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Effect of feeding low protein diets supplemented with selected essential amino acids on growth performance and gut health in early weaned Large White Yorkshire piglets#

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Abstract

A study was conducted to assess the effect of feeding low protein diets supplemented with selected amino acids on growth performance and gut health in early weaned Large White Yorkshire piglets. Thirty Large White Yorkshire female piglets belonging to Centre for Pig Production and Research, Mannuthy, weaned at 21 days of age were allotted to three dietary treatment groups (T1, T2 and T3) with five replicates for each treatment. Piglets were fed pre-starter ration till they gained a body weight of 5 kg and then on starter ration from 5 to 18 kg body weight. The dietary protein in treatments T2 and T3 were reduced by 15 and 25 per cent respectively than that of control treatment (T1). Five amino acids viz., lysine, methionine, threonine, valine and isoleucine were supplemented to all treatment groups to meet the requirements as per NRC (1998). Body weights of animals were taken at weekly intervals. There was no significant difference in average body weight among three treatment groups from first to third week. From fourth to eleventh week, the average weekly body weight of T1 was significantly higher than T3. The average daily gain was significantly different among three treatment groups with highest values observed for group T1, followed by T2 and T3. The feed conversion efficiency was statistically similar among treatment groups. Total viable count and coliform count were significantly lower in the faeces of piglets fed with low protein diets, indicating a reduction in microbial population in faeces of piglets in relation to reduction in

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dietary protein level. From the overall results, it could be concluded that reducing the dietary protein levels had resulted in reduced growth performance in early weaned piglets, in spite of being supplemented with selected essential amino acids

Keywords: *LWY piglets, early weaning, low protein diet, growth performance, faecal microbial count*

Swine farming has gained momentum and the intensification had transformed it into an industry (Keyho *et al*., 2018). Qualities like better-feed conversion efficiency, early maturity, short generation interval, high fecundity, relatively smaller capital investment and a faster economic return to the farmers make commercial piggery lucrative to farmers (Ambily *et al*., 2021). Weaning is an important period in the growth and development of pigs because it could help to improve pig production efficiency and to increase the number of piglets produced per sow per year (Pettigrew *et al*., 1994). During weaning, piglets are separated from their mother, taken to a new environment and switched from a liquid diet to a compounded dry feed (Varley and Wiseman, 2001). Hence, the piglets experience significant physiological, environmental and social challenges during weaning that can predispose them to subsequent diseases and other production losses (Williams, 2003; Lalles *et al.,* 2007). Stress associated with weaning, especially early weaning results in intestinal, immunological, and behavioural changes also. The post weaning period is often characterised by suboptimal growth performance and higher incidence of diarrhoea, which in turn can cause morbidity and mortality, leading to huge economic loss to farmer. Development of normal intestinal structure and function is a vital determinant in reducing incidence of diarrhoea and development of strategies to mitigate various stresses associated with weaning to avoid production loss is the need of hour.

Nutrition can play an important role to alleviate metabolic and physiological distress by providing the essential nutrients in the diet that are fundamental to ensure optimum growth, feed efficiency, health and profitable piglet production. Protein is one of the costlier components in diets of pig and lowering of protein reduces feed cost. High-protein diets led to excretion of excess nitrogen in faeces and urine, resulting in lowered efficiency of nitrogen utilisation. Moreover, fermentation of protein in lower gastro intestinal tract can damage the gut health. Hence, lowering of dietary protein is one among the nutritional strategies suggested to minimise the stress associated with weaning. But low dietary protein results in lesser growth performance raising concern among pig farmers and industry.

Amino acids such as lysine and methionine are commonly added in the piglet diet to ensure their growth performance. Threonine, valine and isoleucine are involved in gut development and health of piglets (Wang *et al*., 2018). The present study envisaged in evaluating the combined effect of dietary supplementation of these five essential amino acids on growth performance and gut health in early weaned piglets fed low protein diet which may help in developing feeding strategies to maximise the health and growth performance of early weaned piglets.

Materials and methods

Thirty Large White Yorkshire female piglets, weaned at 21 days of age belonging to Centre for Pig Production and Research (CPPR), Mannuthy were used as the experimental animals. The piglets were randomly allotted to three dietary treatment groups (T1, T2 and T3) with five replicates for each treatment. Piglets were fed pre-starter ration till they gained a body weight of 5 kg and later fed with starter ration up to 18 kg body weight (NRC, 1998). The dietary protein levels in pre-starter and starter ration in treatments T2 and T3 were reduced by 15 and 25 per cents, respectively than that of control treatment (T1). All the diets were also made iso-caloric (3265 kcal ME/kg feed) as per NRC (1998). Five essential amino acids *viz*., lysine, methionine, threonine, valine and isoleucine were supplemented to all treatments to meet their requirements as per NRC (1998). All the animals were maintained under uniform management conditions. The animals were fed twice daily *ad libitum* for one hour and the left over feed, if any was collected and weighed after each feeding. Daily feed intakes were recorded throughout the experimental period; up to attainment of 18 kg body weight. Experimental animals were weighed at weekly intervals. The feed samples were analysed for crude protein (AOAC, 2012).

Faecal microbial analysis

Fresh faecal samples were collected randomly at 20 days of feeding trial from animals belonging to the three dietary treatment groups. The samples were processed upon arrival in the laboratory and subjected to microbiological analysis on the same day of collection. Nine grams of samples were homogenised in 90 mL of phosphate buffer saline (PBS) and this formed the initial test sample. Further tenfold serial dilution was prepared by transferring one mL of inoculums in nine mL of the diluent. All aseptic precautions were taken during collection and processing of samples.

Total Viable Count (TVC) of all samples was estimated by pour plate technique, as described by Morton (2001). From the selected ten-fold dilution of each sample, one mL of inoculum was transferred into duplicate petri dishes of uniform size. To each of the inoculated plates, about 10-15 mL sterile molten standard plate count agar (HiMedia) maintained at 45 °C was poured and mixed with inoculums by gentle rotary movement i.e., clockwise, anticlockwise, forward and backward. The inoculated plates were left at room temperature and allowed to solidify and were incubated at 37 °C for 24h. At the end of incubation, plates showing colonies between 30 and 300 were selected and counts were taken with the help of a colony counter. The number of colony forming units (cfu) per mg/ml of sample was calculated by multiplying the mean colony count in duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu per g.

Coliform count per mL of samples was estimated according to the procedure described by Kornachi and Johnson (2001). From the selected ten-fold dilution, 0.1 mL of inoculum was inoculated onto duplicate plates of Violet Red Bile Agar (VRBA) (HiMedia) and was uniformly distributed with a sterile 'L' shaped glass rod. The inoculated plates were incubated at 37 °C for 24 h. At the end of incubation, purplish red colonies with a diameter of at least 0.5 mm, surrounded by a reddish precipitation zone were counted as coliforms. The number of organisms was estimated by multiplying the mean count in duplicate plates with the dilution factor and expressed as $\log_{10}$ cfu per g.

Data collected on various parameters were analysed by completely randomised design (CRD) method as described by Snedecor and Cochran (1994). Means were compared by Duncan multiple range test (DMRT) using statistical package for social studies software (version 24).

Results and discussion

The ingredient compositions of pre-starter and starter rations are given in Tables 1 and 2, respectively. The three experimental pre-starter rations T1, T2, and T3 had crude protein (CP) content of 22.51, 18.88 and 16.74, per cents, respectively while the starter ration groups T1, T2 and T3 had CP content of 20.40, 17.65 and 15.08, per cents respectively.
The data on body weight of piglets maintained

Table 1. Ingredient composition of pre-starter rations, %

Ingredients	Pre starter rations (%)		
	T1	T2	T3
Yellow maize	54.50	59.00	62.00
Wheat bran	--	5.00	8.00
Soyabean meal	25.50	20.00	15.00
Unsalted dried fish	8.00	4.00	3.00
Whey powder	10.00	10.00	10.00
Salt	0.50	0.50	0.50
Mineral mixture	1.50	1.50	1.50
Total	100.00	100.00	100.00
To the above mixture following ingredients were added			
Lysine, g	30	320	477
Methionine, g	2	40	72
Threonine, g	-	120	209
Valine, g	-	50	150
Isoleucine, g	-	-	38
Hyblend AB_2D_3K,g	2	2	2
Zinc Oxide, g	20	20	20
Cost of feed (Rs/kg)	53.96	54.16	56.41

Table 2. Ingredient composition of starter rations, %

Ingredients	Starter rations (%)		
	T1	T2	T3
Yellow maize	69.00	72.00	75.00
Wheat bran	--	5.00	10.00
Soyabean meal	25.00	17.00	9.00
Unsalted dried fish	4.00	4.00	4.00
Salt	0.50	0.50	0.50
Mineral mixture	1.50	1.50	1.50
Total	100	100	100
To the above mixture following ingredients were added			
Lysine, g	132	319	506
Methionine, g	2	2	23
Threonine, g	-	90	192
Valine, g	1	109	217
Isoleucine, g	-	38	162
Hyblend AB_2D_3K,g	2	2	2
Zinc Oxide, g	20	20	20
Cost of feed (Rs/kg)	27.05	27.83	30.93

on three experimental rations T1, T2 and T3 recorded at weekly intervals are presented in Table 3. The initial body weight of piglets did not differ significantly among three treatment groups. There was no significant difference in average body weight among three experimental groups from first to third week post weaning. From week 4 to week 11, the average weekly body weight of T1 was significantly higher than that of T3, while no significant difference was observed between T2 and T3, except at tenth week. The average body weight of T1 was significantly higher than that of T2 except for sixth and eleventh weeks post weaning.

From the results of present study, it was found that the body weight gain of piglets has a direct correlation with level of CP in diet, with lesser body weight recorded in piglets fed diet containing lower levels of dietary CP, in spite of being supplemented with selected essential amino acids. Similar observations were also made by Nyachoti *et al*. (2006) that the average daily weight gain of piglets had reduced when CP level was lowered to 19 or 17 per cent than control diet (23 per cent).

Data regarding average daily feed intake of piglets maintained on three experimental rations at weekly intervals are presented in Table 4. There was no significant difference in average daily feed intake between groups from first to three weeks post weaning. From week 4 to week 11, the average daily feed intake of T1 was significantly higher than T2 and T3, except for sixth, tenth and eleventh week. In the current study, the average daily feed intake reduced linearly when dietary crude protein was decreased from 20 to 15 per cent during starter period. Similar observations were also made by Nyachoti *et al*. (2006) that the average daily feed intake was similar between high and low protein groups during initial 3 weeks. Zheng

Table 3. Weekly average body weight of piglets maintained on three experimental rations, kg

Week	Treatments[1]			p- value
	T1	T2	T3	
0	3.93±0.15	4.05±0.10	4.03±0.10	0.86^{ns}
1	4.23±0.16	4.13±0.10	4.14±0.10	0.65^{ns}
2	4.78±0.25	4.72±0.20	4.55±0.29	0.79^{ns}
3	5.79±0.17	5.49±0.16	5.25±0.17	0.14^{ns}
4	7.32^{b}±0.19	6.12^{a}±0.91	5.70^{a}±0.57	0.03^{*}
5	8.51^{b}±0.35	7.19^{a}±0.48	6.33^{a}±0.60	0.003^{**}
6	10.20^{b}±0.50	8.59^{ab}±0.87	7.23^{a}±0.71	0.006^{**}
7	11.74^{b}±0.75	9.90^{a}±0.62	8.28^{a}±0.55	0.003^{**}
8	14.13^{b}±0.64	11.93^{a}±0.69	10.30^{a}±0.73	0.006^{**}
9	16.46^{b}±0.80	13.70^{a}±0.91	11.76^{a}±0.94	0.005^{**}
10	18.78^{b}±0.84	17.06^{b}±0.97	14.98^{a}±1.01	0.02^{*}
11	22.91^{b}±1.06	20.05^{ab}±1.06	17.87^{a}±1.04	0.02^{*}

[1]Mean of five observations with SE; Means with different letter as superscripts within the same row differ significantly at 0.05 level *(P<0.05); ** (P<0.01); ns- Non significant (P>0.05)

Table 4. Weekly average daily feed intake of piglets maintained on the three experimental rations*, kg

Week	Treatments[1]			p- value
	T1	T2	T3	
1	0.07±0.01	0.06±0.01	0.05±0.02	0.35[ns]
2	0.10±0.01	0.11±0.01	0.11±0.01	0.20[ns]
3	0.14±0.01	0.13±0.01	0.13±0.01	0.97[ns]
4	0.17[b]±0.01	0.17[b]±0.01	0.15[a]±0.01	0.03*
5	0.25[b]±0.02	0.20[a]±0.04	0.21[a]±0.02	0.03*
6	0.31[b]±0.28	0.25[a]±0.21	0.25[a]±0.08	0.028*
7	0.39[b]±0.39	0.30[a]±0.33	0.28[a]±0.30	0.01**
8	0.53[b]±0.02	0.42[a]±0.07	0.35[a]±0.06	0.003**
9	0.60[b]±0.34	0.49[a]±0.39	0.41[a]±0.31	0.01**
10	0.63[b]±0.03	0.58[ab]±0.08	0.50[a]±0.06	0.03*
11	0.75[b]±0.04	0.71[ab]±0.05	0.62[a]±0.09	0.049*

*****on dry matter basis

[1]Mean of five observations with SE; Means with different letter as superscripts within the same row differ significantly at 0.05 level *(P<0.05); ** (P<0.01); ns- Non significant (P>0.05)

Table 5. Feed intake, average daily gain and feed conversion efficiency of piglets maintained on three experimental rations

Parameters	Treatments[1]			p- value
	T1	T2	T3	
Average initial body weight, kg	3.93±0.15	4.05±0.10	4.03±0.10	0.87[ns]
Average final body weight, kg	22.91[b]±1.06	20.05[ab]±1.06	17.87[a]±1.04	0.02*
Total weight gain, kg	18.98[b]±1.14	16.00[ab]±1.84	13.84[a]±2.66	0.03*
Average daily gain, g	246.49[c]±6.95	207.79[b]±7.84	179.74[a]±8.25	0.02*
Total feed intake, kg	55.38[b]±1.09	48.22[a]±2.98	43.38[a]±3.44	0.014*
Average Feed conversion efficiency	2.92±0.06	3.01±0.26	3.13±0.10	0.86[ns]

[1]Mean of five observations with SE; Means with different letter as superscripts within the same row differ significantly at 0.05 level *(P<0.05); ns- Non significant (P>0.05)

et al. (2001) found that reducing the crude protein content from 20 to 16 per cent resulted in significant reduction in feed intake and daily gain. However, pigs fed with lower protein diets along with supplemented amino acids had comparable growth performance with those fed with higher protein diet.

Data related to average daily gain and feed conversion efficiency of piglets maintained on three experimental rations T1, T2 and T3 are presented in Table 5. Total weight gain and average final body weight were significantly higher in T1 than T3, while no significant difference was observed between T2 and T1 or T3. Furthermore, total feed intake was significantly higher in T1 than T2 and T3, whereas the average feed conversion efficiency did not differ significantly among three treatment groups.

The results of present study indicated that the average daily gain and final body weight were significantly lower in piglets fed diet containing lower levels of CP supplemented with selected essential amino acids. Similar observations were also made by Opapeju *et al*. (2008) that average daily gain and feed conversion ratio were negatively affected by reduction in dietary CP. On contrary to present results, Yue and Qiao (2008), reported that piglets fed diet containing 17.2 per cent crude protein had the poorest average daily gain and feed gain ratio compared to piglets fed with higher levels of dietary CP.

The total viable count in the faecal content of piglets maintained on three experimental rations T1, T2 and T3 were 11.7, 11.29 and 11.12 log_{10} cfu per g, respectively

Table 6. Faecal microbial count of piglets maintained on three experimental rations, (log_{10} cfu/g)

Parameters	Treatments			P -value
	T1	T2	T3	
Total viable count(log_{10} cfu/g)	11.70±0.13^{b}	11.29±0.07^{a}	11.12±0.12^{a}	0.01*
Coliform count (log_{10} cfu/g)	6.61±0.14^{c}	6.35±0.16^{b}	5.85±0.15^{a}	0.014*

Bacterial numbers are expressed as log10 colony forming units per gram.

[1]Mean of five observations with SE; Means with different letter as superscripts within the same row differ significantly at 0.05 level **(P<0.01)

and coliform counts were 6.61, 6.35 and 5.85 log_{10} cfu per g for T1, T2 and T3, respectively. Faecal microbial count of piglets maintained on three experimental rations are shown in Table 6. The total viable count was significantly higher in the faecal samples of T1 than T2 and T3. However, the coliform values differed significantly among three treatment groups with highest value recorded for T1 and lowest value for T3. From the present results, it could be found that total viable count and coliform count were significantly lower for low CP containing diet, supplemented with selected essential amino acids. In the present study, the faecal consistency had improved linearly with reduction in dietary CP. When the faecal samples contained lower counts of pathogenic bacteria, such as coliforms, and a higher ratio of lactobacilli to coliforms, it is indicative of a decreased risk for the development of post weaning diarrhea (Wellock *et al*., 2006). Similarly, Yue and Qiao (2008) found that reducing the CP level in piglet feed had resulted in an improvement in faecal consistency scores, which is in agreement to the present study. The observations made on coliform count in the present study was also in agreement with those reported by Nyachoti *et al*. (2006), where the reported values ranged from 6.16 to 6.59 log_{10} cfu per gram for treatments groups, when CP levels were reduced from 23.0 to 17.0 per cent. Similarly, Luo *et al*. (2015) noted reduced numbers of Firmicutes and Clostridium clusters in the caecal digesta of piglets, when CP levels were reduced from 20.0 to 14.0 per cent. Rist *et al.* (2013) opined that excessive fermentation of dietary protein in the lower intestine might lead to growth of potential pathogenic bacteria such as *Clostridium perfringens* and the reduction of faecal counts of beneficial bifidobacteria.

Conclusion

In the present study, there was no significant difference in average body weight among treatment groups from first to third week. From fourth to eleventh week, the average weekly body weight of T1 was significantly higher than T3. The average daily gain was significantly different among three treatment groups with highest values observed for T1, followed by T2 and T3. Total feed intake of T1 was significantly higher than T2 and T3, while it was statistically similar between T2 and T3. The feed conversion efficiency was statistically similar among treatment groups. Total viable count and coliform count were significantly lower in the feces of piglets fed with low protein diets. From the overall results, it could be concluded that reducing the dietary protein levels resulted in reduced growth performance in early weaned piglets, in spite of being supplemented with selected essential amino acids.

Conflict of interest

The authors declare that they have no conflict of interest.

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