

Review

Application of biologics to feedstuff

Tzu Tai Lee¹ and Bi Yu^{2*}

¹Department of Biotechnology, Ming Dao University, Changhau, Taiwan 52345.

²Department of Animal Science, National Chung Hsing University, Taichung, Taiwan 40227.

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The application of biologics additives from enzyme, prebiotics and probiotics to improve the feedstuff utilization and animal growth is an aim for safety and hygiene animal products. These additives are gradually replacing the antibiotics. As biotechnology develops to meet animal demand, high bioavailability and low production cost technological products will be novel feed additives, including high production efficiency transformed microorganism and probiotics of transformed enzyme gene or raw material of feedstuff using biotechnology (e.g. fermentation) to enhance nutritive value. However, the availability of these biotechnological products shall be proved by the animal feeding trial.

Key words: Enzyme, feed additives, fermentation, probiotics.

INTRODUCTION

Biotechnology is extensively used throughout the entire agricultural production, including breed improvement, disease and pest control, nutrients improving, etc. Animal production also uses multi-aspect biotechnology to improve animal growth, carcass quality, multiplication, nutritional improvement, feed efficiency, animal health and benefit, and even reuses resources to reduce environmental pollution (Lee et al., 2012a,b). In terms of the feedstuff industry and animal nutrition, biotechnology can increase the nutritive value of raw materials of feedstuff or multifunctional microorganisms by developing novel feed additives and using transgene technology (Yu et al., 2008a,b). Due to the requirement of animal products safety, the utilization of antibiotics has been gradually restricted; therefore, improving animal growth and other technological products has gained attention. Microorganisms spread over the environment, as there are great varieties and various characteristics, and more diversified biologics have been effectively developed using microorganisms and various technologies. Biologics refer to the metabolites of microorganisms and the substances beneficial to microbial growth, which can improve animal growth. This paper summarizes the enzyme and probiotics for feedstuff as follows:

ENZYME

The enzyme is a sort of natural organism, and is a natural catalyst. With the development of applied microbiology, the varieties and characteristics of enzyme increase, and the proportion of enzymes used in the feedstuff industry increases. The exogenous enzymes for application to the component (including anti-nutritive factors) of feedstuff include protein hydrolysate, starch, non-starch polysaccharides, phytic acid, etc. The enzyme is added into feedstuff to:

1. Remove anti-nutritional factors;
2. Increase nutrient digestibility;
3. Increase non-starch polysaccharides (NSP) digestibility; and
4. Supplement the activity of endogenous enzyme (Chen et al., 2011; Chen et al., 2010; Pettersson et al., 1990).

With the improvement of culture screening and fermentation technology, bacteria with higher enzyme activity can be widely created; thus, the application of the enzyme has extended from fowls with shorter intestinal tracts and chyme retention time to pigs. In recent years, it is applied to ruminants (Hristov et al., 1998a,b). However, these extensively used enzyme characteristics vary with the adopted culture (strains), culture substrate, and conditions for enzyme production; thus, the enzyme activity/capacity is usually doubted for application.

*Corresponding author. E-mail: byu@dragon.nchu.edu.tw. Tel: +886-4-22860799. Fax: +886-4-22860265.

As the raw material of feedstuff derived from plants is the major part of feedstuff for livestock and poultry, the cell wall structure and composition of the raw material influence the digestibility of intra-cellular components. As the intestine of simple stomach animals is restricted to the secretion of polysaccharide hydrolytic enzyme such as glucanase, xylanase and mannase, it cannot make the most of the cell wall constituent, and influence the utilization of intra-cellular components. Therefore, the key point of the enzyme industry has turned from traditional protein and starch production to the development of polysaccharide hydrolytic enzymes, such as applying glucanase to barley and oat containing glucan and xylanase to wheat and rye containing araboxylan (Yu et al., 2007; Wu et al., 2011).

Recently, composite enzymes with xylanase, protease, and amylase have also markedly increased the digestibility of low glutinousness raw materials, such as corn and grain sorghum (Yu et al., 2007; Liu et al., 2007; Pack et al., 1998). Since the starch granule in corn is surrounded by the protein matrix, the combination of two enzymes can significantly improve the digestibility of exogenous amylase. The protease not only hydrolyzes the storage protein, but also has more significant effect on the degradation of allergic proteins (glycinin and β -conglycinin) of soybean meal, when the alimentary tract of pup has not yet developed completely (Chen et al., 2011; Chen et al., 2010). The lectin and anti-trypsin factor in Leguminosae seeds are destroyed, which is helpful to the utilization of storage proteins. The phytic acid enzyme not only improves the utilization of phytic acid derived from plants, but also reduces the pollution of phosphorus from excrement (Yu et al., 2004).

The *Aspergillus oryzae* extracts or polysaccharide - degrading enzyme is the most extensively used enzyme for ruminants. The *Aspergillus oryzae* extracts have esterase and split folic acid and p-coumaric acid bonding araban, which is helpful to the effect of rumen microorganism; therefore, it is indicated that the synergism of extraneous enzyme and rumen microorganism accelerates the hydrolysis in rumen, but does not increase digestibility (Hristov et al., 1998a,b). As the hydrolysis rate in rumen is increased, the chyme through rate and feed intake are increased accordingly. The effect of extraneous enzyme on rumen may be confined to partial enzymes. Hristov et al. (1998a) indicated that extraneous or endogenous cellulase was not activated due to pH and pepsin, thus, only the xylanase had activity in the small intestine. Literature reports and practical applications show the inconsistent effects of additional enzymes, thus, the effect is doubted. The activity of commercial enzyme is measured under the simple effects of a laboratory; however, the enzyme will be influenced by multiple factors in practical application, such as the source of target feedstuff, feed processing (temperature and moisture content), feedstuff composition (e.g. mineral substance), and the intestinal bacterial

phase of the host related to endogenous enzyme content. Therefore, the enzyme shall be adopted appropriately to exert its effect. As the enzyme can be extensively used, the enzyme industry shall aim to develop novel enzymes, increase the activity and yield of the enzymes, and reduce production costs (Yu et al., 2008ab; Yu et al., 2007).

PREBIOTICS AND/OR PROBIOTICS

Low dose antibiotics added in feedstuff can promote growth and result in residual antibiotics in livestock meat and microorganism drug-resistance, and high density retention or other factors press animals, causes unbalanced microbiofacies in intestine and alimentary tract diseases, thus, more antibiotics are used. However, restricting the utilization of antibiotics has been a method for improving the livestock product quality in global animal production. Therefore, in recent years, prebiotics or probiotics have become another biologics for improving animal production, which adjusts intestinal bacterial phase to improve animal health (Hsueh et al., 2010). The former is the substance of non-feed nutrient content, e.g. oligosaccharide beneficial to multiplication of intestinal beneficial microorganisms, while the latter is an additive of viable bacteria for improving the intestinal bacterial phase, thus, the extraneous beneficial bacteria become advantageous intestinal bacteria. Both reduce the multiplication and healing of pathogenic bacteria and enhance disease resistance by adjusting the intestinal bacterial phase, which can improve animal growth.

Generally, the effect of the two additives is doubted for tremendous changes. Since the intestinal environment is complex, the prebiotics composition or the culture of probiotics can be retained in intestine, and the bioactivity becomes the key point of this type of products; thus appropriate timing, such as nascent pup and changes in environmental conditions, are the factors influencing the effect.

Probiotics for livestock products refers to using viable bacteria to improve the intestinal bacterial phase of the host (Fuller, 1992). The active mechanisms include:

1. Inhibit formation of poisonous or cancerogenic metabolites.
2. Stimulate detoxification related enzyme reaction.
3. Stimulate or supply the formation of enzyme for digestion of anti-nutrient substance.
4. Synthesize vitamins or other nutrient substances lacking in food.
5. Secrete bacteriostatics or create a bacteriostatic environment to prevent pathogenic bacteria from multiplication.
6. Enhance the immunity of the host animal.

However, these active mechanisms are an integrated result, and they cannot be provided by the specific culture

Table 1. Common probiotic microorganisms.

Lactic acid bacteria	Other probiotic organism
<i>L. acidophilus</i>	<i>Bifidobacterium bifidum</i>
<i>L. bulgaricus</i>	<i>Bacillus subtilis</i>
<i>L. plantarum</i>	<i>Torulopsis spp.</i>
<i>L. casei</i>	<i>Aspergillus oryzae</i>
<i>L. reuteri</i>	
<i>Streptococcus thermophilus</i>	
<i>Pediococcus pentosaceus</i>	
<i>Enterococcus faecium</i>	

of probiotics; however, the culture characteristic of probiotics is the determinative factor. The cultures of probiotics shall be GRAS (Generally Recognized as Safe) bacteria, the main candidate cultures are as shown in Table 1.

There are numerous strains created for choosing; therefore, screening for potential performance strains is the major factor determining the effect of additional probiotics. Basically, a probiotic strain shall meet the following conditions:

1. Good storage activity.
2. Good processing stability.
3. Good tolerant of gastric acid and bile salt.
4. Good resistant to antibiotics.
5. Good adhesion to epithelia of host.
6. Antagonistic to other microorganisms.
7. High multiplication rate.

Many reports have indicated that probiotics can improve the body weight gain and the feed efficiency, the laying performance (Abdulrahim et al., 1996) and reducing mortality (Jin et al., 1998a,b). The strains used include *Lactobacillus* species separated from the chicken (Jin et al., 1998a) and *L. casei* (Yeo and Kim, 1997); the mixed strains combined with *L. lactis* and *Streptococcus thermophilus*. Jin et al. (1998a) indicates that the effect of probiotics was influenced by the culture, bacterial count, and method of application, and is relatively related to chicken environments and pressure degrees, such as high temperature, humidity, feedstuff changes, and transportation. The addition of probiotics can maintain the intestinal bacterial phase by means of competitive exclusion and antagonistic activity of pathogenic bacteria. The *in vitro* analysis shows that the antagonism of probiotic strain for pathogenic bacteria is related to the metabolites of organic acid, H₂O₂, and bacteriocins. The reduction of pathogenic bacteria, such as coliform in intestine and the exclusion of *Salmonella* infection are related to the adsorbability of the strain for the host intestine cell, which capacity has host specificity (Jin et al., 1997a,b; Hejlícek et al., 1995). In addition, the agglutina-

bility of the strain for pathogenicity can effectively exclude pathogenic bacteria (Reid et al., 1988). The competitive exclusion of probiotics is related to the aforesaid strain specificity, high multiplication rate, or components beneficial to growth. For example, the Fructo-oligosaccharides or lactose is not utilized by *E. coli* or *Salmonella*, it can be a nutrition source for lactic acid bacteria (Edens et al., 1997), and helping these beneficial strains become advantageous strains in intestine.

Diarrhea is the first cause of death for nascent pigs to weaning pigs, and higher ratio came from infection by *E. coli* (enterotoxic) (Huang et al., 2012). It is indicated that besides providing appropriate feedstuff (including reducing indigestible protein) and good management in this period, providing probiotics can reduce the enteritis incidence rate. However, at the growing to finishing pig stage, the probiotics promote growth, but do not prevent bacteria/virus infection (Campbell, 1988). There is no difference in the characteristics of probiotics between pigs and chickens, and the provided strains are still required to be intestinal advantageous bacterium. Once the strain with host specificity adheres in intestinal cells, it will be exclusive of extraneous strains. As the alimentary tract of newly born pigs or chickens, the probiotic strain shall be provided as early as possible and shall survive in the intestine, thus, probiotics can be effective.

APPLICATION OF TRANSFORMED PRODUCTS

In order to improve the applicability of the aforesaid additives, in addition to traditional technology, molecular biology is used in this type of research, transferring the target gene to the microorganism to increase the efficiency of biologics. The screened microorganism is used as the main culture for producing enzyme or probiotics, while production efficiency or biological availability of the product is limited to the culture characteristics. Therefore, combining the requirements of culture and target product in order to produce transformed microorganism is very promising to improve the utilization of nutrients, intestinal tract development, and animal health (Yu et al., 2008a,b).

The rumen microorganism is the main source of phytic acid or the polysaccharide-degrading enzyme, thus, it is regarded as the research subject; however, the rumen microorganism is inapplicable to practical production for the anaerobic environment of rumen. Choi et al. (1998) separated cellulase and xylanase genes from rumen microorganism (*Fibrobacter succinogenes*), and transferred to *Sacchaomyces cerevisiae* which could secrete multi-enzyme. On the other hand, the transformed Lactobacilli (*L. plantarum* or *L. bulgarius*) containing glucanase gene or cellulase gene can be completely applied to the fermentation of silage (Choi et al., 1998; Hazzlewood et al., 1993). As the Lactobacilli species are intestinal beneficial bacteria, the multi-functional transformed microorganism is developed by screening lactic acid bacteria culture multiplied in the intestinal tract, including that with anti-gastric low pH, bile salt, intestinal protein, and by the transferring enzyme, in order to create enzymes and additives for novel feedstuff in the future.

APPLICATION OF FERMENTED FEED

In recent years, in regard to safety problems and shortage of proteins from animals, the demand from plants were increased. Thus, fermentation technology is applied to improve the nutritive value of feed such as soybean meal etc (Chen et al., 2011; Chen et al., 2010). Nkonge and Balance, (1984) indicated that a higher protein and amino acid availability could be obtained using the appropriate protein enzyme to the protein structures of various grains. The rat fed with the hydrolysate of fish meal processed by appropriate proteolytic enzyme is lower than 10–15% of casein in protein nutrition value (Rebeca et al., 1991). In order to improve the nutritive value of soybean meal using microorganism such as *Aspergillus oryzae* or *Rhizopus oligosporus*, for fermentation can increase the consumption of feedstuff for weaning pigs (Chen et al., 2011; Chen et al., 2010; Zamora and Veum, 1988). The specific proteolytic enzyme can be used for processing in order to effectively reduce the allergic proteins in soybean meal (Yamanishi et al., 1996). Hirabayashi et al. (1998), used *Aspergillus usamii* for fermentation; the bioavailability of phosphor could also be increased in soybean meal.

CONCLUSION

According to the global demand for animal products without residual antibiotics and environmental protection, the biologics, including the probiotics and enzyme products beneficial to the intestinal tract environment of animals, will replace traditional growth promoter antibiotics, and they can be used in the raw materials of feedstuff to increase nutritive value. The characteristics of this type of biologics shall be completely known, and biologics shall be appropriately used, in order to render such additives effective.

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