SPECIALTY

The benefits of low CP diets and protease using

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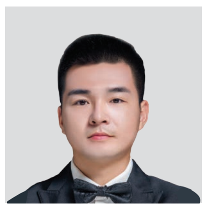


Abstract

It's witnessed that the benefits of reducing dietary crude protein (CP) in swine industry with free amino acids (AA) supplementation, including saving protein costs, reducing nitrogen (N) excretion and keeping intestinal health. Different with AA supplementation, protease can improve the inherent ileal amino acid digestibility (from 2.68% Glu to 5.64% Thr) and reduce undigested proteins moving to the intestinal backend. Overall, this review gives a better understanding of this nutritional strategy.

Background

Protein is one kind of expensive but essential nutrients for animals. Due to some reasons, the SBM price got a sharp increase and reached around 5000 RMB / metric ton in China (by 2nd March, 2022). To minimize feed costs and environmental pollution caused by the excretion of excessive nutrients, low CP diets are getting more and more attention (Yuming and Junyan et al., 2018). A series of policies related to low CP diets were published in last two years in China and also in South Korea (Standardization Administration, 2020; MAFRA, 2021).

Advantages of Low CP diets

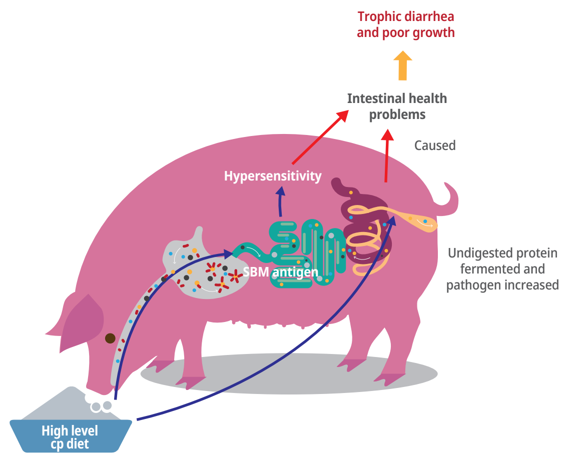
Low protein diet has already been studied for more than 30 years and is used regularly in the European Union. The advantages of low protein are summarized as below (Yuming and Junyan et al., 2018);

1) saving protein ingredients and reducing feed costs

2) reduce nitrogen emission

3) gut health benefits: lowering nutritional diarrhea incidence, intestinal morphology and microbiota and immune response

While reducing the protein level, it also should meet animals' demand for AAs.(Sutton and Kephart et al., 1999; Nahm, 2002; Rotz, 2004). An alternative to improve the nutritive value of feedstuffs is to supplement exogenous enzymes, especially protease. Different from adding crystalline amino acids, the aim of adding protease is to increase the inherent digestion efficiency of amino acids that are naturally present in feed (Bedford and Partridge, 2010).



① High CP level diet contains high level antigens which can induce hypersensitivity in small intestinal.
② The undigested protein is fermented in the intestinal backend and pathogen increased.
→ Both of them can cause intestinal health problems, like trophic diarrhea and poor growth.

Figure 1.

High level CP damaged intestinal health

Improving inherent amino acid digestibility

The essence of an animal's demand for protein is the demand for amino acids. A high-protein diet can meet the demand for restricted amino acids of animals, but at the same time, it is easy to cause other excess amino acids to be consumed and wasted by animals through deamination. As shown in Table 1, a total of 25 independent experiments were analyzed by meta-analysis in a total of 804 data points (Aaron and Franz, 2013). It summarized that the inherent apparent illegal digestibility of different amino acids has great differences, ranging from 72.7% for Cysteine (Cys) to 85.4% for Glutamic acid (Glu). Protease effects over control on amino acid digestibility are positive. Threonine (Thr) improved 5.64% which is the highest, and Glu improved 2.68% which is the lowest. It's reported that protease is more effective when the digestibility of amino acids in the diet is low and for every 10% decrease in ileal amino acid digestibility the efficacy of protease doubles (Aaron and Franz, 2013). Protease is irreplaceable in the process of degrading inherent protein to peptide or free amino acids. But we should know that the improvement is in a limited range.

Table 1. Means of feed intake, average daily gain and feed conversion ratio as a response to addition of isoleucine.

Amino Acid	Control Digestibility %	S.E.	Protease effect over control %	N	S.E.	P value
Ala	78	1.82	3.99	34	1.232	<0.01
Arg	83.4	1.72	3.55	51	1.2	<0.01
Asp	77.4	1.71	3.68	54	1.193	<0.01
Cys	72.7	1.7	5.36	53	1.195	<0.001
Glu	85.4	1.8	**2.68**	40	1.222	<0.05
Gly	73.7	1.82	4.35	38	1.228	<0.001
His	81.7	1.73	3.17	46	1.202	<0.01
Ile	80.7	1.71	3.2	54	1.193	<0.01
Leu	82.3	1.8	3.31	40	1.222	<0.01
Lys	80.1	1.7	3.77	55	1.191	<0.01
Met	84.2	1.71	3.25	54	1.193	<0.01
Phe	82.9	1.8	2.9	40	1.222	<0.05
Pro	79.3	1.82	4.09	38	1.228	<0.001
Ser	79.3	1.71	3.71	53	1.195	<0.01
Thr	75	1.71	**5.64**	54	1.193	<0.001
Trp	80.2	2.47	2.76	11	1.458	NS
Tyr	81.4	1.84	3.28	35	1.237	<0.01
Val	79.5	1.71	3.87	54	1.193	<0.01

Degrading anti-nutritional proteins

The β-conglycinin (7S globulin) and glycinin (11S globulin), the major storage proteins (accounting for about 70% of the total soy proteins) in soybean, are also major allergens that induced anaphylactic reaction in the intestinal mucosa (Robert, 2000; Wang and Li et al., 2014). Their antigenicity still persisted through feed production process and the induced anaphylaxis will be magnified in antibiotic-free diets (Yin and Zhang et al., 2021). They have good thermal stability and relatively pepsin and trypsin tolerance (E. and Niusa et al., 2003; 2015; Panda and Tetteh et al., 2015). Compared with protease released within

the gut of animals, supplemented protease derived from microorganisms can hydrolyze soybean 11S more easily. Yin reported alkaline protease from ***Bacillus subtilis ACCC 01746*** increased the degree of hydrolysis of β-conglycinin and reduced antigenicity (Yin and Zhang et al., 2021) and Wang et al. reported that protease improved the degradation of soy protein over trypsin treatment (Wang and Li et al., 2014). And many successful commercial pro- teases already showed the effect on the two anti-nutritional proteins.

Reducing undigested protein fermentation and improve gut health

Animal nutritionists have been working to improve the digestibility of proteins. However, even with the highest quality diet, not all dietary protein is digested by the host in the small intestine (J. and K., 2016). A high level of bypass proteins had impacts on the intestinal backend and even on animal health. The bypass proteins interacted with the intestinal microbiota. Such functional bacterial groups included putrefactive or protein-fermenting bacteria, which produced toxic end-products in their protein metabolism (Figure. 2; J. and K., 2016). Bypass protein in caecum is a potential target for protein-fermenting bacteria. When there is an excess of protein at the back of the intestine, deamination produces more ammonia than it's absorbed by bacteria. And more ammonia has some adverse effects on epithelial cells, including altering epithelial morphology, metabolism and even DNA synthesis (Clausen and Mortensen, 1992; Mary and John, 1995; J. and K., 2016).The use of protease can reduce undigested protein, reduce the production of harmful substances such as ammonia, and improve intestinal health.

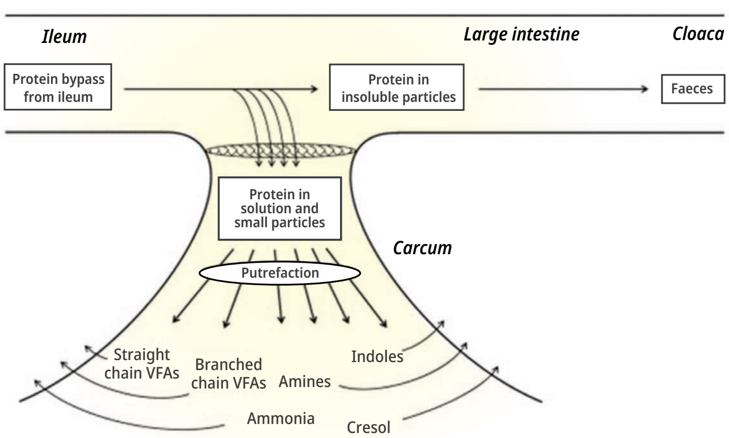


Figure 2. **Protein fermentation of ileal bypass protein in the caecum. From the terminal ileum, protein can either enter the caecum or pass across the ileocaecal junction to the large intestine. Certain levels of indoles, amines, ammonia, and cresol will probably cause endothelial injury. (J. and K., 2016).**

Reducing nitrogen excretion

Reducing N excretion is one of the aims of pushing low CP diets. N excretion would decrease due to lower crude protein in diet. According to one report, every 10 g/kg reduction of dietary CP could decrease ammonia emission from feces and urine by 8% to 10% (Yuming and Junyan et al., 2018; Figure. 3). In addition to the reduced N content of the low CP diet itself, the use of protease further reduced N content in feces probably because of increasing N retention. It was reported that adding 300 ppm protease got the same results in low CP diets (CP 15.1%) and high CP diets (CP 17.3%) for growing pigs (Kim and Kim et al., 2020). The N concentrations in urine and feces significantly decreased (P < 0.05; Figure. 4A - 4B) and there was an increasing trend for N retention (P = 0.061, % of N intake; Figure. 4C). Protease in low CP diets helps in higher nitrogen reduction and lowers fecal and urine nitrogen excretion.

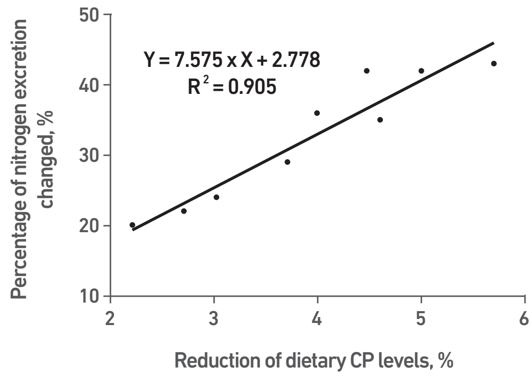


Figure 3.

The linear relationship between the reduced percentage of nitrogen excretion and dietary CP reduction levels for pigs summarized from 7 published articles by (Yuming and Junyan et al., 2018)

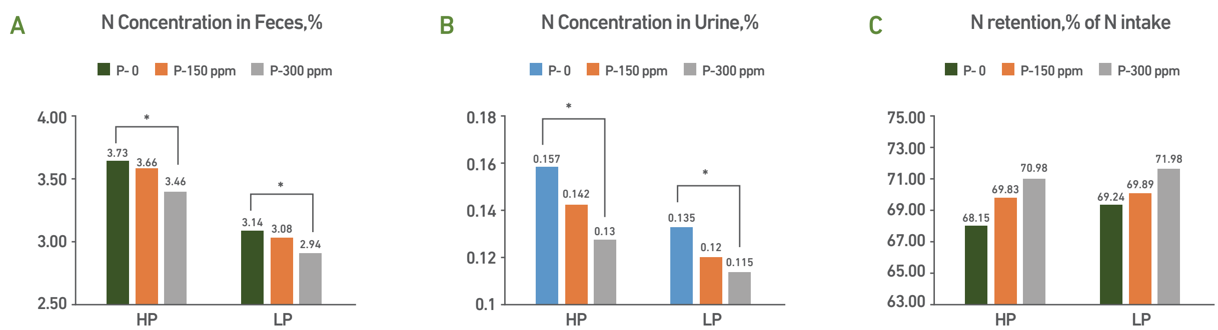


Figure 4. **Effects of protease on N concentration in feces (A), urine (B) and N retention (C). HP, high level protein (17.3%); LP, low level protein (15.1%); N, Nitrogen; P, Protease; * means the significant difference (P < 0.05) (Kim and Kim et al., 2020).**

Conclusions

Protease can improve inherent amino acids digestibility in low CP diets and maximize utilization of amino acids present in feed. Protease reduces undigested protein fermentation in the intestinal backend and builds a better gut environment. Meanwhile, nitrogen excretion is reduced and environmentally friendly.

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