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Role of nutrients in the intestinal health of swine

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Abstract
Nowadays weaning of piglet's poses a stressful condition under environmental, nutritional, psychological and immunological conditions leading to a gut associated epithelial damage along with damage to intestinal barrier function. Focus should be diverted to develop alternate strategies to develop gut and maintain it's health. Dietary intervention is one of the feeding strategies to achieve this goal which cover feeding optimum quantum of protein, less amount of soluble NSP, required quantities of minerals like Calcium, Phosphorus, Zinc and Iron, feeding of fermented liquid feeds. Excessive protein for the weaned piglets leaves undigested protein molecules which act as substrates for pathogen proliferation producing mucosal irritants. On the other hand, feeding of soluble NSP containing high arabinose and xylose caused a higher incidence of non-specific enteritis and could change the Sacchyrolytic: Proteolytic bacteria ratio. However insoluble NSP checked the growth of pathogensas it decreases the digesta retention time in the gut and it was also demonstrated that insoluble NSP attracts more Sacchyrolytic bacteria instead of Proteolytic. Another strategy to reduce Post Weaning Diarrhoea (PWD) is to feed fermented liquid feed(FLF).Steeping causes the lactic acid bacteria proliferation producing Lactic acid which lowers the p H and thus act as the first line defence against possible pathogen infections. Minerals like Zn, Ca, P, Fe also play a pivotal role in maintaining the gut health. High levels of dietary ZnO causes reduced *lactic acid bacteria* with an increase in Coliforms and *Enterococci*. Excess availability of Ca, P and Fe also will have detrimental effects on the gut health. Probiotics, prebiotics also have a major role in gut health as the former eliminates the pathogens by competitive exclusion and the latter acts as a substrate for the proliferation of *bifidobacteria* and *Lactobacilli.* It is concluded that during post weaning, growth check and enteric diseases including PWD cause a major loss to the swine industry for which a number of feeding strategies have been suggested like low dietary protein, dietary fibre, optimum level of inclusion of minerals and their salts (ZnO), use of probiotics, prebiotics, acidifiers. However use of minerals should be judicious as excess dietary inclusion leads to environmental pollution.

Keywords: Weaning, enteric diseases, dietary nutrient intervention, fermented liquid feed, feeding strategies

Introduction

Animal production systems are now based on consumer preference. These are influenced by consumer views, environmental conditions and public health concerns. When the consumer preference is taken into consideration care should be taken to give utmost importance to animal health also. Various feed nutrients are being fed to swine to obtain productivity. Since recently, use of antibiotics in the feed was mandatory since these prevent the pathogenic bacteria in addition to the maintaining the animal immunity and normal balance of intestinal flora. But in view of a ban on the use of dietary antibiotics by the European Union, other non-nutrient substances like feed additives are being explored which improve the animal productivity by increasing the feed nutrient utilization in the gut.

The association between nutrition and health of the animals is gaining importance in the recent years. Here when we speak of health and its maintenance, it not only implies to pathogenic bacteria, but also to the feed nutrients. The role of feed nutrients plays a pivotal role especially when the animals land in stress and thus causing the pathogen proliferation. Elimination of pathogen from the gut can be achieved in many ways and one of the useful sense of achieving it is through *nutritional interventions*. This can be better explained in weaned piglets. Pigs immediately after weaning undergo a stressful condition of changing the intestinal barrier function. During suckling period, easily and readily fermentable liquid feeding is replaced by a total solid high protein feed posing for gut digestible problem [1]. Milk acts as a ready source of energy for the maintenance of integrity of gut epithelium [2]. It impairs the digestibility due to impaired digestion and the undigested feed particles become substrates for the pathogen proliferation.

When the integrity of the epithelium is lost due to insufficient supply of energy, it causes damage by two fold-it increases the pathogen attachment to the gut mucosa [3] and it leads to the production of epithelial irritants like ammonia, biogenic amines [4].

The intestinal epithelium is the main barrier to transport the absorbed nutrients from the gut to the blood pool. The intestinal epithelium is protected with a mucous layer which allow only nutrients and not the macro molecules like enzymes [5] and antigens in addition to not allowing the pathogenic bacteria to colonize on the gut epithelium for absorption into the blood pool and so it was reported to supplement certain of the beneficial bacteria like *Lactobacillus spp* and *Bifidobacteria spp* which colonize on the gut epithelium and eliminates the pathogens by competitive exclusion competing for the absorptive sites. Since, the focus is on the effect of dietary nutrients on the gut health especially in weaner pigs, they remain highly susceptible to a number of bacterial and viral diseases. The most predominant bacteria that affects the gut health are *E.coli* and *Salmonella* causing Post Weaning Diarrhoea (PWD). So efforts are to be put forth to reduce the incidences of PWD for which the dietary nutrient interventions are to be adopted and are discussed under the following headings…..

Dietary protein intervention in gut health

The digestibility of protein is important for the growth of the animals on various factors like pH in the gut, pancreatic enzyme secretions and the production status of the proteolytic enzymes. Low gastric pH in the stomach facilitates the conversion of *inactive pepsinogen* to active pepsin [6] for its action on proteins. When the proteins are not digested properly the undigested particles will remain in the gut and act as substrates for the pathogenic proliferation and these produce mucosal irritants like ammonia and biogenic amines [7]. Thus low gastric pH eliminates [8] pathogens and so high gastric pH favours [9] the growth of *E. coli* and *Coliforms* leading to Post Weaning Diarrhoea (PWD). The availability of undigested feed particles can change Sacchyrolytic: Proteolytic microbe's ratio (Sacchyrolytic microbes ferment carbohydrates whereas proteolytic microbes ferment proteins) which leads to the production of potentially harmful irritants to the intestinal epithelial cells and these effect the intestinal permeability or barrier function. It was reported by [10] that the increase in the dietary protein significantly reduced the *Lactobacilli* to *Coliform* ratio. Based on all these works it can be concluded that lower dietary protein either remains unaltered in terms of *Sacchyrolytic: Proteolytic* microbes ratio or changes to a little extent without affecting the intestinal barrier functions. It was reported that pigs fed higher proportion of protein would have higher risk of gut infection rather than with lower quantum as it stimulates the production of proteolytic microbes such as *E. Coli* and *Clostridium perfringens* [11]. One remedy to control PWD is to supply the low level of dietary protein along with the supplementation of crystalline or synthetic amino acids like Lysine [12, 13]. Feeding a lower protein (173 g CP/kg) diet after weaning reduced indices of protein fermentation with an associated decrease in diarrhoea, and without adverse effects on production [14] compared with feeding a higher protein (243 g CP/kg).

Dietary carbohydrate intervention in gut health

Carbohydrates are naturally occurring organic compounds and the inclusion of carbohydrates with prebiotic properties in pig diets to stimulate establishment of beneficial enteric micro-organisms may limit the proliferation of enteric pathogens and decrease formation of toxic fermentation products [15]. When the availability of carbohydrates for microbial fermentation is limiting, undigested protein can be deaminated, used as an energy source, and yield potentially harmful compounds. On the other hand, microbes can utilize ammonia and amines to synthesize microbial protein, provided that sufficient fermentable carbohydrates are available. This would suggest there is an optimum balance between fermentable protein (FP) and fermentable carbohydrates (FC)

During the process of weaning, the changes in the GIT can be categorized into 2 phases, *active phase* which takes about 5-7 days in which the weaned piglets epithelium react to the solid feed and *adaptive phase* which lasts for 7-14 days and this is the period where in the weaned piglets adapt to the dry matter intake [16].

The carbohydrate fraction can be divided into sugars, oligosaccharides and poly saccharids (Starch and non-starch polysaccharide) as per the glycosidic linkages. The digestion of carbohydrates depends on the way in which the alpha linkages are broken down in the gut. It is to be noted that the carbohydrates are divided into simple sugars which are easily fermentable and complex polysaccharides which are easily fermentable and complex with alpha linkages which are to be degraded to simple sugars by the endogenous enzymes secreted by the gut. Other carbohydrate with beta linkages cannot be degraded by the gut endogenous enzymes but by the microbial enzymes to simple sugars on various metabolic pathways to yield the energy.

Define intestinal to epithelial health

While maintaining the gut health, three factors need to be considered viz-diet, mucosa and gut microflora. The mucosa consists of the gut epithelium, gut associated lymphoid tissue (GALT) and the mucous overlying the epithelium. All these three components along with the bacterial flora work together to keep the gut epithelium healthy ensuring efficient absorption of all the nutrients into the blood stream. In order to maintain the gut integrity, feed plays a major role in maintaining the balance of the microbiota.

Thus to maintain the gut health the dietary intervention is mandatory. This was reported by [17] using 2 prebiotics viz-insulin and alginate fed to piglets and found that the intestinal microbial eco physiology was affected with these two feed additives.

The digestion of starch changes with the number of increased days of weaning because at about 3 weeks after weaning, there will be decreased or insufficient production of pancreatic enzymes required for starch digestion [18]. As the age of the pig advances and in growing pigs the starch is well digested after being gelatinized [19].As discussed above it takes at least 5-10 days for the intestinal flora to adjust for the diet fed. The complex microbial community in the GIT consists of different groups of microbes including bacteria, anaerobic fungi, ciliate and flagellate protozoa [20]. However it was reported that during the first 11 days of weaning, the growth of lactic acid will be more due to the predominant presence of *Lactic acid bacteria*. It is to be noted that the maintenance of gut health is complex and depends on the balance between the diet, commensal microbiota, the mucosa and the gut epithelium.

The digestibility and the absorption of nutrients depend on various techniques like processing of feeds (pelleting, reduction in the particle size), supplementation of exogenous enzymes and other physical methods [21]. Enzyme supplementation has added advantages in releasing the entrapped nutrients from the feed resources and also in changing some of the anti-nutritional factors like viscosity and water holding capacity of NSP.

Immediately after weaning up to a period of about 3 weeks, the carbohydrate digestion will be lowered owing to a decreased or insufficient production pancreatic enzyme to digest starch. It was reported [18, 19] that for the starch to get digested, the enzymes that are present active at about 10 days weaning are *maltase* and *glycoamylase* which gelatinize the starch for increasing the interactions between the gut endogenous enzymes and also to increase the surface area for enzymatic action. These incidences are in contrast to those that occur in growing and adult pigs where the gelatinized starch is completely digested. These reactions in the small intestines have a profound influence on the type of substrate available for microbial fermentation in the large intestines. Unlike in growers and adult pigs, starch is the main substrate for microbial fermentation in 10 days weaned piglets.

At the time of weaning, the GI tract of piglets is densely populated with disorganized bacterial communities of which the predominant species will be *Lactobacillus* producing *Lactic acid* leading to a drop in pH. Unlike in 10 day weaned piglets [17, 22] in growing pigs other than *Lactobacilli*, other gram positive bacteria like *Streptococci*, *Clostridia* will also find their existence**.** However, the microbiota in the gut depends on the availability and type of substrate or feed given. When sufficient substrate is available for bacteria, they ferment and yield organic acid compromising predominantly lactate, acetate, propionate and butyrate. These organic acids not only reduce the gut pH, but also stimulates epithelial cell proliferation [23]. Acidic condition in the gut kills all the enteric bacteria like *E.coli*, *Salmonella*, *Clostridium* etc. [24]. All this process mentioned here will happen when the dietary fibre is included in the diet. But when soluble NSP are added in the diets they can promote the proliferation of commensal gut microbiota [25] and on the other hand inclusion of insoluble NSP reduce the transit time of the feed in the gut providing a good substrate for pathogenic bacterial proliferation [26]. Feeding viscous NSP like guar gum increases the proliferation of *Enterotoxegenic E.coli* [27].

It is to be noted that bacterial fermentation produces SCFA whose concentration increases in the colon as seen in adult and growing pigs [9, 28]. As discussed earlier, the concentration of SCFA depend on the type of substrate used for fermentation. If starch is more, high butyrate concentrations are seen [29].

Immediately after weaning, there is every possibility for the piglets to develop enteric disorders due to many reasons as depicted here under…

1. Change of feed from a liquid easily digestible milk to a complex solid feed
2. Change of environmental temperature and there by physiological changes
3. Changes in the structural epithelium
4. Changes in the microbial community
5. Immune system of the gut mucosa is at primitive stage favouring the growth of enterotoxegenic bacteria.

In a conclusion about carbohydrates, they form the largest source of energy for pigs and their inclusion in different forms change the gut morphology and SCFA ratio. Since the occurrence of enteric diseases is also dependent on these, utmost care needs to be taken, while feeding the piglets and finally we can say the carbohydrate feeding intervention is critical.

Dietary fermented liquid feed intervention in gut health

Though fermented liquid feed (FLF) is not an additive, the use of these has 2 main advantages [30].

1. During weaning, simultaneous provision of feed and water may also reduce the time spent to find both sources of nutrients.
2. Feeding of FLF with low pH will reduce the stomach pH and thus eliminates the pathogens as the first line of defence [31].

Now a days FLF has gained popularity due to the below said explanations

- Decrease in the use of antibiotics in pig production
- Change of feeding strategy by feeding cheaper liquid feed ingredients
- Greater demand for biofuels
- Reduction in the waste disposal to prevent environmental pollution.

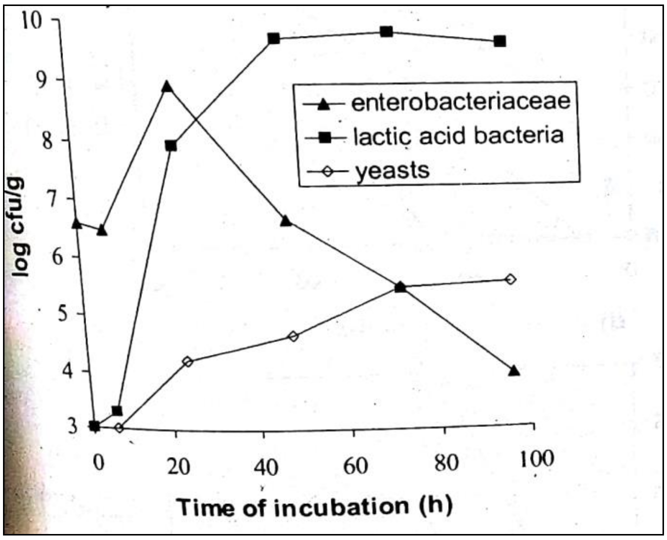
When compound feed is simply soaked in water for hours or for a certain length of time, *Lactic acid bacteria* and *yeast* naturally occurring in the feed proliferates and produces mainly lactic acid, acetic acid and ethanol resulting in a reduction of pH to about 4.5 in the fermented liquid mixture. This pH prevents the proliferation of spoilage organisms and food borne pathogens. Another advantage of lower pH is that better protein hydrolysis occurs in the diet. Thus the ability of LAB to inhibit the growth of various gram negative bacteria especially pathogenic *E. coli* is well documented both *in vitro* [32] and *in vivo* conditions [33]. Here is the table which shows the average different bacterial counts for 28 days weaned piglets at different segment levels of the gut viz-stomach, first 3 meters of the small intestines SI_1, last 3 meters of the small intestines SI_2, caecum and last 25 cm of the colon. The table shows the different bacterial counts for two groups of piglets fed with dry and FLF [30].

Bacteria spp	Dry feed FLF	Stomach	SI_1	SI_2	Caecum	Colon
Total anaerobic count	Dry FLF	8.1 ± 0.8^a 8.8 ± 0.2^a	7.7 ± 1.1^a 8.1 ± 0.5^a	8.7 ± 0.5^a 8.9 ± 0.5^a	9.5 ± 0.9^a 10.0 ± 1.2^a	9.1 ± 0.4^a 8.9 ± 0.4^a
Lactobacillus spp	Dry FLF	7.8 ± 1.0^a 8.7 ± 0.3^b	6.5 ± 1.3^a 7.9 ± 0.5^b	7.3 ± 0.7^a 8.5 ± 0.4^b	8.3 ± 0.6^a 8.9 ± 0.5^a	8.4 ± 0.3^a 8.5 ± 0.5^a
Bifido bacteria spp	Dry FLF	7.6 ± 1.2^a 7.0 ± 1.1^a	5.9 ± 1.2^a 5.3 ± 1.1^a	6.9 ± 0.9^a 5.8 ± 1.1^b	7.7 ± 0.7^a 6.6 ± 1.1^b	8.1 ± 0.7^a 6.8 ± 1.0^b
Streptococcus spp	Dry FLF	7.1 ± 0.5^a 7.9 ± 0.4^b	6.1 ± 0.8^a 7.2 ± 0.6^b	7.4 ± 0.9^a 8.1 ± 0.7^b	8.0 ± 0.8^a 8.5 ± 0.7^b	8.1 ± 0.9^a 8.0 ± 0.7^b
Coliforms	Dry FLF	3.5 ± 0.3^a	3.1 ± 1.0^a	4.7 ± 1.4^a	5.9 ± 1.1^a	5.6 ± 1.0^a

		4.0±0.5^b^	3.2±0.5^a^	4.8±1.4^a^	5.6±1.4^a^	5.4±1.1^a^
Yeast	Dry FLF	3.3±0.5^a^ 4.6±0.8^b^	3.1±0.8^a^ 4.2±1.1^b^	3.8±0.4^a^ 4.9±0.9^b^	3.9±0.2^a^ 5.5±0.8^b^	2.9±0.5^a^ 5.1±0.4^b^

[ab]means within same column and species counts with different superscripts differ significantly (P<0.05)

As seen from the table, in FLF fed pigs the concentration of the *Lactobacilli* and the *Yeast* has increased. (Source: Canibe *et al*., 2012)



Nutritional changes in FLF

Change in the amino acid lysine

The main nutritional value shift will be observed for the amino acid lysine. During the fermentation process, lysine loses its efficacy mainly due to decarboxylation producing *Cadaverine* which is a foul smelling diamine. Incubation of feed with liquid feed releaved that the loss of lysine and production of *Cadaverine* is due to metabolism of *E.coli* [34].

Change in the nutrient composition

Soaking in water or any fermented liquid will bring in changes in some of the nutrient composition which depends on the period of incubation. Soaking (also called steeping) is usually practiced to soften, cleanse or to extract some active principles in the feed stuffs [35]. reported a drastic decrease in the sugar concentrations of wheat and barley grains when fermented. The ill effect of fermentation is that it produced biogenic amines, the percentage of which decides the health of the animal. However, no changes in the total non-starch polysaccharides and starch during fermentation were observed.

Mobilization of phosphorus from phytates

It was reported that soaking initiates mobilization of phosphorus from the phytates that are present [36, 37] in the grains. However mobilization depends on the source of phytase in the grains.

Improving the digestibility of soaking

Fermentation leads to an improved ileal digestibility of organic matter, protein, amino acids and calcium [37]. Increase in protein digestibility can be attributed to the reason that due to the production of *lactic acid*, lowering of the pH and stimulating the proteolytic activity. It was also reported [38] a reduced the anti-nutritional principle Isothiocyanatesupto 17% in rape seed meal when fermented for one day. Soaking also reduced the concentration of some of the anti-nutritional factors like saponins, tannins, phytates, alphagalactosides.

Effect of minerals in controlling PWC and on intestinal barrier function

Ban on the use of antibiotics lead to the use of some of the minerals and their salts in therapeutical doses exhibited bacteriocidal effects. Especially Zinc oxide arrests the growth of *E.coli* and *Enterococcus* [39]. ZnO is supposed to reduce the incidence of Post Weaning Colibacillosis and this activity is achieved by 2 ways.

1. By reducing the *Enterogenic E.coli* to the mucosa,
2. By reducing the intestinal permeability to these pathogens

However usage of higher levels of ZnO is not advisable as its excretion in the faeces leads to environmental pollution and hence European Union has banned the use of higher doses of ZnO in diets. As an alternate, micro encapsulated Zinc Oxide is being used nowadays.

The other minerals iron, calcium and phosphorus have also a major role in pathogen proliferation in the gut. An increase in the serum iron level proliferate the bacterial growth and being changed in the intestinal barrier function [40]. Dietary phosphorus and its availability in the intestines is another important feature to be discussed since phosphorus is the fundamental component for the formation of bacterial cell membranes. A study conducted by [41 in cannulated pigs revealed that reducing the intestinal availability of phosphorus for the bacteria reduced their activity in the gut and increase intestinal calcium availability decreased the number of gram positive bacteria.

Conclusion

Weaning is complex physiological phenomenon where in the pigs have to face immunological nutritional and psychological stresses. All these could damage the intestinal epithelium and thus affect the intestinal barrier function. When this property is lost to the intestinal epithelium, pathogen entry into the blood pool becomes easy to cause diseases. Most commonly seen condition is Post Weaning Diarrhea. These ill conditions can be checked by 2ways viz. either by elimination of gut pathogen or by preventing their proliferation. The activity of pathogens can be controlled by nutrient dietary interventions of carbohydrates, proteins, minerals and also by feeding FLF. Reducing the protein content up to last 10 days of weaning, limiting the intake of soluble NSP and limiting the use of iron are some of the factors of prime importance to reduce the incidence of PWD and to restore intestinal barrier function.

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