

# EFFECT OF DIETARY SUPPLEMENTATION OF ARGININE AND GLUTAMINE ON GUT HEALTH IN EARLY WEANED LARGE WHITE YORKSHIRE PIGLETS

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## ABSTRACT

An experiment was conducted to study the effect of dietary supplementation of functional amino acids on gut health in early weaned Large White Yorkshire (LWY) piglets. Forty LWY piglets weaned at twenty one days of age were randomly divided into four groups and allotted to four dietary treatments. T1 - (Control ration as per NRC, 1998), T2 - (Control ration + 0.7 percent Arginine +1 percent Glutamine), T3 - (Control ration + 0.35 percent Arginine + 0.5 percent Glutamine), T4 - (Control ration + 0.18 percent Arginine + 0.25 per cent Glutamine). At the end of the experimental trail of 64 days, faecal samples were collected from animals belonging to the four dietary treatment groups and were processed for determination of total viable count and coliform count. From the results, it could be observed that the total viable count in the faecal matter was similar in all the treatment groups and ranged from 11.70 to 11.98 log<sub>10</sub> cfu per g. But the coliform count was lower (P<0.05) in T4 group (6.65) compared to T1 (6.72), T2 (6.98) and T3 (6.75).

**Keywords:** Arginine, early weaning, glutamine, gut health

## INTRODUCTION

The consumption of pork has increased during last decade which resulted in increased pork production through intensive rearing system. Weaning of piglets is done at eight weeks of age in traditional pig production which has been brought down to three weeks in intensive rearing system. So piglets have to deal with many physiological, environmental and social challenges which subsequently predisposes to many diseases and other production losses. During weaning, piglets are subjected to a number of stressors, such as an abrupt separation from the sow, transportation, handling, sudden withdrawal of sow milk, a different food source, social hierarchy in the litter, co-mingling with piglets from other litters, a different physical environment, increased exposure to pathogens and dietary or environmental antigens. Improvement in the gut health depends on intestinal environment and gut microflora, which protect the animal from oral pathogens. Nutrition is a tool

required to control immune sensitivity to pathogens by providing substrate for immune cells or pathogens, protecting animal against immunopathology, influencing gut microbial populations and the hormonal environment. Arginine and glutamine are examples of functional amino acids with well-defined functions and roles in maintaining gut microflora. Nitric oxide plays a very important role in the destruction of some pathogenic microbes by neutrophils and macrophages. Dietary supplementation of arginine decreases coliform count of small intestine by producing nitric oxide. Hence this study is aimed at evaluating the gut health in early weaned piglets by supplementation of arginine and glutamine in diets of pure bred LWY piglets.

#### **MATERIALS AND METHODS**

Forty LWY piglets of either sex, weaned at three weeks of age belonging to Centre for Pig Production and Research, Mannuthy were used as experimental animals. Piglets were divided into four groups as uniformly as possible with regard to age, sex and weight. There were five replicates for each treatment with two piglets in each replicate. All piglets were maintained under uniform management conditions throughout the experimental period of 64 days. Piglets were fed with pre-starter ration (22 per cent CP and 3265 kcal ME) upto body weight of 5.5 kg and starter/creep ration (20 per cent CP and 3265 kcal ME) from 5.5 to 18 kg body weight. The piglets were randomly allotted to four dietary treatments as follows, T1 - control ration (as per NRC, 1998), T2 - Control ration + 0.7 percent Arginine + 1 percent Glutamine, T3 - Control ration + 0.35 percent Arginine +

0.5 percent Glutamine, T4 - Control ration + 0.18 percent Arginine + 0.25 percent Glutamine.

#### **Collection and processing of samples**

Fresh faecal samples were collected randomly towards the end of feeding trail from animals belonging to the four 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 homogenized in 90 ml of phosphate buffer saline (PBS) and this form the initial test sample. Further tenfold serial dilutions were 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**

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 the each sample, one ml of inoculum was transferred into duplicate petridishes 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 gram.

### Coliform count

Coliform count per ml of samples was estimated according to the procedure described by Kornacki 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 plate were incubated at 37°C for 24h. 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 gram.

Data collected on various parameters were statistically analysed by Completely Randomized 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 data on faecal microbial count is

presented in Table 1. The total viable count in the faecal content of piglets maintained on four experimental rations T1, T2, T3 and T4 was 11.89, 11.70, 11.98 and 11.82  $\log_{10}$  cfu per g, respectively and coliform count was 6.72, 6.68, 6.75 and 6.65  $\log_{10}$  cfu per g for treatments T1, T2, T3 and T4, respectively.

The statistical analysis of the data revealed similar observations among the treatments in total viable count whereas coliform count in T4 was statistically significant and showed lower value than T1 and T3 whereas T1, T2 and T3 were similar among them.

These results are in contrary to Oso *et al.* (2017) who reported an increase in coliform count of small intestine by dietary supplementation of arginine 0, 0.5, and 1.0 g per kg in growing turkeys. Shakeri *et al.* (2014) reported no effect on caecal coliform count of broiler chickens fed with 0.5% mixture of l-glutamine and l-glutamic acid.

### CONCLUSION

An evaluation of the results obtained in the current experiment indicates that dietary supplementation of functional amino acids like arginine and glutamine at 0.18 and 0.25 per cent level (T4) reduces the coliform count in the intestine, and

**Table 1.** Faecal microbial count of piglets maintained on four experimental rations, ( $\log_{10}$  cfu per gram)

Parameter	Treatments				P value
	T1	T2	T3	T4	
Total viable count	11.89±0.12	11.70±0.11	11.98±0.08	11.82±0.04	0.21 <sup>ns</sup>
Coliform count	6.72±0.02 <sup>a</sup>	6.68±0.02 <sup>ab</sup>	6.75±0.02 <sup>a</sup>	6.65±0.03 <sup>b</sup>	0.03*

<sup>1</sup>Mean of five observations with SE;

Means with different superscripts within the same row differ significantly (P<0.05)

hence can be included in the diet of early weaned LWY piglets for better nutrient utilization and performance.

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