.19%, neutral detergent fibre 15.40%, acid detergent fibre 3.45%, hemi-cellulose 11.95%, calcium 3.12% and total phosphorous 0.75% (On dry matter basis). The calculated metabolizable energy, lysine and methionine were 3312 kcal ME/kg, 1.16% and 0.45%, respectively on dry matter basis.

Fermentation is a cost-effective way of improving the nutritive value of feeds. Feeding fermented feed can enhance gut health, improve nutrient absorption, and support better overall performance (Anonymous 2025) [2]. Understanding the relationship between pH and fermentation is crucial as it ensures the production of high-quality fermented product. Fermented feed having a low pH, typically below 4.5 to 4.6, indicates successful fermentation (Mwangi 2025) [12]. In the present study, pH of fermented feed inoculating with *Lactobacillus acidophilus* and *Enterococcus faecium* were within the range of 4.28-4.45 and 4.31-4.43, respectively. This might be the indication of satisfactory fermentation till 7 cycles of fermentation. There was no significant difference (p>0.05) in changes of pH of fermented feed between fermentation cycles (Figure 1).

The numbers of LAB in both the fermented feed inoculated with *Lactobacillus acidophilus* and *Enterococcus faecium* increased from 1st to 7th fermentation cycles. The LAB count varied from 8.69 to 7.30 CFU $\log_{10}$/ml in fermented feed inoculated with *Lactobacillus acidophilus* collected in different fermentation cycles. The respective count for fermented feed inoculated with *Enterococcus faecium* varied from 8.78 to 6.47 CFU $\log_{10}$/ml collected in different fermentation cycles (Figure 2). No significant different (p>0.05) was recorded in different fermentation cycles. The *E.coli* count progressively decreased form fermentation cycle 1 to 7, but was statistically non-significant. *Salmonella* was not detected in any samples of fermented feeds in all the fermentation cycles.

The dry matter contents of fermented feeds from 1st to 7th cycles of back-slopping ranged from 20.66-28.93% and 22.72-28.08%, respectively for fermentation by *Lactobacillus acidophilus* and *Enterococcus faecium*. These variations might be for manual back-slopping process of fermentation with every possibility of minimal differences of feed and water ratio in each cycle. Comparatively increasing trend was observed for crude protein (%) levels from 1st to 7th collection for both *Lactobacillus acidophilus* and *Enterococcus faecium* inoculation, but reverse was recorded for ether extract (%) in the fermented feeds varying from 5.13 to 3.04% (on dry matter basis). The level of other proximate principles were variable but without any significant difference (p>0.05). Xu *et al*. (2023) [19] also reported insignificant changes of nutritional composition of fermented feed compared to basal unfermented feed in chicken.

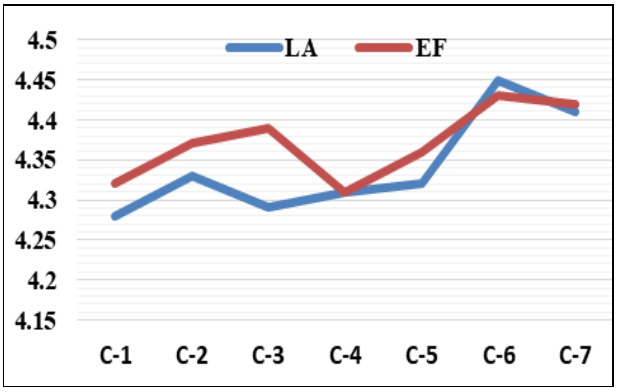

Fig 1: Change in pH of fermented feed

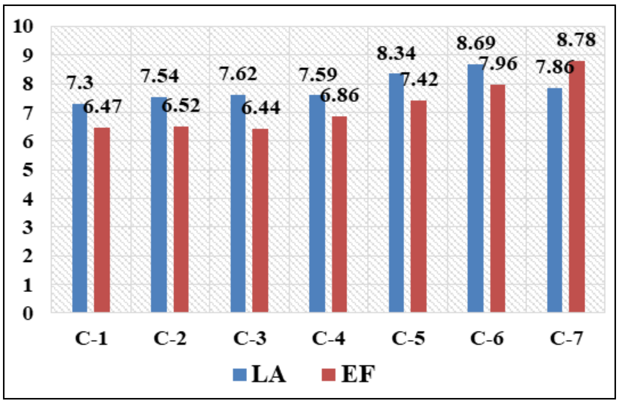

Fig 2: LAB counts (CFU log10/ml) in fermented feed

4. Conclusion

The present study standardised a method of fermentation of compound pig feed by *Lactobacillus acidophilus* and *Enterococcus faecium* (LAB-8.69 to 7.30 CFU $\log_{10}$/ml) and provided baseline information on effects of fermentation on pH, microbial profile and proximate composition. The findings recommended feeding of fermented compound feed to pigs than following the conventional dry or liquid feeding methods for better performance and productivity.

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Conflict of Interest

Not available

Financial Support

Not available

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