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Meta Analysis to Draw the Appropriate Regimen of Enzyme and Probiotic Supplementation to Pigs and Chicken Diets*

Sang Jip Ohh**

College of Animal Life Sciences, Kangwon National University, Chuncheon 200-701, Korea

ABSTRACT : Along with the recent changes in animal feed supply circumstances, many enzyme and probiotic feed supplements have been introduced and applied to pigs and chicken diets. Therefore, both selection of the appropriate feed supplements and their proper supplementation becomes critical to justify the supplementation. Meta-analysis was proposed as an appropriate tool to assess the large amount of relevant information. In this review, reliable data from recent publications was compounded then analyzed to determine the best practice of effective enzyme supplementation from the perspectives of animal species, age, characteristics of feed, target substrates, optimum multi enzymes combination and intended objectives. The results of the analysis suggested pratical methods of probiotic supplementation regarding intestinal microbiota, physiological limitation of probiotics, maximization of the probiotic benefit and synergism with prebiotic supplements. (**Key Words :** Enzymes, Probiotics, Pigs, Chicken, Feed Supplementation)

INTRODUCTION

Application of biotechnological feed supplements to animal feeds has been increasingly focused recently. This focus became imminent concern since the worldwide enforced ban on AGP (antibiotic growth promoters) and tightened supply situation of conventional feed ingredients. Among those biotechnological feed supplements, both enzyme and probiotic (direct-fed microbial) have drawn more attention due to several practical reasons.

In this respect, feed formulators and animal feeders are forced to adventure feed enzyme and probiotics although they are uncertain for the benefits. Since it is still unsuccessful to find the dependable alternatives that could replace AGPs and to maintain the nutritional quality of diet against the limitation of rather good quality ingredients, this uncertain but unavoidable adventure would not be ceased. It is certainly a waste of various expenses for the animal industry but in reality, the supplements become more confusing in types, sources and their proposed efficacies. In addition, those confusing process has resulted many inefficient and even wrong-directed usage of these expensive biotech feed supplements.

Therefore, this article is intended to infer the best possible application guide of the feed enzyme and probiotics to pig and chicken diets. Although there have been so many technological renovation and its related data accumulation during last several years, the several inferences by this review should remain in the presumptive stage. The front part of this review infers practically effective ways to apply feed enzyme whereas the later part focuses on the effective ways for the dietary probiotic application.

EFFECTIVE USE OF FEED ENZYME

Exogenous enzymes supplemented to feed are theoretically capable for improving digestibility of feed by hydrolyzing the substrates that hinders digestion and specific anti-nutritional factors. This rationale is usually confirmed by *in vitro* hydrolysis and even by *in vivo* animal experimentation. However, in reality, the similar next practices do not exert the theoretically expected and previously appeared benefits. Therefore, the field users are hesitating and confusing upon applying feed enzymes. In addition, it is even more complicating since the type and numbers of feed enzymes and target feed ingredients in the market is continuously increasing as listed in (Table 1).

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** Corresponding Author : Sang Jip Ohh. Tel: +82-33-250-8631, Fax: +82-33-255-5482, E-mail: sjohh@kangwon.ac.kr

Even amidst all the intensified regulations, 40 feed enzymes have achieved the EU approval as feed additive (Brufau et al., 2006).

Therefore, it is now needed to suggest the convenient guidelines for the field user using feed enzyme more efficiently. By far, this guideline could be extracted from the meta-analysis of the recent reports on feed enzyme application. Several meta-analyses indicated there could be the potential ways to improve and maximize the benefits of enzyme. In this review, the author focuses on type and age of animal, quality of feed ingredients including presence of target substrate and optimization of multi-enzyme combination upon using feed enzymes.

Scrutinizing the synergism of multi-enzymes application

Multi-enzymes application has been increased as the numbers of formulated ingredient, enzyme type and their products have increased. It was hypothesized that the multi-enzymes exert an enhanced animal performance, and an improved nutrient utilization. It is also documented from several studies that the use of an effective blend of enzymes exerted the relatively better responses compared to single enzyme or no enzyme supplementation regardless of cost-effectiveness. However, several other studies indicated there would be more cost-effective way to maximize the benefit of multi-enzyme supplementation.

A study (O′Connell et al., 2005) on wheat and barley based diet demonstrated that the combined use of both xylanase and glucanase was more effective for the viscosity reduction, the cell wall polysaccharide depolymerization and the release of protein that are complexed or enclosed by the cell wall structure thus poorly available for pigs and chicken. In the enzyme compatibility study, activity of xylanase and amylase were not affected by the addition of other enzyme preparations while the glucanase activity was declined when it was used in combination with protease (Mathlouthi et al., 2002). However, these xylanase and glucanase combined supplementation was not beneficial for corn or sorghum based diet that does not cause severe intestinal viscosity problem. Even other enzyme combinations were practically behind expectation once applied to corn-soy diet.

Response of poultry to dietary enzyme supplementation is a little bit different from pigs. Body weight gain and feed intake of broiler were significantly increased by addition of NSP-degrading enzyme, but were not affected by supplementation of phytase in the diet (Ghorbani et al., 2009). NSP degrading xylanase and glucanase supplementation increased mainly starch digestibility in the small intestine of pigs. Since chicken have relatively smaller size digestive organ and less water consumption compared to pigs, a detrimental impact of NSP such as intestinal viscosity would be greater. This indicates the benefit would be greater when the enzymes are supplemented to chicken diet compared to pig diet.

The increase in small intestine starch digestibility due to the enzyme supplementation indicates there may be a shift of the digestion site from the large intestine to the small intestine. From the view of energy utilization efficiency, this shift would be beneficial since direct absorption of starch as glucose from the small intestine is more effective than its absorption as volatile fatty acids from the large intestine. On the other hand, digestion and absorption of nutrients anterior to the large intestine would rather limit proliferation of the microbial population in the lower gut, therefore decreasing digestive turmoil.

There have been more varied responses when NSP hydrolyzing enzymes are supplemented to corn-soy diet, especially in the view of animal performance. Several experiments with corn-soy diet focusing on utilization of NSP indicated that the performance of broilers is not affected by supplementation of various NSP degrading enzymes (cellulose, xylanase, pectinase and α-galactosidase etc.). This insignificant effect indicates that the enzymes at the given concentration did not elicit any beneficial response on the utilization of NSP (Jackson et al., 2004).

Table 1. Notable substrates and their respective feed enzymes

Notable substrates	Enzyme	Notable Ingredients
Protein	Protease, peptidase	Beans and oilseeds
Starch	Amylase	Grains
Lipids	Lipase, phospholipase	Oilseeds
Phytate	Phytase	Refer to Table 3
Hemicellulose	Hemicellulase	
Pentosans (xylose, arabinose)	Pentosanase (Xylanase)	Rye, wheat
β-Glucan	β-Glucanase	Barley, oat
Mannan	Mannanase	Soybean, palm, copra
Pectin	Pectinase	Beans
Galactan	α-Galactosidase	Beans
Cellulose(plant cell wall)	Cellulase, cellobiase	Forages, brans

* Combined from Brufau et al. (2006), Park (2005) and Bedford (2003).

They also mentioned that the benefit of enzyme become remarkable with increased dosage although the dose-response relationship was not extended up to its maximum.

However, with closer look for the related results, 7 multi- enzymes in corn-soy diet was generally effective for improving only F/G, but the improved F/G was not resulted in any improvement in weight gain (Zakaria et al., 2008). Effect of multi-enzyme supplementation improved F/G but reduced an intake therefore resulted no benefit on growth (Ragland et al., 2008). These results indicate the different taste of each enzyme may have some possibility to decrease intake. Another possible explanation for the decreased intake may be directed to the changes in intestinal viscosity (Brufau et al., 2006) or VFA level (Awad et al., 2009; Lynch et al., 2009). Since it is almost impossible to quantify the level of the change due to numbers of enzyme and substrate, it is already predicted to have such varied responses, case by case and time to time. This means, in another words, if the intake can be increased, there would be a benefit on growth.

Although, the most of studies witnessed, at least, an improvement in F/G by enzyme supplementation, the improvement in F/G could be disappeared when the digestibility of diet are already high enough without any help from exogenous enzyme (Juanpere et al., 2004). For corn-soybean meal diets, combined supplementation of five different enzymes did not exert benefits on digestibility and fecal output of pigs (Wubben et al., 2000).

Consideration of the animal age upon enzyme application

It was theoretically hypothesized that the additional enzymes may be necessary to replenish the insufficient secretion of endogenous enzyme to anticipate an improvement in nutrient digestibility. This hypothesis has been more frequently proposed to justify enzyme supplementation to early-weaned pigs and young poultry. However, several recent studies indicated that this approach could be more objective by focusing on the type of enzymes and its suitability to the age of animal.

Feed intake and feed conversion ratio was significantly improved in 1-28 d broilers fed NSP-degrading enzyme supplemented diets. However, the enzyme supplementation could not improve feed intake, body weight gain and resulting feed efficiency after 28 days (Nadeem et al., 2005). A similar result was also published by Jhori (2006). The author reported that an increase in body weight gain by dietary xylanase and pectinase supplementation was only remarkable for birds below 28 d of age but this benefit had disappeared subsequently after then. These observations indicate that the chicken at the younger age are not able to utilize NSPs like xylan and pectin and thus requires exogenous supplementation of the respective enzymes to hydrolyze these compounds. That could be a possible reason why NSP degrading enzyme supplementation is beneficial for younger chicken. In other words, this observation also indicates that the anti-nutritional impact of NSP may be declined as the bird is getting old.

Effect of age would be different by the type of enzyme or substrate. Vieira et al. (2008) reported the efficacy of amylase and beta-glucanase supplementation to corn-soy broiler diet was demonstrated after 21 d of age. Phytase supplementation was also beneficial at 4-6 wks on broiler gain and feed intake. Benefit of phytase supplementation was also tended to increase when the pigs getting older. The reason why the benefit of phytase supplementation is more remarkable with older chicken and pig is not known. Mannanase supplementation was also relatively more effective for 3-6-wk old broilers than 0-3 wk (Jackson et al., 2004; Khanongnuch et al., 2006; Zou et al., 2006; Lee et al., 2008; 2009).

These results indicated some of the anti-nutritional NSPs including glucan, phytate and mannan may not be hydrolyzed by the respective enzyme alone. Phytate was known to be better solubilized in the acidic condition like stomach (Olukosi et al., 2007b; Wyatt et al., 2008). Since the younger age animal was also immature to secret sufficient amount of pepsin as well as gastric acid, it could be a reason why phytase was more efficient with maturation. However, the effect by increasing maturation was not extended to finisher pigs (Olukosi et al., 2007b). β-mannanase supplementation to corn soy diet was effective for improving F/G in nursery and growing pig but not(less) for finisher pig. The similar response was observed with benefit for 4-6 week broiler but not for other period (Zou et al., 2006). Considering the above practical results, it is definitely worth to consider the age of recipient animals before supplementing enzymes.

Selective application of enzyme considering the characteristic of feeds

Careful focus should be directed to the physico-chemical characteristic of target feed ingredients before applying enzymes to feed. Physico-chemical properties like major ingredients, target substrate and its amount, and physical structure has influenced the efficacy and onset of the enzymatic hydrolysis. By far, the varied enzyme efficacy due to ingredients cannot be simply explained. Amount and characteristics of NSP (as shown in Table 2), anti-nutritional factors, oligosaccharides and/or other components, physical structure of mainly starch and protein and the degree of feed processing have been compounded to represent the efficacy of supplemental enzymes.

Benefit of dietary enzyme supplementation has been

Table 2. Concentrations* of major NSP, total NSP, soluble and insoluble fiber (F) of selected feed ingredients

Ingredients	Major NSP (%)	NSP (%)	Soluble F (%)	Insoluble F (%)
Wheat middlings	Xylan 15.5, glucan 2.4	37	6.7	32.5
Oats	Xylan 8.0, glucan 3.0	31	1.1	24.0
Barley	Xylan 7.0, glucan 5.0	18	4.5	14.0
Soybean meal	Pectin 4.8, xylan 4.9	17	1.6	31.5
Wheat	Xylan 6.0	10-12	0.8	9.3
Corn	Cellulose	10	0.9	6.0

* Combined and pooled from Park (2006), Hogberg (2003), Meng and Slominski (2005) and Molist et al. (2009).

maximized when the enzyme was supplemented to the lower-quality feed (Preston et al., 2001). There has been a negative relationship between available nutrients, especially energy and amino acid, contents and the efficacy of supplemented enzyme. This inverse relationship could be related to the maximum capacity of animal to exploit available nutrients for growth (Meng and Slominski, 2005). With sufficient supplying situation in the body pool of available nutrients, the further release of available nutrients by enzyme could not be a help for the performance. Therefore, the recent enzyme supplementation was more focused on both reduced level diet and lower quality feeds.

F/G improvement by enzyme addition was achieved when the diet were prepared with appropriate lysine to ME ratio. Enzyme supplementation was proposed not only to improve overall nutrient digestion but also to reduce endogenous amino acid losses. Thus, a possible increase in energy digestibility might not be matched by improved lysine and methionine digestibility, since these two amino acids are not present at high levels in endogenous protein. This change may affect the lysine to energy ratio in the diet referring to the non-supplemented feed (Hruby et al., 2002).

In reality, a mannanase supplementation was more effective with reduced energy diet (Jackson et al., 2004; Lee et al., 2008). Performance was not improved by mannanase when there formulated more than 15% copra meal, which is a poor quality ingredient (Khanongnuch et al., 2006). The copra meal primarily caused a decrease in feed intake (Sundu and Dingle, 2002). If intake is reduced regardless of mannanase supplementation, it is difficult to recover the growth deficit. Although the feed intake is reduced by copra meal included diet but FCR was not different compare to control by enzyme supplementation (Khanongnuch et al., 2006). Therefore, it is justified to say that the cost effectiveness of diets containing rather poor quality ingredients can be mostly enhanced by the use of an appropriate enzyme supplementation.

Physical quality of the ingredients and feed is also known to affect enzyme efficacy. Strong cell wall structure which limit enzymatic hydrolysis need to be properly processed before consumption. Extra fine grinding of flax, extrusion (Costa et al., 2008) ahead of enzyme supplementation helped energy digestion and enzymatic hydrolysis. Dietary Mn, Fe, Cu could inhibit the activity of mannanase (Magalhaes, 2009).

Even within the same enzymes, the optimal pH and temperature can be different due to the type of fermentation microorganism. Therefore, it is needed to consider optimal pH and major action intestine of the supplemental enzyme before selection. Nutrients released by exogenous enzyme to upper gut could affect the host utilizability of the diet more remarkably whereas the nutrients released to hind gut could be used, with more magnitude, for intestinal microbiota.

Enzyme supplementation aiming for the specific substrate

An enzyme, in theory, is responsible for the hydrolysis of its respective substrate. This means when there is a respective substrate, the responsible enzyme supplementation is able to anticipate relatively more efficient response compared to general purpose supplementation. There has been a debate how the general purpose multi-enzyme supplementation may respond when the multi-enzyme actually does not include a respective enzyme responsible for the primary target substrate. Recently, several substrates are under reconsideration along with enzyme supplementation.

Beta-mannan is a polysaccharide commonly found in feed ingredients such as soybean meal, palm kernel meal, copra meal, guar gum meal and sesame meal. The mannans have been primarily focused due to its anti-nutritional effect, especially in the view of animal performance. Therefore, the incorporation of beta-mannanase into these diets was mainly evaluated by the degree of decreased intestinal viscosity and resulting performance. However, there has been another approach to handle this problem by more optimized way. Sub-adequate level of mannanase supplementation to soybean based diet did not exert benefit although the sufficient level did exert benefit (Jackson et al., 2004). This suggests there is a precise enzyme-substrate relationship that affects the effectiveness.

Corn has been considered as the best quality grain especially for pig and chicken. Therefore, the response and its degree due to dietary enzyme supplementation have been negligible or relatively less to other grains (Kidd et al., 2001; Vieira et al., 2007). Since the primary NSP in corn is cellulose, a structural insoluble fiber, enzymes hydrolyzing soluble fiber did not respond well upon supplementation. Therefore, cellulase and xylanase supplementation to poultry diet was proven more effective compared to other enzymes without cellulase (Sundu and Dingle, 2002; Wyatt et al., 2008). The similar response was also observed by xylanase + protease supplementation to wheat-based diet in broiler (Costa et al., 2008). However, mannanase-only supplementation to corn based diet was failed to improve F/G, but the F/G was improved by multi-enzyme supplementation including cellulase as well as mannanase in nursery pig (Ragland et al., 2008) and in broiler (Sundu and Dingle, 2002; Zakaria et al., 2008). On the other hand, mannanase supplementation to DDGS included pig diet was effective to improve ADG (Yoon et al., 2010), probably due to relatively higher mannan in DDGS than corn grain.

For finisher pig study, both glucanase + xylanase improved digestibility of barley based diet but not of wheat based diet (O′Connell et al., 2005). There is a similar response in broiler study that glucanase and pentosanase was more effective for barley based diet not for wheat based diet (Senkoylu et al., 2004). Barley has been notorious for its higher amount of glucan inclusion, which is the primary target substrate. In wheat, however, xylan has been considered as the primary anti-nutritional substrate.

Soybean has been known to contain higher amount of pectin (Table 2) as well as significant amount of mannan (1.3% from Jackson et al., 2004). Pectinase and mannanase combined supplementation was more effective for soybean than corn (Jackson et al., 2004).

Magnitude or level of the target substrate could be a threshold factor to present a positive response by enzyme. There was a research that the oligosaccharides raffinose and stachyose in soybean do not pose any significant nutritional concerns, therefore, the α-galactosidase enzyme to enhance their digestibility did not produce a beneficial effect in chick performance. When the target substrate was significant in nutrition, a significant depolymerization of the cell wall polysaccharides in canola, soybean and peas was achieved by using a combination of carbohydrase enzymes (Mathlouthi et al., 2002; Meng and Slominski, 2005).

More reasonable use of phytase

Among the feed enzymes, phytase has been a leading enzyme that has been practiced early and therefore documented more especially from pigs and chicken studies. However, the originally intended objective of phytase supplementation and its significance has been slightly changed and redirected recently. Appearance of lesser phytin ingredients (Israel et al., 2007) and higher intrinsic phytase ingredients as shown in (Table 3) has alleviated the burden of phytase but the increasing use of relatively poor quality byproduct ingredients has compounded the role of phytase. Therefore, dietary application of phytase is still increasing and is expanded to the combination use with other enzymes.

Effect of microbial phytase on performances of animal: There has been little argument on the effectiveness of supplemental microbial phytase for improving the P availability. The dietary phytase supplementation has been also documented with increased body weight gain and feed intake in broiler chicken. However, the most of reports indicated that phytase supplementation had no significant effect on feed efficiency, probably due to a simultaneous increase in feed intake along with body weight gain (Viverous et al., 2002; Liu et al., 2007, 2008). It indicates that any improvement in growth should be directed to other factor such as an improved utilization of dietary phosphorus and resulting structural growth. Since an *in vitro* study has shown that phytate-protein complexes are insoluble, any improved body weight gain by phytase supplementation is hardly resulted from an indirect increase of digestibility. There was no benefit of phytase alone supplementation at all on body weight gain and feed intake of broiler (Ghorbani et al., 2009).

Response to the phytase supplementation has been, however, also affected by the chemical characteristics of the feed. Since rye, wheat and its byproducts usually contained relatively significant level of intrinsic plant phytase, the benefit by microbial phytase supplementation was expected to increase with corn-soy diet, which usually carry little plant phytase (Park, 2005). In practical feeding studies, the addition of dietary phytase to nutritionally adequate corn-soybean meal diet did not exert an improvement in either body weight gain or feed intake in pig (Kies et al., 2006; Liao et al., 2005; Radcliffe et al., 2006; Pomar et al., 2008; Veum and Ellersieck, 2008).

It may partly be attributed to the limit of phytase (Juanpere et al., 2004) that substitute the role of dietary inorganic P but also to the activity of plant phytase *per se* that certainly need to be reconsidered (Dhawan and Jagdeep, 2007).

Supplementation of phytase to layer feed differs from that to broiler feeds. The supplemental phytase may not be as effective in presence of high (3.5%) dietary Ca as the case of low (1.0%) dietary Ca such as broiler diet. Higher dietary Ca may induce intestinal pH increase and therefore, decrease the efficacy of the phytase (Beaulieu et al., 2005). A few workers could not find any improvement in the production performance of laying birds (Liu et al., 2007). However, they still demonstrated the potential to eliminate dietary inorganic P supplementation without affecting laying performance.

Effects of phytase on organic nutrients digestibility:

Table 3. Phytate phosphorus contents and phytase activities of plant feed ingredients

Ingredients	Phytate P (%)	Phytate P (as % of total P)	Phytase activity (units/kg)
Cereal			
Maize	0.20 (0.17-0.25)	72 (66-85)	15 (0-46)
Barley	0.24 (0.19-0.33)	64 (56-70)	582 (408-882)
Wheat	0.27 (0.17-0.38)	69 (60-80)	1,193 (915-1,581)
Oats	0.29 (0.22-0.35)	67 (59-78)	42 (0-108)
Rye	0.22 (0.20-0.23)	61 (56-66)	5,140 (4,132-6,127)
Sorghum	0.22 (0.17-0.28)	66 (64-69)	24 (0-76)
Foxtail millet	0.19	70	-
Finger millet	0.14	58	-
Rice	0.27 (0.25-0.28)	77 (74-81)	-
Rice, polished	0.09 (0.04-0.17)	51 (49-55)	-
Cereal by-product			
Rice bran	1.41 (0.70-2.42)	80 (72-86)	122 (108-135)
Wheat bran	0.92 (0.88-0.96)	71 (70-72)	2,957 (1,180-5,208)
Rice polishing	2.04	89	
Oil seed meal			
Soybean meal,	0.39 (0.37-0.45)	60 (57-61)	8 (0-20)
Cottonseed meal	0.84 (0.75-0.90)	70 (70-71)	-
Peanut meal	0.48	80	3 (0-8)
Rapeseed meal	0.70 (0.54-0.78)	59 (43-70)	16 (0-36)
Sunflower meal	0.89	77	62 (0-185)
Coconut meal	0.29 (0.26-0.33)	49 (43-56)	24 (0-80)
Sesame meal	1.02 (1.0-1.06)	81 (77-84)	-

* Combined from Park (2005), Selle and Ravindran (2007).

There has been a lot of controversy on phytase associated digestion of other organic nutrients such as protein, amino acid and energy. Some recent report has focused on the apparent and ileal digestibility of protein and several amino acids as well as dietary energy (Kies et al., 2006). But the results are also varied due to several unexplainable reasons.

Improvement in energy utilization by dietary phytase supplementation was explained by either reduction or termination of anti-nutritional binding by phytate, such as starch-phytate and amylase-phytate complexes (Liao et al., 2005b). Thereby, it was proposed the supplemented phytase may release starch as well as alpha-amylase. And this release was proposed to be responsible for the improvement in energy utilization as shown in (Table 5) by broilers. But the most of the improvements were only numerical advantages and were not steadily reproducible.

For the protein and amino acids, the recent results are again varied due to unknown reasons. Since the most of phytate in plant are located in protein body at aleurone layer or near cell wall by making protein-phytate complex, initial hydrolysis of phytate by phytase may facilitate the release of protein and their amino acids. This release has been a proposed background of the improvement in protein and amino acid digestibility.

But the recent meta-analysis data in (Table 4) and (Table 5) indicate that the expectation for an improved digestibility of organic nutrients is relatively low. This means it is not sufficient and concrete enough to explain by the above theories. The varied responses suggest there should be other factor that intervene the proposed theoretical action. Dietary chemical characteristic was proposed as one of the intervention. Diets contain relatively higher phytate but less intrinsic phytase responded better by dietary phytase supplementation than other diets. It was confirmed by

Table 4. Meta-analysis of the recent studies[1] (since 2000) for the effect of phytase supplementation to pig diet

Responses over control	No (proportion, %) of observation			
	ADG	Feed intake	F/G	Organic nutrient digestibility
Significantly improved	15 (79)	9 (53)	5 (31)	6 (38)
Not different	4 (21)	8 (47)	11 (69)	10 (62)
Significantly worse	0 (0)	0 (0)	0 (0)	0 (0)

[1] References involved in this meta-analysis could not be shown in this table due to the many numbers. Refer to the overall literature cited.

Table 5. Meta-analysis of the 19 recent references for phytase on energy utilization in broiler chickens

Diet-base	No. of reference	% Improvement
Corn-soy	3	1.1
Sorghum-soy	3	1.9
Barley	1	2.7
Wheat-soy	3	3.4
Wheat-sorghum	5	3.0
Corn-soy+others*	4	5.2
Overall	19	2.9

* Others include rice bran, rapeseed meal peanut meal.

animal studies with wheat-soybean meal-canola meal diets (Liao et al., 2005a; 2005b), corn-wheat-soya-canola diet (Olukosi, 2007a, 2007b) and corn-barley-soya-sunflower meal diet (Kies et al., 2006). Since there were no such a response in animal studies with corn-soy, wheat-soy and barley soy diets (Liao et al., 2005a; 2005b; Veum and Ellersieck, 2008), dietary phytate level can be proposed as a factor that affect the threshold of enzymatic hydrolysis. There was a report that plant phytase are not effective as microbial phytase.

The other authors suggested a higher amount of protein (amino acids)-phytate-P complex as an explanation for the varied response. It has been evident that phytate can be solubilized in the relatively acidic region of the gut. Since this is also the region where either endogenous or exogenous protease including pepsin could impact the activity of phytase (Kies et al., 2006), it is expected to have a compounded response resulted from factors including degree of phytate hydrolysis and protein including enzyme break down and amount of newly formed protein-phytate P complex. Therefore, it needs a very careful approach to decide the type of phytase and its addition level.

EFFECTIVE USE OF PROBIOTIC FEED SUPPLEMENTS

Probiotics are used to influence the microbial flora in the gut and are usually defined as live microbial feed supplements that have a beneficial effect on the host animal by improving its intestinal microbial balance. Therefore, the probiotics have been intentionally supplemented to animal diet to rehabilitate or recover normal intestinal flora that were disturbed by several reasons. In addition, the probiotic supplementation draws more attention recently due to anticipation to substitute the function of AGPs. However, there have been so many *de facto* variations in animal responses when the probiotic are practically supplemented to animal diets.

Such variations on the response of animals to probiotics feeding have been attributed to several factors such as microbial strains and their combination, mode of administration, presence of prebiotics, age of the animal, stress and environmental condition and animal species. Therefore, this review intended to suggest several practical guidelines from compromising the recent results with dietary probiotics as well as prebiotics upon pigs and chicken.

There are so many probiotics products in the market and under investigations. These products contain single or multi species of the followings: *Lactobacilli* (*L. lactis*, *L. bulgaricus*, *L. bifidus*, *L. brevis*, *L. cellobiosus*, *L. fermentum*, *L. sporogenes*, *L. acidophillus*, *L. plantrum, L.cremoris*, *L. cellinoides, L. salivarius*, *L.reuteri*); *Streptococcus (S. faecium*, *S. lactis, S. thermophilus*); *Pdiococcus* (*P. halophilus*, *P. pentosaccus), Bfidobacterium spp. and Saccharomyces* (*S.cerevisine*, *S. boulardii*), *Bacillus* (*B. cereus, B. subtilis*), *etc.* However, the microbial species also needs to be approved by the regional authority (for instance, like EU, FDA-USA and Korea as shown in Table 8) before selection to make a commercial probiotic products.

Understanding of the intestinal microbiota

To make a suitable probiotics, it has been a basic approach to understand the status of the normal or typical microbiota of each gastro-intestinal tract of animals. Both (Table 6) and (Table 7) summarized the microflora of each compartment in poultry and pigs, respectively. The microbial flora in the ileum of matured broiler chicken is related to those of the Gram-positive *Lactobacilli* and *Streptococci*, while in the ceaca, 65% is related to *Clostridia*. During the first 14 d of age, the ceacal microflora is similar to those of the ileum. At the young age, *Latobacillus* is abundant in the ileum and then, shifts to *L. crispatus* at a later age. In the ceaca, there is a shift from *Lactobacillus* to *Clostridia*, then *Eubacteria* and *Fusobacterium* species with increasing age. All these species are Gram-positive and sensitive to many AGPs that are now banned.

A recent 16S rDNA technology enables us to identify the intestinal microbiota more specifically. Among about 4,000 16S genes in pig intestine, 81% belonged to gram-positive bacteria (*Streptococcus*, *Staphylococcus*, *Bacillus*, *Clostridium*, *Lactobacillus*) and 11.2% belonged to *Bacteriodes* and *Prevotella* group (Kelly et al., 2008). Therefore, the ideal probitics and prebiotics are presumed to carry some functions to maintain or resume the already stabilized microbiota.

However, the unique microbial community at a very young age suggests that the early bacterial community is relatively transient and is replaced by a more stable community later in life. This suggests that influencing the bacterial community by probiotics or feed is probably most successful at a very young age in the proximal part of the

Table 6. Microflora, pH and digesta residence time in the compartments of avian gastro-intestinal tract

Gastro-intestinal tract	pH	Residence time (min)	Microflora, before 21d	Microflora, adult
Crop	4.5-5.3	45	*Streptococci*[1] *Coliformi*[1] *Lactobacilli*[1]	*Streptococci*[2] *Coliformi*[3] *Lactobacilli*[3]
Proventriculus and Gizzard	2.0-4.5	70	*Streptococci*[1] *Coliformi*[1] *Lactobacilli*[1]	*Streptococci*[2] *Coliformi*[3] *Lactobacilli*[3]
Ileum	5.6-7.9	160-200	*Streptococci*[1] *Coliformi*[1] *Lactobacilli*[1]	*Streptococci*[1] *Coliformi*[1] *Lactobacilli*[1] *Bacteroides*[1]
Cecum	5.8-6.8	120	*Streptococci*[1] *Coliformi*[1] *Lactobacilli*[3]	*Bifidobacteria*[1] *Peptostreptococci*[1] *Clostridia*[1] *Propionic bacteria*[1] *Eubacteria*[1]
Colon and Cloaca	6.3-7.7	30-50	*Streptococci*[1] *Coliformi*[1] *Lactobacilli*[3]	Mixture of ileal cecal *bacteria*[3]

[1] Dominant. [2] Predominant. [3] Significant.

gastro intestinal tract. Since the supplemented probiotic species do not become established members of the normal microbiota but persist only during period of dosing or for a short period there after (Corthesy et al., 2007), it would be practically more reasonable to manipulate intestinal microbiota before stabilization.

Efficacy of probiotics for the manipulation of intestinal microbiota

It has been generally accepted so far that the primary response of dietary probiotic supplementation has been effective for adjusting intestinal microbiota intentionally. There have been so many reports available that witnessed the benefit as shown in (Table 9). The pulled data revealed almost 85% of the pigs were changed in their intestinal microbiota upon dietary supplementation with probiotics. This kind of response was also recognized as a reduction in diarrhea especially with nursery pigs. Therefore, there is little argument against the supplementing probiotics for beneficially manipulating the microbiota of animal, especially of pigs. But there could be proposed several additional approaches for improving efficiency of probiotics manipulation further.

Since feed ingredients could include, more or less, the problematic substrates that are responsible for the increase in mucosal viscosity and/or the structural enclosure of nutrients, the feed enzyme supplementation could be proposed as an inducer for changing the intestinal microbiota by the respective hydrolysis. Bedford (2003) suggested that ingredient composition could have an important effect on intestinal microflora and enzyme

Table 7. Major microbiota and pH at each compartment of gastrointestinal tract of pigs

Gut	pH	Microbiota
Stomach	3.2	*Lactobacillus*[3] *Streptococci*[3]
Fore SI	3.2-4.0	*Lactobacillus*[3] *Streptococci*[3]
Ileum	6.7-7.0	*Lactobacillus*[2] *Streptococci*[2]
Cecum	5.8-6.3	*Lactic acid bacteria*[2] *Bacteriode*[1] *Fibrobacter*[1] *Clostridium spp*[1]
Colon	6.0-6.9	*Streptococus*[1] *Lactobacillus*[2] *Eubacterium*[3] *Fusobacterium*[3]

[1] Dominant. [2] Predominant. [3] Significant.

Table 8. Numbers of feed probiotic microorganisms that were approved by EC, FDA-USA and Korea

Genus	No. of species		
	EC	FDA	Korea
Bacillus	3	5	3
Lactobacillus	4	8	4
Bacteriodes	0	4	0
Bifidobacterium	0	6	2
Enterococus	1	6	0
Pediococus	1	3	1
Saccharomyces	1	1	1
Propiomibacterium	0	2	0
Leuconostoc	0	1	0
Total	10	35	11

Table 9. Pulled response of probiotic supplementation to pig diet from 4 meta-analyzed results

Responses	No. of responses (proportion, %)		
	Intestinal microflora	Growth	Feed efficiency
Beneficial	29 (85)	48 (39.7)	30 (28)
No influence	5 (15)	72 (59.5)	77 (72)
Not beneficial	-	1 (0.8)	-
No. of observations	34 (100)	121 (100)	107 (100)

* Pulled from Kwon and Chae (2006), Doyle (2001), Damgaard and McLaren (2006) and Simon et al. (2007).

supplementations may bring an additional benefit when antibiotic growth promoters are not used. This suggests that the viscosity reduction observed in wheat-based diets may not be the main mode of action for the enzyme affects on microbial population in corn/soy-based diets. It is very likely the level of challenge will influence the response to the enzyme addition.

In addition, enzyme application is likely related to an increase in the rate of diet digestibility and resulting short chain sugars. Those resulting sugars and other hydrolyzed byproducts were suggested as nutrients available to the intestinal microflora. Therefore, the simultaneous or prior enzyme application has suggested as an encouraging way of microbiota manipulation. This could be a background to propose that the improved animal performance by enzyme is related to the changes in the intestinal microflora, rather than to a direct effect of the enzyme *per se* on diet digestibility. This also could explain why the response to enzymes may be more remarkable in the absence of AGPs than in their presence.

With closer look for the enzyme effect, many (Pierce et al., 2006; Lee et al., 2008) reported an increase in VFA concentration in the enzyme supplemented diets where the increased VFA served to make unfamiliar habitat against the potentially pathogenic bacteria. Choct (2009) reported that NSP9+enzyme combined supplementation has increased cecal VFA more than NSP alone supplementation. However, there was no such an increase in ileal VFA, thereby has improved starch digestion in the ileum. Moreover, several reports indicated the enzyme only supplementation without probiotics was effective to reduce the number of *Salmonella* (Jackson et al., 2003, 2004; Khanongnuch et al., 2006). In addition, those results were generally affected by either the release of prebiotic substances or reduction of pH mainly caused by an increased VFA production.

That also explain how factors like the management practices, disease challenge, types of ingredients, immune status, baseline levels of beneficial and potentially pathogenic bacteria, presence or absence of antibiotics, other feed additives (e.g. copper sulfate), and coccidiostats that affect the enzyme response also affect microbial population changes. Therefore, considering the all the rationale by far, it could be recommended to add the responsible enzyme when there apply probiotics to manipulate microbiota of animal. This recommendation could be more practical with NSP included diets.

Possible adverse effects by the intestinal microbiota

Use of probiotics especially as an alternative of AGP was always challenged from the field whether the probiotics is able to improve feed deficiency that was typical by AGP supplementation. One of the hypothesized theories to expect an improvement in F/G by the probiotic has been an indirect effect from the improved microbiota. Improved microbiota could reduce a turbulence of animal digestive system, thereby result an increase in growth as well as feed conversion ratio. To achieve a good feed efficiency, a sound balance between the host and its microbes is important, meaning that the number of commensal bacteria should be kept quite low.

A relationship between microbial flora and F/G was examined by relative quantity of DNA from *Lactobacillus acidophilus* (LA). In feeding trials in broilers, using LA probiotics, there was a frequent shift within the bacterial community in the gut. The LA becomes more dominant when the intestinal fermentation was stimulated. This was proved by the increase of 16S ribosomal RNA genes for LA in the total bacterial mass. The feed conversion ratio becomes worse when there are high numbers of LA in the gut (Newman, 2007). This could be an explanation, even in pig, why more numbers of studies as shown in (Table 9) had failed to have an improved feed efficiency regardless of the status of microbiota (Bikker et al., 2006).

The reason why there was no significant effect by probiotics on F/G as well as growth has not been elucidated yet. Competition for nutrients by intestinal commensal bacteria with the host animal was proposed as one of the reason. Portions of the hydrolyzed nutrients would be consumed by bacteria in the crop and fermented mainly to volatile fatty acids (VFA) and used for bacterial protein synthesis. Although the end products from this fermentation could, more or less, be utilized by the host, the indirect digestion is definitely less efficient and decreases the utilization of the feed. So, the more intensive this fermentation is, the lower the utilization of the feed becomes.

The pH or concentration of VFA is often used as an indicator for quantifying the fermentation. Another phenomenon is the deconjugation of bile salts by the microbial flora. An increase in microbial activity in the intestine is associated with a deconjugation and loss of bile salts resulting in the less digestion of fat, particularly long saturated fatty acids, followed by rather poor feed conversion (Brufau et al., 2006).

It has been widely accepted that the one of the advantage of probiotics is the stimulation of the animal immune system. Cell wall components and the DNA of even probiotic bacteria have been known to stimulate the immune system (Szabo et al., 2009). However, this stimulation, thereafter, can cause a lower feed intake, an increased production of white blood cells and the formation of acute phase proteins. Extra protein required for this formation need to be originated mostly from the already reserved proteins. This means that the metabolism of the animal becomes catabolic. This would be another explanation for the decreased daily gain of the animal and loss in feed conversional efficiency (Fatufe and Matanmi, 2008).

Maximizing the benefit by the probiotics supplementation

A healthy intestinal microflora is important to prevent animal from being infected with external pathogens. The commensal bacteria were known to stimulate the development of the immune system of the host and compete for nutrients and attachment sites with pathogenic bacteria. In addition, there is also a direct interaction and molecular level communication between bacteria and the host. These are the background ideas behind the development of practical probiotics. The first-generation probiotics were mainly based on one lactic acid producing species such as *Lactobacilli* and *Bifidobacteria* whereas the recent products are generally a mixture of several species including *Bacilli, Streptococci* and *Clostridiaceae.* The main objective of these new probiotics is to keep out pathogens and to improve intestinal health more effectively than before. This mean there could be a more effective way to apply probiotic expecting the evident benefits.

Recently, the scientist has advanced to categorize probiotic species that can be stabilized microbiota more effectively. Most of probiotics are, more or less, able to inhibit the growth of intestinal microbes, but this function was stronger to the microbes similar to the strain of probiotics (Szabo et al., 2009). There has been also an improvement in developing probiotic that are active against a wider range of condition. Multiple-species probiotics have not been successful by far, but the better combination is now developed. Several specific species probiotics are also under development to control the specifically pathogenic bacteria like *Salmonella, Clostridia* and *E. coli* and to develop immuno-competence against enteric infections.

There was also a scientific realization that probiotics can not substitute AGP in animal under serious pathogenic infections. This indicates the probiotics are recommended for restoring the normal bacterial population that was obstructed mainly by nutritional and physiological imbalances.

Reported improvements in the performance of birds by probiotic supplementation has usually been realized with young chicken (Islam et al., 2004; Racevicute et al., 2007), and more so under the unhygienic housing conditions. The feeding of probiotics in UV irradiated feed exerts more gains in broiler chickens. The probiotics appear to colonize the GIT of the chicks of a particular strain and also show proper compatibility only with a combination of suitable microbes.

The above findings suggest that it needs very careful approach as well as prior examination of the intestinal status of the host animal to get the maximum benefit from the intended probiotic supplementation.

More focus on prebiotic supplementation

There has been a never-ending controversy whether a dietary modification of intestinal microbiota is realistic in the practical feeding condition. A recent report (Corthesy et al., 2008) confirmed the supplemental probiotics with fairly foreign species could not become the stabilized members of normal microbiota eventually. Therefore, the recent focus is more directed to prebiotics instead of probiotics.

While there is still much work to be carried out to demonstrate the probiotic effect in all species, there is a growing recognition that non-digestible oligosaccharides are more than an energy source for the hindgut microflora. They also play a vital role in cellular metabolism, protein structure and function, cell-to-cell communication and host immunity (Corthesy et al., 2007). In animals, the dietary inclusion of prebiotics has been demonstrated to have a broad range of physiological responses through modification in gastrointestinal tract activity, which can influence elsewhere, energy and lipid metabolism and immune status (Awad et al., 2009).

Prebiotic inulin and β-glucan was effective for improving intestinal microbiota (O′ Doherty, 2008). Inulin supplementation increased *Lactobacillus* population but decreased *Enterobacteria spp.* And branched chain VFA production in pig by soluble NSP (Pierce et al., 2006; Lynch et al., 2007, 2009; Molist et al., 2009). Villus/crypt ratio in broiler chicks is well related to growth, FCR, and NE score once challenged *C. perfringense* (Choct, 2009). Probiotic and prebiotic combined supplementation increased the villus/crypt ratio in broiler (Awad et al., 2009).

It can be speculated that manno-oligosaccharides, mannotriose and mannobiose as well as a small amount of mannose are generated when mannanase is supplemented to the diet. Among those, only a small proportion of mannose is likely to be absorbed and used as energy in the intestine of broilers (Dhawan and Jagdeep, 2007). Therefore, the production of mannobiose and manno-oligosaccharides are, to some extent, useless. However, the manno-oligosaccharides were beneficial to improve intestinal health (Khanongnuch et al., 2006) by increasing the population of beneficial bacteria such as *Bifidiobacteria*. This carbohydrate can be a nutrient for the bacteria in the cecum and thus suppressing the pathogenic counter parts. In fact, mannan-oligosaccharides are added to the poultry diets for this purpose and the use of mannanase in poultry diet has been increased (Lee et al., 2008; Lee et al., 2009).

Intestinal numbers of both *Salmonella* and *E. coli* was decreased by mannanase treated copra meal (Khanongnuch et al., 2006) and by fructo oligosaccharides (Newman, 2007). *E. coli* attach to mannose, more likely to oligosaccharides (mannan oligosaccharide), is proposed to prevent pathogen from binding to the intestine. Increasing fermentable carbohydrates (FC) alter microflora and reduce ammonia emission. This mode of action by mannan-oligosaccharide could be different from that by fructo oligosaccharides (FOS). The FOS was known to supply nutrients for beneficial bacteria and these beneficial bacteria can exclude harmful bacteria by competition (Newman, 2007).

Meta-analysis data indicates the dietary prebiotic supplementation improved the growth of pig by 4%, in average although there was no explanation how the growth was improved (Pettigrew, 2008). This beneficial effect of prebiotic supplementation to pig becomes more evident by another meta-analysis as shown in (Table 10). Lower guts FC were proposed to be responsible for improving ADG and ADFI (Bikker et al., 2006). But there was no such an improvement by too much enzyme supplementation, which has released more FC. Too much hydrolysis of NSP to monosaccharide was again proposed not to be helpful for the manipulation of intestinal microbiota (O'Doherty And branched, 2008). Therefore, it could be important to be extra careful for deciding the supplementation level of prebiotic which is related to intestinal viscosity increase.

Table 10. Pulled meta-analysis for the recent reports about the effect of dietary prebiotic supplementation on pig performance and intestinal microbiota

Responses	No. of observation (proportion, %)	
	Growth	Microbiota
Improved	24 (66.6)	4
Not improved	12 (33.3)	0
No. of observations	36 (100)	4

* Pulled data from Miguel et al. (2006) in Pettigrew (2008), Patterson (2005), Lynch et al. (2007, 2009), Bikker et al. (2006) and Pierce et al. (2006).

CONCLUSIONS

A good balance between digestion capacity and feed supply and that between the host animal and the intestinal microbiota are known to be essential for a healthy and economical production of animal. To maintain the above good balances, both dietary enzyme and probiotics have been widely employed especially for pig and chicken diets. In this review, the author proposed several prior considerations including age and species of animals, residing level of the target substrate and the quality of feeds and appropriate combination of different enzyme before applying feed enzymes. For probiotics, prior examination of intestinal microbiota status and practical realization of primary objective using the probiotics should be ahead of probiotic supplementation. In addition, the author suggests prebiotic supplementation could be more practical than probiotic only supplementation since the field studies with probiotic only supplementation has failed to restore the normal microbiota. This could be alternatively achieved by enzyme supplementation to the feed included the potentially beneficial NSP. Above all, the prior careful evaluation of the several decisive factors and the following precise prescription should be the pre-supplementation routine to maximize the benefit of valuable feed enzymes and probiotics.

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