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Evaluation of encapsulated probiotics containing *Pediococcus and Lactobacillus* strains on nutrient digestibility of pig finisher ration[#]

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Abstract

A nutrient digestibility trial was conducted to evaluate the effect of feeding encapsulated probiotics on the nutrient digestibility of pig finisher ration. A total of eighteen Large White Yorkshire pigs were randomly assigned into three treatment groups having three replicates with two piglets per replicate. Encapsulation of probiotics ($1x10^{11}$CFU/ml) was done by extrusion process using sodium alginate and calcium chloride. The encapsulated probiotics Pediococcus acidilactici (NCIM 5721) and Lactobacillus plantarum (NCIM 2374) were given separately mixed with fermented maize for T2 and T3 group pigs, respectively, and supplemented from 42nd day of its age and pigs in the control group (T1) received only fermented maize. The pigs in all groups were fed with pig grower and finisher ration as per ICAR (2013) recommendations. One pig from each group was selected randomly for a nutrient digestibility trial at the 213th day of its age using Titanium dioxide (TiO_2) as an external marker mixed at 5 g/kg of feed and fed for six days. On day six, faecal content was collected and Titanium dioxide was estimated. The results revealed supplementation of encapsulated probiotics

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showed a significant (P<0.01) increase in dry matter, ether extract, crude fibre, crude protein and organic matter digestibility in encapsulated probiotic supplemented group pigs compared to the control. Further, it was concluded that encapsulated L. plantarum (NCIM 2374) supplemented T3 group showed better digestibility of nutrients than encapsulated P. acidilactici (NCIM 5721) (T2) group animals.

Keywords: *Encapsulated probiotics, P. acidilactici, L. plantarum, digestibility, pig finisher ration*

In recent years, there has been considerable interest in developing alternatives to antibiotic growth promoters like probiotics (bacterial cultures), prebiotics (oligosaccharides), yeast, essential oils, plant extract and yucca products for pig production and shown variable results also. Meanwhile, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO, 2009). The gut microbiome plays fundamentally crucial functions in the health of animals and the well-being of their host. The probiotics are made up of particular live bacteria that, when given to animals orally, proliferate in their digestive tracts and compete with microbial pathogens for nutrients and intestinal binding sites.

Probiotic microorganisms isolated from the intestinal microflora of the host had more efficiency to establish the gut microbial balance, when animals are confronted with stress (Cho *et al*., 2011). Commonly, lactic acid bacteria (LAB) probiotics are considered as natural microflora of the gut and supplemented as growth promoters. In general, pig feed with probiotics showed positive results on nutrient digestibility (Vanbelle, 2001) and varied on different strains (Weichselbaum, 2009). Further, growth performance and nutrient digestibility were not consistent in grower and finisher pigs, because many probiotic bacteria could not survive in adequate quantities during passage through the gastrointestinal tract and the efficiency of these helpful bacteria is reduced (Gracia *et al*., 2004). It is also reported that survival of *Lactobacillus* strains in the stomach and intestinal juices has been improved by encapsulation techniques using alginate (Nualkaekul *et al*., 2013). Moreover, studies conducted to evaluate the nutrient digestibility of finisher rations using encapsulated probiotics were scanty. Hence, the present study was envisaged to ascertain the effects of encapsulated probiotics containing *Pediococcus* and *Lactobacillus* strains on the apparent total nutrient digestibility of pig finisher ration.

Materials and methods

The experimental protocols were approved by the Institutional Animal Ethics Committee of Kerala Veterinary and Animal Sciences University (Ref Number: IAEC/COVAS/PKD/15/2021)

Source and encapsulation of probiotics

The freeze-dried cultures of *Lactobacillus plantarum* NCIM 2374 and *Pediococcus acidilactici* NCIM 5721 in glass ampoules were purchased from the National Collection of Industrial Microorganisms (NCIM), Pune. The glass ampoules were opened and two millilitres of de Man, Rogosa & Sharpe (MRS) broth (Himedia M369) solution was mixed with the freeze-dried cultures and then transferred to 20 mL of MRS broth solution as slant and kept for 24 h at 37°C and marked as master culture separately. The probiotic cultures of *L. plantarum* (NCIM 2374) and *P. acidilactici* (NCIM 5721) were encapsulated separately using sodium alginate and calcium chloride by extrusion method (Nualkaekul *et al*., 2013).

Experimental design, animals and study material

Eighteen weaned male Large White Yorkshire (LWY) crossed piglets with the same body weight (8.77 kg) at six weeks of age were selected from Pig Farm, Instructional Livestock Farm Complex (ILFC), College of Veterinary and Animal Sciences, Pookode. The selected piglets were randomly distributed to three treatment groups *viz* T1, T2, and T3 and had three replicates with two animals in each group.

The encapsulated probiotics *P. acidilactici* (NCIM 5721) (1×10^{11} CFU/mL) and

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L. plantarum (NCIM 2374) (1 $\times 10^{11}$ CFU/mL) were mixed with 100 g fermented maize for T2 and T3 group pigs separately, respectively and pigs in the control group (T1) given a basal diet and 100 g fermented maize powder without any probiotics supplementation. The experimental period started on 42nd day of its age, and continued for 168 days. The grower ration was given up to 50 kg of the body weight of animals from the day of the experiment and the finisher ration was fed above 50 kg body weight animals. The pigs in all groups were fed with grower and finisher ration as per ICAR (2013) recommendations. All rations were made iso-caloric and iso-nitrogenous. The ingredient composition (% as fed basis) and chemical composition (% as dry matter basis) of finisher ration for experimental animals are presented in Table-1.

Table 1. Ingredient composition (% as fed basis) and chemical composition (% of experimental animals

Item	Finisher ration
Ingredient composition	
Maize	28.00
Corn gluten meal	20.00
Tapioca starch waste	2.00
Fish meal (unsalted, dried)	4.00
Soya bean meal	6.00
Wheat Bran	15.00
De-oiled rice bran	14.50
Rice polish	9.00
Calcite powder	0.50
Mineral mixture	0.50
Salt	0.50
Chemical composition*	
Dry matter	91.27± 0.04
Crude protein	16.30± 0.02
Ether extract	4.29± 0.01
Crude fibre	6.90± 0.01
Nitrogen free extract	65.69± 0.02
Total ash	6.81± 0.02
Acid insoluble ash	0.94± 0.01
Calcium	0.74± 0.01
Phosphorus	0.55± 0.01
Organic matter	93.19± 0.02

*Each value is a mean of three observed values with standard error

Nutrient digestibility trial

During the last week of the experimental period, one pig from each group was selected randomly for a nutrient digestibility trial at the 213th day of its age using Titanium dioxide (TiO_2) as an external marker mixed at 5 g/kg of feed and fed for six days. On day six, faecal content was collected and Titanium dioxide (TiO_2) estimated with a spectrophotometer at 410 nm (Short *et al*., 1996; Myers *et al*., 2004).

The dry matter content, crude protein, crude fat, and crude fibre were analysed as per AOAC (2016). The digestibility coefficient (%) of nutrients was calculated based on the formula given below:

Digestibility coefficient (%) = 100 - (100 x % of marker in feed/ % of marker in faeces x % of nutrient in faeces/ % of nutrient in feed)

Statistical analysis

The experimental data collected on nutrient digestibility parameters were analysed statistically for analysis of variance by One-way ANOVA for linear terms. The other data means were compared for their significance at a 99.5 per cent confidence level by Duncan's multiple range tests using the General Linear Model (GLM) of multivariate in the statistical package IBM SPSS 20.0.

Results and discussion

In the current study, supplementation with encapsulated *L. plantarum* (NCIM 2374) strains had improved (P<0.01) dry matter, crude protein, ether extract and crude fibre digestibility compared to *P. acidilactici* (NCIM 5721) and control group animals. The effect of encapsulated probiotics supplementation on nutrient digestibility in finisher pigs is presented in Table-2.

Similar to the current study, Joysowal *et al*. (2018) conducted an experiment in pigs and compared the effect of host probiotic and milk origin probiotics without encapsulation and detailed that supplementation of *L. acidophilus* NCDC15 a conventional dairy-

Table 2. Effect of encapsulated probiotics supplementation on nutrient digestibility (%) in finisher pigs

Items	Groups			SEM	P-value
	Control (T1)	*P. acidilactici* (NCIM 5721) (T2)	*L. plantarum* (NCIM 2374) (T3)		
Dry matter	86.19[a]	87.21[b]	89.98[c]	0.19	0.00**
Ether extract	73.89[a]	80.06[b]	83.34[c]	0.07	0.00**
Crude fibre	58.87[a]	59.74[b]	64.13[c]	0.06	0.00**
Crude protein	64.63[a]	73.10[b]	77.18[c]	0.05	0.00**
Organic matter	70.67[a]	70.99[a]	74.49[b]	0.09	0.00**

[a, b, c] *Means with different superscripts within a row differ significantly*

***Significance at P < 0.01*

origin probiotic and *P. acidilactici* FT28; swine-origin probiotics had not improved the total tract apparent digestibility of DM, organic matter, EE, CF, and nitrogen free extract (NFE) and not differ significantly between the groups.

Balasubramanian *et al*. **(**2018) opined that apparent total tract digestibility of dry matter and nitrogen significantly improved probiotics-supplemented piglets in the 6th week of their age compared to the control group pigs, but similar results were recorded in the 16th week of its age. Similarly, Hou *et al*. (2021) reported that dry matter digestibility was similar between probiotic supplemented and other control group pigs during the early weaning period, but a significant increase in crude protein digestibility was found. The result of an increase in crude protein digestibility was reported by Mun *et al*. (2021) while they implemented a combination of *Bacillus subtilis* and *Bacillus licheniformis* at 0.01 per cent as probiotics in weaned pigs. However, in the present study, crude protein digestibility was studied in the finishing stage of pigs while other reports pertain to the studies in the growing stage of pigs. Many researchers reported that the efficiency of apparent total tract dry matter digestibility varied in probiotic supplemented group pigs at different doses, ages and strains (Giang *et al*., 2011**;** Yan and Kim, 2013; Yang *et al*., 2020).

In agreement with the current findings, Nguyen *et al*. (2019) recorded improved dry matter digestibility in spray-dried multi-species probiotics supplemented group of finisher pigs, whereas digestibility values were comparatively lower than the reported values in the present study. On the contrary, Wang and Kim (2021) reported apparent total tract nutrient digestibility co-efficient values for all the nutrients were similar between control and spray-dried *Lactobacillus plantarum* BG0001 (1.30 x 10^7 CFU/g) supplemented at 0.1 and 0.2 per cent, respectively. The dry matter digestibility values ranged from 80.20 to 82.05 per cent.

The improvement in nutrient digestibility in the present study might be due to the effect of encapsulated probiotics, and the efficiency of the encapsulation process. While comparing encapsulated probiotics supplemented in this study *L. plantarum* (NCIM 2374) showed significantly improved nutrient digestibility coefficient values compared to *P. acidilactici* NCIM 5721 strain. Those pigs supplemented with *L. plantarum* showed significantly (P< 0.01) better organic and crude protein digestibility compared to *P. acidilactici*. This better performance of *Lactobacillus* spp populations may be due to an increase in lactic acid production in the gut and a drop in pH might improve the ability to digest nutrients and the survivable rate of probiotics during the encapsulation process.

Conclusion

The supplementation of encapsulated probiotics significantly improved (P< 0.01) the digestibility of nutrients which could help in better nutrient absorption and growth in Large White Yorkshire piglets.

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Conflict of interest

The authors declare that they have no conflict of interest.

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