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〈Review〉

사료 제조에서 발효 가능한 탄수화물 이용과 가금 사료에서 효소의 처리에 관한 연구

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Utilization of Fermentable Carbohydrates in Feed Manufacturing and in Enzyme of Poultry Feed

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ABSTRACT Improvements in understanding the effects of dietary fermentable carbohydrates and their interaction with supplemental feed enzymes and the feed manufacturing process may lead to reductions in volatile organic compound (VOC) emissions from poultry manure. Starch digestibility has been improved by replacing ground wheat or barley with whole wheat or barley, but there was no consistent effect of cereal species or feed form on the pH value of the gizzard contents. Pelleting results in improvements in feed conversion from 0 to 12%. Starch digestibility has been reported to account for up to 35 % of the improvement in available metabolic energy as a result of xylase supplementation. Factors which affect starch utilization and non-starch polysaccharide (NSP) absorption include the presence of anti-nutrient factor (ANF) in grains, the nature of grain starch, NSP and the digestive capacity of animals. Improvements in feed production technology have been made in enzyme stabilization, allowing some dry enzyme products to be pelleted after conditioning at up to 87.69℃ and liquid enzymes to be stored in the feed mill for up to four months prior to use. The soluble NSP, arabinoxylans and beta-glucans are partially degraded into smaller fragments by enzymes. With fragmentation, the water holding capacity is decreased, which leads to a reduction in digesta moisture, wet feces, and dirty eggs from hens fed diets containing viscosity-inducing ingredients.
(Key words: fermentable carbohydrates, volatile organic compound, non-starch polysaccharide, anti-nutrient factor, pellet)

Introduction

Ideally, there would be a balance among the dietary levels of nutrients that poultry need for growth, maintenance or productive functions, so minimal poultry waste would result and there would be few environmental concerns about pollution. Unfortunately no perfectly balanced diet exists nor are there highly digestible feed groups for animals.

A major aerial pollutant from poultry manure is atmospheric ammonia (Kristensen and Wathes, 2000). Changes of the ratio of N excreted in urine or feces by the addition of fermentable carbohydrates hold a strong possibility to reduce the emission of NH_3. Reduction in NH_3 volatilization has been achieved by reducing the N excretion in urine as urea and shifting the N excretion in feces in the form of bacterial protein (Sutton et al., 1999).

Reduction in N excretion has been seen with the reduction of antinutritional factors in feed through selection of specific ingredients (Jongbloed and Lenis, 1991; Schulze, 1994). Although pelleting involves a high initial capital investment among feed processing methods, it also provides various benefits such as 1) a more uniform and desirable appearance, 2) increased density, 3) less dust, 4) ease of handling, 5) reduced segregation and 6) reduction of waste (Nahm and Chung, 1995).

Exogenic enzymes (enzymes) are protein catalysts that aid the biochemical reactions that occur during digestion. Enzymes are heat sensitive, since they are proteins. So its enzymes are subjected to the heat and pressure of a pellet mill, they are

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likely to be cooked and their ability to assist the digestive process will be impaired. Many enzyme producers recommend adding their products post pelleting for this reason (Lobo, 1999). The major reason for post pelleting liquid addition is heat, but pressure and friction may also negatively impact enzymes and other feed additives (Lobo, 2001).

The following discussion is a summary of research on the potential impact of fermentable carbohydrate sources used in the poultry diets on the environment.

Feed Manufacturing

1. Effect of Particle Size and Pelleting on Starch Digestibility

It has been reported (Bustany, 1996) that pelleting significantly increased live weight by 7.8%, feed intake by 5.1% and improved the feed conversion ratio by 3.2% in broiler. Beyer et al. (2001) said that pelleting has been known to result in improvements in feed conversion from 0 to 12%. Research in the past, however, has focused little on the effect of feed size on nutrient requirements.

Scientists (Jensen et al., 1965) reported many years ago that pelleted feed increased lysine requirements in growing turkeys compared to turkeys fed similar diets in mash form, especially when diets were formulated with marginal lysine levels. Amino acid bioavailability may be increased when corn or soybean meal is expanded under different pressure. When pressure was increased, there were general increases in true metabolizable energy (TME), protein solubility and increased starch gelatinization. Under pressure or at high temperature, the interaction of protein, starch and fat particles will likely be interactive (Beyer et al., 2004).

A study was conducted on the effects of whole wheat, oats and barley at different inclusion levels on performance, starch digestibility, duodenal particle size distribution, gizzard weight and pH of gizzard conditions for broiler chickens (Hetland et al., 2002). Starch digestibility was improved by replacing ground wheat or barley with whole wheat or barley, but there was no consistent effect of cereal species or feed form on the pH value of the gizzard contents. There was a significant interaction between the diet type and the soy particle size when the corn-soybean meal diets were fed, whereas there was little effect when the semipurified were fed (Kilburn and Edwards, 2004). This finding suggested that large particle size soybean meal (SBM) may be more efficiently utilized than fine particle size SBM. Scientists (Bruggeman et al., 2005) reported that feeding slowly digestible starch improved protein and energy utilization in broiler chickens with no effect on chick's quality. Digestion of the major part of starch occurs in the upper part of the small intestine. Plasma glucose levels may be elevated by diets with rapidly digestible starch when other nutrients have not been absorbed, and this may affect protein utilization. It was reported (Bruggeman et al., 2005) that broilers fed diets containing slowly digestible starch had better feed conversion than chickens fed diets containing rapidly digestible starch. Diets in which all starch is digested in the upper small intestine will not provide glucose to the lower part needed for its energy demands.

More amino acids may be oxidized in that case for energy. Amino acids may be spared from oxidation by diets containing starch that is partly digested in the lower small intestine (slowly digestible starch) and supply that part with glucose (Fleming et al., 1991; Weurding et al., 2003). Another experiment was conducted to study causes of low ileal starch digestibilities when broiler chickens were fed on wheat-based diets (Svihus and Hetland, 2001). Ileal starch digestibility increased significantly from 0.79 to 0.95, 0.93 and 0.91, respectively, when the diet was crushed and fed in a mash form, was diluted with cellulose prior to pelleting, or when parts of the wheat were fed as whole grains. Correspondingly, random variation between individual birds was reduced. They indicated that an overload of wheat starch in the digestive tract may be the cause of poor digestibility for some broilers in a flock. Grinding of the wheat may also influence starch digestibility.

It was reported (Carre et al., 2005) that individual starch digestibilities were negatively correlated with wheat hardness, particle size of wheat flour before pelleting, and pellet durability. Wheat lipase activity, however, was positively correlated with individual starch digestibility, which was the reverse of a result obtained in a previous experiment. These scientists reported that durum wheat (*Triticum durum*) showed the highest protein content and the lowest content of water-insoluble cell- wall among all wheat samples. Starch digestibility of durum wheat tended to be lower than that of other wheats (0.916 vs 0.936).

Svihus et al. (2004) indicated that replacement of ground wheat with whole wheat increased AME content and starch digestibility measured at the ileal as well as fecal level, while weight gain and feed conversion efficiency (FCE) were not affected. Jejunal chime showed increased amylase activity and bile salt concentration. Fine maize diets (Kilburn and Edwards, 2001) had higher ME values and there was a significant interaction between maize particle size and feed form as pelleting improved the ME value of coarse maize diets but not fine maize diets. In another experiment only pelleting of the factors studied improved the ME value of the diets (Kilburn and Edwards, 2001).

Pelleting contributes 187 kcal/kg of diet at 100% pellet quality (PQ) and the effective caloric value (ECV) declines curvilinearly as PQ falls. Birds were observed eating less and resting more as PQ increased, suggesting that ECV of pelleting is mediated by energy expenditure for activity (McKinney and Teeter, 2004).

Gous and Morris, 2001 showed interesting results when the scientists tested the hypothesis that delayed sexual maturity in pullets reared on very short (4 hours) constant photoperiods might be partly attributable to limitation of feed intake and that offering a pelleted feed might circumvent this effect. They concluded that constant short photoperiods during rearing cause delayed sexual maturity entirely due to the effect of light on gonadal development and that limitation of feed intake is not a factor in this response. The effect of grain form (whole, mash, or pelleted) on the live performance of broiler chickens was determined (Bennett et al., 1995). They found that feeding whole-grain and mash supplements caused at least a temporary loss in growth rate and feed efficiency but in some causes improved bird health. Broiler chickens fed whole-wheat diets had the same market weight and feed conversion as birds fed pelleted wheat diets (Rose et al., 1995). In two trials conducted by scientists (Svihus et al., 1997), broiler chickens fed whole barley grew at the same rate but with a higher feed : gain ratio than those fed roller barley.

Feeding of mash has been shown to increase the weight of the broiler digestive tract (Choi et al., 1986) and the length of the jejunum and ileum (Nir et al., 1994). Feed particle size strongly influences the development of the gizzard. Gizzard weight is significantly increased by feeding of coarsely ground feed or whole grains (Nir et al., 1994; Svihus et al., 1997). Taylor and Jones, (2004b) reported that whole grain inclusion in pellets reduced proventriculus proportional mass and increased gizzard proportional mass with no apparent effects of exogenous feed enzyme addition. Relative ileal mass was reduced by enzyme inclusion in a wheat diet and by inclusion of whole barley in the pellets. They concluded that evidence of a complex interaction between higher viscosity and pH being involved in differences in ileal relative mass was found through significant relationships being produced by enzyme use on the wheat diet alone. Other scientists (Wu et al., 2004) found that improved performance observed with post-pelleting inclusion of whole wheat was associated with increased size of the gizzard and improved AME. The gizzard development hypothesis, however, will not explain the improvements observed with pre-pelleting inclusion of whole wheat, suggesting the involvement of other factors. They also reported that xylanase supplementation reduced the relative weight of the pancrease, but neither xylanase supplementation nor whole wheat inclusion influenced the relative weight and length of the small intestine.

Gizzard size increased with inclusion of oat hulls, whole wheat, wood shavings and grits, and starch digestibility was significantly increased by inclusion of oat hulls for broilers, and by wood shavings for layers (Sutton et al., 1997; Hetland et al., 2003). They conluded that all particles of each feed ingredient are ground to a certain critical size before leaving the gizzard.

2. Digesta Viscosity and Starch Digestibility

Feeds which contain ANF have viscosities that disturb starch digestion and utilization. Soluble cell wall polysaccharides are the primary factors affecting starch digestion, but they also impede digestion of all nutrients. Starch utilization is affected by factors including presence of ANF in grains, the nature of grain starch and the digestive capacity of the animal (Classen, 1996).

The effect of enzyme supplementation (ES) on intestinal viscosity, nutrient availability and feed conversion would be greater with heat processing (HP) than with raw cereals. ES reduces the weight of digestive organs and increases crypt depth and villus height in the small intestine (Maneewan and Yamauchi, 2004; Svihus et al., 1997), but there is scarce information published on the effect of ES and HP of barley on the size and morphology of the digestive organs.

Heteropolymers such as beta-glucan and arabinoxylan (pentosan) form most of the cell wall carbohydrates. Most grains contain these substances, but their total amount and proportions vary considerably. Barley and oats contain predominately beta-glucans, while wheat, rye and triticale contain higher levels of arabinoxylans. Phenolic acids such as ferrulic acid are primarily esterified to the arabinoxylans (Classen and Bedford, 1991). The soluble fractions of beta-glucan and arabinoxylan in cereal grains are of major importance when determining the utilization of nutrients such as starch by poultry. The effects of these fractions are divided into two major mechanisms of action. When beta-glucan and arabinoxylan solubilize after ingestion, there is an increase in viscosity (Teitge et al., 1991). The increase in viscosity of the digesta is a major factor influencing the nutritional value of rye, barley and oats, and recently similar evidence has become available for wheat (Bedford and Classen, 1992). The second mechanism is that cell walls prevent or slow access of endogenous enzymes to nutrients (Hesselman and Aman, 1986).

The effects of ANF can be summarized as follows:

(1) Local or intestinal effects caused by

- a) increased osmolarity resulting in increased liquid retention,
- b) decreased convectional motility
- c) increased viscosity and
- d) decreased hydrolysis and absorption of nutrients due to the above mentioned effects.

(2) Systemic effects due to increased volume of intestinal contents that results in stimulation of intestinal motility and alterations in intestinal flow rate (Bedford and Classen, 1992). As a consequence of all of these mechanisms, nutrient absorption is decreased.

NSP is the main ANF in grains (Lobo, 1999). NSP includes all of the polysaccharides, with the exception of alpha-glucans (starch). NSP can roughly be separated into soluble and insoluble fractions. The soluble portion of NSP appears to have anti-nutritive characteristics caused by the effect that the solubility of some large polymers (though not all) has on viscosity. The insoluble portion of NSP has no negative nutritional effect on chickens. Cellulose, a linear homopolymer of 1-4-glucose units is a major part of the insoluble NSP component. The insoluble portion of NSP showed no detrimental effect when up to 21% cellulose was added to poultry diet (Choct, 1997). While they are not soluble in water, insoluble NSP components have the ability to absorb large quantities of water, a characteristic which maintains normal gut motility and fecal consistency.

Insoluble fiber such as NSP and lignin affects gut function and modulates nutrient digestion (Hetland et al., 2004). These scientists indicated that moderate levels of insoluble fiber in diets improve digestibility of starch and increase digesta passage rate. Insoluble fiber affects gut function through its ability to accumulate in the gizzard, which regulates digesta passage rate and nutrient digestion in the intestine. When added up to 10% of the diet, oat hulls may have a positive effect on starch digestibility of wheat-based diets (Hetland and Svihus, 2001; Hetland et al., 2003). In another study (Hetland et al., 2003), positive effects were seen when 3~4% wood shavings were added to wheat-based diets for layers. This study also showed that the amount of bile acids in the gizzard increased in proportion to the amount of gizzard contents. Bile acid levels in the gizzard give a good indication of gastroduodenal reflux since bile acids enter the intestine in the junction between the duodenal loop and jejunum. These results indicate that inclusion of insoluble fiber increases chime reflux between the gizzard and duodenum.

The soluble components of NSP are primarily responsible for the anti-nutritive effects. It is generally accepted that the viscosity of these polysaccharides is the major detrimental factor of NSP. The solubility and molecular weight of the NSP determines the viscosity caused by the NSP. Hind gut viscosity reduces the rate of diffusion of substrates and digestive enzymes, hindering their effective interactions at the mucosal surface. In monogastric animals (poultry and swine), NSP (especially water-insoluble NSP) is known to be rather indigestible (Carre et al., 1990) and to contain anti-nutritional properties which depress animal performance.

Since most common feed ingredients contain a certain percentage of NSP, the energy values of these ingredients could be significantly increased if these compounds could be metabolized to produce energy, or simply inactivated, and therefore commercial feeds could be formulated at a substantially lower cost at the same nutrient density (Puchal and Mascarell, 1999).

Supplementation of diets with appropriate enzymes may minimize intestinal viscosity. Enzymes partially degrade the soluble NSP, arabinoxylans and beta-glucans into smaller fragments

(White et al., 1983). Fragmentation results in a decreased water holding capacity, which leads to a reduction in digesta moisture (Almirall et al., 1995), wet feces (Gohl et al., 1978), and dirty eggs from hens fed diets containing ingredients which increase viscosity (Graham et al., 1993). Enzyme supplementation has been shown to decrease intestinal viscosity by 85%(Ward, 1996).

Enzyme Utilization

1. Feed Manufacturing

Since enzymes are proteins, they are heat sensitive. Processing enzymes through the heat and pressure of a pelleting mill cooks them, and impairs their ability to assist in the digestive processes. Many enzyme producers recommend adding their products post-pelleting for this reason.

Prior to application, most liquid forms of enzymes are diluted in water. After application to feed, the enzymes absorb or adhere to different particles (Lobo, 1999). Since enzymes are heat sensitive proteins, the heat and the time they are exposed to the heat determine if the enzyme are cooked and reduced in effectiveness. Enzymes are exposed to heat for about 1 second if they are injected into the line just before application. The maximum exposure is usually less than 2 seconds. There is little damage to the enzymes ability to work when fat is applied at 60℃. Even the application of fat at temperature up to 100℃ caused few negative effects on the enzymes because they are exposed to the hot fat for a short period of time (Lobo, 1999).

Non-starch polysaccardie, pelleting and expansion have previously been shown to increase the response of birds fed xylanase-supplemented diets (Teitge et al., 1991; McCracken et al., 1993). These technological treatments are known to result in cell wall damage or perforation, but increase intestinal weight in birds fed these diets (Teitge et al., 1991; McCracken et al., 1993). These observations show that the integrity of the cell wall is not the problem in wheat, but dissolution of arabinoxylans is, which favors a viscosity based mechanism. Improvements in production technology have been made in enzyme stabilization, allowing some dry enzyme products to be pelleted after conditioning at up to 87.69℃ and liquid enzymes to be stored in the feed mill for up to four months prior to use (Graham, 1994). One report (Angel, 2003) indicated that microbial phytases tend to have pH optima in the range of 2～6, while plant phytases have a pH optima of 5. Enzyme stability has been shown to be affected very little by vitamins, minerals, trace elements and other oxidizing agents found in concentrated premixes (Inborr, 1990). Enzymes added to the feed have to withstand the manufacturing, process, acidic conditions in the stomach and proteolytic attack in the small intestine (Classen et al., 1991).

Enzyme supplementation with the enzyme preparation Roxazyme G (0.15 g kg^{-1} diet, supplied by Hoffman-La Roche, Switzerland) was more effective on the mash than on the pelleted diets for improving the feed conversion ratio and reducing the frequency of sticky droppings, as indicated by the significant interaction between type of diet and enzyme supplementation (Bustany, 1996).

2. Poultry Feed

The use of exogenous enzymes in poultry diets in Europe is now almost universal. The reasons why they are used are manifold and include: 1. To increase the feeding value of raw materials, 2. To reduce the variation in nutrient quality of ingredients, 3. To reduce the incidence of wet litter (Bedford, 2000). Enzymes are added to rations of poultry and swine to enhance digestion and absorption of nutrients and reduce pollution (Swick, 1991).

In poultry nutrition today, most enzymes can be classified as carbohydrases (beta-glucanase, xylanases, hemicellulase, pentinase, alpha-galactosidases, inulase)-which are enzymes that act on different carbohydrates, particualy the types of carbohydrates (hemicellulises, oligosaccharides etc.) that have been identified as components of the group known as NSP (Puchal and Mascarell, 1999).

Use of supplemental enzymes to reduce the dry matter content of the digesta in the intestinal tract has a marked impact on excreta volume and composition. When broilers were offered wheat or wheat/barley based diets supplemental with enzymes, fresh excreta weights were reduced 17～28%, with a reduction in dry matter output of 12～15%. This has important implications for nitrogen output from broiler units, since enzymes provide additional improvements in protein digestibility (Wyatt and Harker, 1995). It was reported (Auclair and Larbier, 2000) that AME nitrogen corrected (AMEn) were significantly increased for pelleted wheat gluten feed (+ 75 kcal/kg), rice bran (+ 50 kcal/kg), sunflower meal (+ 74 kcal/kg) and, pea (+ 63 kcal/kg) by enzyme supplementation. AMEn of other raw materials (pelleted cassava, and rapeseed meal) were not significantly improved by enzyme addition (xylanase preparation-SAFIZYM XP 20, SAF-ISIS, France).

The mechanism of enzymes in improving digestibility values of specific cereal grains has been proposed to be cell wall disruption and the release of cell bound nutrients (Wyatt and Harker, 1995) and NSP. Bacterial activity and the composition of the bacterial population within the gastrointestinal tract of the bird may be impacted by the feed enzyme mechanisms involved in viscosity and viscosity-reduction. The bacterial population in different regions of the intestinal tract may be indirectly altered by enzymes digesting the long-chain carbohydrate molecules utilized by some bacteria to colonize the tract (Morgan, 1995).

It has been found that the nutritive value of barley could be improved by either a simple water treatment (Fry et al., 1957) or supplementation with enzymes (Jensen et al., 1957). These observations have been confirmed and expanded upon by two scientists (Anderson, 1958; Arscott, 1958). Other investigations (Hamm, 1958; Gerry, 1950) have failed to improve the nutritional value of barley by addition of enzyme supplements to the feed or water treated barley.

The geographical area of production of barley affects its nutritional value with enzyme supplements, water-treatment and heat-treatment (Willingham et al., 1960). As measured by chick growth, barley grown in the mid-west and east generally did not respond significantly to enzyme supplements as did barley grown in the west. A significant response was obtained by water-treating all the samples, but the magnitude of response was greater for western barley. Nutritional value of a blend of eastern barley was markedly lowered by wet-autoclaving and drying at 95℃. Addition of enzymes to this autoclaved blend gave a highly significant response in chick growth. Wheat and barley are used in some areas of the world to provide much of the energy an some of the protein in a diet (Silversides and Bedford, 1999).

The use of xylanase-based enzyme in a wheat-based feed would increase starch digestibility more than that of any other nutrient if its function were simply to break down endosperm cell walls. The data does not support this observation, even with French wheat which contains a very low soluble arabinoxylan (Carre et al., 1992). As a result of xylase supplementation, starch digestibility has been reported to account for to 35% of the improvement in available metabolic energy, while fat accounts for 35% and protein accounts for 30% (Carre et al., 1992). Decreases in digesta viscosity and better diffusion/digestion are almost entirely responsible for the benefits to starch digestion. This has been proven by studies in which isolated wheat non-starch polysaccharides were added back to sorghum-based diets. The negative effects of viscosity were equally apparent on starch and protein digestion, and more apparent on fat digestion (Choct and Annison, 1992). Thus, no further mechanism (i.e., cell wall degradation) is needed to explain the action of xylanase enzymes.

In the small intestine, the residence time of feed is approximately 2 hours (Isshiki et al., 1989). Based on the quantity of enzyme typically added to poultry feed and the kinetics of the reaction involved, there is likely to be sufficient time for a few rather than many of the xylan bonds to be hydrolyzed by xylanase. This mechanism favors the function as a viscosity reducing enzyme (only 4～5 random catalytic events per high molecular weight xylan are needed to reduce viscosity) rather than a cell wall puncturing enzyme (many coordinated catalytic events are needed to digest cell walls and create holes large enough to allow ingress of amylase molecules). This argument has been presented elsewhere in greater detail (Chesson, 1994).

The digestibility of poorly digested cereals is more greatly increased by enzymes than that of well digested cereals (Lobo, 2000). For the feed compounder, enzyme addition has been shown to have two consequences: 1) There is a reduction in variation between the best and worst samples of a given grain, 2) The feed formulation nutrient matrix values may be elevated by addition of enzymes (Bedford, 2000).

Conclusion

ANF is a broad category of nutrients that includes NSP, but is not limited to NSP. Arabinoxylans and beta-glucans are the major categories of NSP that are responsible for increased digesta viscosity, leading to wet litter and other environmental problems. Enzyme supplementation to feed reduces the water-holding capacity of the digesta so the moisture content of the manure is reduced by this decrease in digesta viscosity. A researcher (Ward, 1996) has shown that enzyme supplementation can reduce intestinal viscosity by 85%.

Poultry diets containing barley, rye, wheat, triticale and oats are commonly used, especially in Europe, but these ingredients are responsible for increased intestinal viscosity. Enzyme supplementation may be utilized with these feed ingredients to improve poultry production. The most common enzymes utilized are carbohydrases (beta-glucanase, xylanase, hemicellulase, pectinases, alpha-galactosidase, and inulinase), but proteases and lipases are also used to increase digestibility of feeds.

적 요

발효가 가능한 탄수화물을 이용하거나 효소 이용 및 사료 가공 등에 관한 이해를 증진시키는 길은 닭의 분중 휘발성 유가물질의 감소를 가져올 수 있다. 전분질의 소화는 가루 사료를 (밀과 보리) 알곡으로 대치하게 하였다. 그러나 근위의 pH로 볼 때 사료의 종류나 형태는 연속성이 떨어진다. 펠렛으로 만든 사료는 사료 요구율이 0～12% 증진된다. 전분질의 소화는 xylase를 첨가하였을 때 대사 에너지(ME) 가는 35% 증진 효과가 잇는 것으로 보고되었다. 전분질의 이용과 전분질이 아닌 다당류(NSP)의 이용은 전분질을 포함한 알갱이 사료의 존재 즉 비 영양소 물질(ANF)의 포함 여부에 달려 있다. 사료 생산 기술의 증가는 33℃에서 만들어지는 펠렛 사료에 이용될 수 있는 건조된 상태의 효소나 액체상태의 효소 생산 기법에 달려 있다. 수용성 NSP나 arabinoxylans 또는 beta-glucan 등은 부분적으로 효소 가격이 크기로 나누어지는 정도에 따라 달라진다. 적은 크기는 수분 흡수력이 감소되어야 하는데 만약 수분 흡수력이 지나치면 소화물에 수분이 너무 많아지게 되어 분에 수분 함량이 많아지며 사료 곡물 중에 비스코시티 현상이 생겨서 계란 껍질이 지저분해진다.

(Key words: 발효 가능한 탄수화물, 휘발성 유기 물질, 전분질이 아닌 다당류, 비영양소 물질, 펠렛)

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