

Review

# Effect of common feed enzymes on nutrient utilization of monogastric animals

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Some nutrients in livestock feed may not be fully digested by animals own digestive enzymes, and hence important nutrients are unavailable to the animal. Although, supplementation of enzymes to farm animals has shown to increase the digestibility of poorly digested diets to a much greater extent. By targeting specific anti-nutrients in certain feed ingredients, feed enzymes allow especially pigs and poultry to extract more nutrients from the feed and so improve feed efficiency. Enzymes are most commonly used when the dietary ingredients contain relatively higher amounts of fiber. The classification of enzymes is usually according to the substrates they act upon and the classification can be enzymes that break down fiber, proteins, starch and phytate. Appropriate use of exogenous enzymes in feeds requires strategic reductions in dietary energy and nutrient content, as well as careful choice of feed ingredients to capture economic benefits of the various enzymes. The efficacy of enzymes will vary depending on ingredients because nutrient and energy release caused by enzyme supplementation will depend on the structure of the feedstuff itself. It is important to continue the effort to understand the use and limitations of matrix values of enzymes, which, if inappropriately applied, will result in depressed performance because of inadequacy of diets or will lead to wastage of resources.

**Key words:** Carbohydrate, monogastric, feed enzymes, phytase, nutrient utilization.

## INTRODUCTION

Not all compounds in animal feed are broken down by animals' own digestive enzymes, and so some potential nutrients are unavailable to the animal (McDonald et al., 2010). To alleviate this problem, in the 1950s, pioneering scientists added enzymes called amylases and proteases to the diets of various farm animals and observed benefits in productivity. Such kinds of exogenous enzymes are produced commercially from microbes, fungi and yeasts in highly controlled conditions in

fermentation plants (Fuller, 2004). Their main uses are in the detergent and food industries but significant quantities are manufactured for use in animal diets. As feed additives, enzymes are mainly used in the diets of non-ruminants but are also added to ruminant diets (Fuller, 2004). Among monogastric animals, pigs and poultry are important beneficiaries these days from exogenous enzyme supplementation diets, and are even used extensively for the latter (McDonald et al., 2010).

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Feed enzymes help fundamentally to improve the efficiency of meat and egg production by changing the nutritional profile of feed ingredients (Bedford and Partridge, 2010). Enzymes have clearly been demonstrated to increase the digestibility of poorly digested diets to a much greater extent than well digested diets (Scott et al., 1998). Enzymes are most commonly used when the dietary ingredients contain relatively higher amounts of fiber (Bedford, 2000). For example, the various forms of fiber in the pig's diet will not be well digested by the pig; as a result, a large portion of the fiber in the diet passes through the small intestine intact, and the only breakdown that can occur is through fermentation by bacteria and yeast in the cecum and large intestine. The disruption of the cell matrix of fibrous feedstuffs by exogenous enzymes can lead to easy access of the endogenous proteolytic and cellulolytic enzymes to digest the entrapped protein and carbohydrates. This will consequently reduce the feed cost in animal production. However, the effects of exogenous enzymes can be variable and it depends on a large number of factors such as the age of the animal and the quality and type of diet (Bedford, 2000). Further, feed enzymes allow the feed producer greater flexibility in the type of raw materials that can confidently be used in feed formulation. Another application of enzymes is to break down the phytate molecule that binds phosphorus and some other mineral elements in plant based feedstuffs (McDonald et al., 2004; Fuller, 2004). Based on the fact that a significant portion of phosphorus in the diet of the pig is bound to phytate, it will not be well digested by the pig.

Another area of feed enzyme application in farm animal diet is to supplement the enzyme complement of young animals, in which the rate of endogenous enzyme production may be limiting. The effect of commercially available enzymes on the feeding value of major ingredients is often based on their effect in young chicks less than two weeks of age has been demonstrated by Meng and Soliminski (2005). This is further explained by the fact that in newly hatched chicks, the enterocyte is poorly developed, limiting the bird's digestion and absorption abilities (Lji et al., 2001a). During this maturation period, the gut lacks the competency to fully digest feedstuffs and absorb smaller molecules because of a lack of brush-border enzymes, inadequate maintenance of absorptive mechanisms, and low surface area caused by immature villus height (Van Leeuwen et al., 2004). As the gastrointestinal tract develops, it is able to take advantage of the effects of fibrolytic enzymes. Before this, however, the pancreatic enzymes needed to initiate digestion in the intestinal lumen are limited in both volume and activity (Noy and Sklan, 1995). Thus, they may be unable to utilize substrates made available by a fibrolytic enzyme. Early-weaned pigs have limited amylase, protease and lipase activity, and enhancement of the extent of digestion of nutrients would improve performance and reduce the incidence of the diarrhoea

that results from undigested nutrients reaching the hind gut and being fermented by bacteria.

In a study with finishing pigs, Zhang and Kornegay (1999) reported that the digestibility of all amino acids except proline and glycine increased linearly as phytase supplementation increased. Enzymes are essential for the breakdown of cell-wall carbohydrates to release the sugars necessary for the growth of the lactic acid bacteria. Commercial hemicellulase and cellulase enzyme cocktails are now available and can improve the fermentation process considerably (Hooper et al., 1989). However, prices of these products preclude their viability for farm level application, especially in developing countries. Supplementation of a wheat by-product diet with cellulase increased the ileal digestibility of non-starch polysaccharides from 0.192 to 0.359 and crude protein from 0.65 to 0.71 (McDonald et al., 2010). The diversity of enzyme activities within commercially available enzyme preparations is probably advantageous, in that a single product can target a wide variety of substrates. Enzymes are categorized according to the substrates they act upon (Bedford and Partridge, 2010). Currently, in animal nutrition, the types of enzymes used are those that break down fibre, proteins, starch and phytate.

The objective of this paper was to review the characteristic feed enzymes and their roles in mono-gastric nutrition

## COMMON FEED ENZYMES ON NUTRIENT UTILIZATION AND ANIMAL PERFORMANCE

### Carbohydrases

Studies reported that carbohydrase supplementation improved the digestibility of dry matter (Northey et al., 2007), organic matter (Li et al., 1996), and energy (Yin et al., 2000) in monogastric animal nutrition. Other studies also reported an improved result on the digestibility of amino acids due to carbohydrase-supplemented wheat (Vahjen et al., 2007) and barley-based diets (Li et al., 1996). However, observations of increased digestibility of fiber or non starch polysacchirde components with carbohydrase supplementation emphasize the importance of release of inaccessible nutrients in enhancing amino acid digestibility.

Generally, the importance of enhanced digestibility after carbohydrase supplementation should be considered in evaluating the role of carbohydrases in enhancing nutrient utilization.

In many poultry studies, carbohydrase supplementation has been shown to improve energy utilization in corn-soybean meal diets (Rutherford et al., 2007; Yang et al., 2010). Others noted no improvement in energy utilization in response to carbohydrases (Olukosi et al., 2007b). In diets with cereal grains containing greater quantity of non starch polysaccharides, carbohydrase supplementation

also improved energy utilization (MacLeod et al., 2008). The studies show that carbohydrases often improve energy value of diet or feed ingredients containing increased concentration of non starch polysaccharides. The differences in the effect of the enzymes on energy of feedstuffs or diet may relate to the amount of substrate for the enzyme or availability of energy from the ingredient itself, or both. Similarly, in Adeola et al. (2008) study, carbohydrases improved metabolizable energy in diets with reduced metabolizable energy but not in diets with higher metabolizable energy. There are also reports of improvement in dry matter utilization (Yang et al., 2010), fat (Boguhn and Rodehutsord, 2010), starch (Meng and Slominski, 2005) and minerals (Olukosi et al., 2008b) in response to carbohydrase supplementation. The responses to enzyme supplementation are feedstuff-, diet- and enzyme-dependent. Generally, the feedstuffs with greater amount of non starch polysaccharides, intuitively, respond to a greater extent to carbohydrase supplementation.

There is association between extent of digesta-viscosity reduction and improvement in protein digestibility (Palander et al., 2005). Many others have also reported improvement in N (Yang et al., 2010) and amino acid digestibility (Boguhn and Rodehutsord, 2010) in response to carbohydrase supplementation. However, Rutherford et al. (2007) noted that reduction of endogenous loss by carbohydrases is secondary to improvement in protein hydrolysis. Clearly, there is need to understand why specific amino acids respond to a greater extent and how this can be used to increase the benefit from carbohydrase supplementation.

It is important to note that improvement in nutrient digestibility does not explain all the effects of carbohydrase supplementation on performance. This is demonstrated in Barrera et al. (2004), in which xylanase supplementation to a low amino acid wheat-based diet only marginally improved performance, whereas supplementation of crystalline amino acid improved growth. In the same study, xylanase supplementation improved amino acid digestibility by an average of 11%.

In corn-soybean meal-based diets, Tahir et al. (2005) observed that cellulase, hemicellulase and their combination increased body weight gain without any effect on feed intake in broilers but Cowieson and Ravindran (2008b) observed both increased body weight gain and feed intake in response to supplementation with a mixture of xylanase, amylase and protease. Similarly, Olukosi et al. (2007a) reported a dose-related increase in body weight gain, feed intake and feed efficiency in broilers receiving wheat and rye-based diets with xylanase supplementation. In other studies, there were no responses to supplementation of carbohydrases (Olukosi and Adeola, 2008). According to the authors, part of the differences observed in the various studies can be due to the extent of nutrient density reduction in the control diets.

In comparison with broilers, the effect of non starch polysaccharides-degrading enzymes was smaller for layer pullets (Karimi et al., 2007), although the enzyme reduced digesta viscosity to the same extent in the different chicks. Some studies reported no effects of non starch polysaccharides-degrading enzymes on egg production (Hampson et al., 2002), but at the same time, reported positive effects on specific response criteria related to the quality of eggs produced (Jaroni et al., 1999). Due to variations in treatments and enzyme activities used, it is difficult to make across-study comparisons for the effects of the enzymes.

### ***Effect of carbohydrases on non starch polysaccharides***

The main reason for the use of carbohydrases is to hydrolyze complex carbohydrates that non-ruminant animals are unable to hydrolyze by themselves. Some of these compounds are present as part of the cell wall, thus shielding substrates from contact with the digestive enzymes, or as part of cell content where their presence may interfere with digestion and absorption by their chemical nature. Nitrayová et al. (2009) reported improved ileal disappearance of non starch polysaccharides in diets containing 96% rye for weanling swine; there was a 740% improvement in disappearance for xylose and a 144% improvement in disappearance for total non starch polysaccharides when xylanase was added at the rate of up to 200 mg/kg. These data indicate that non starch polysaccharides removal is one of the critical roles of carbohydrases when added to diets containing non starch polysaccharides. It seems that reduction in digesta viscosity may be one of the most important benefits of carbohydrase supplementation (Vahjen et al., 2007). Several studies have shown that xylanase attacks the arabinoxylan backbone, causing a decrease in the degree of polymerization (Courtin and Delcour, 2002) and thus liberate oligomers. The importance of this hydrolysis is that the direct link between digesta viscosity and animal performance has been demonstrated in several studies (Zhang et al., 2000a). Adeola and Bedford (2004) demonstrated that one of the modes of action of carbohydrases is their ability to reduce non starch polysaccharides-induced digesta viscosity. High-viscosity wheat responded more (in terms of improved nutrient utilization) than low-viscosity wheat when the diets were supplemented with xylanase.

### ***Impact of carbohydrases on energy availability***

Energy digestibility in swine generally decreases with increased fiber intake (Nortey et al., 2008). Explanations for reduced energy digestibility could be increased

endogenous energy loss, reduced digestibility of energy yielding fraction because of impaired nutrient absorption, reduced contact of substrates and digestive enzymes, reduced proportion of energy-yielding fractions in high-fiber feedstuffs, or reduction in feed intake because of bulkiness of high-fiber diets combined with inherent stomach capacity of the animal. Johnson and Gee (1986) observed that feeding of high- non starch polysaccharides diet to rats reduced DNA and protein contents of the brush border. This reduction could be a result of increased cell turnover rate engendered by increased cell proliferation. Therefore, if carbohydrases hydrolyze the non starch polysaccharides fractions, these effects could be reversed and energy utilization should improve. Indeed, there have been observations of increased quantity of mono- and oligosaccharides in the ileum after the use of cellulase or xylanase (van der Meulen et al., 2001) and  $\beta$ -glucanase (Li et al., 1996) or multi-activity carbohydrases (Kiarie *et al.*, 2007). It seems that one of the ways by which carbohydrases improve energy utilization is by shifting production of volatile fatty acid and absorption of energy-yielding monosaccharides to proximal intestine. This is supported by observations of decreased net disappearance of nutrients in the large intestine of swine receiving  $\beta$ -glucanase-supplemented diets (Li et al., 1996). The shift in nutrient utilization to the more proximal intestine would decrease host-microbe competition for nutrients, ensure availability of nutrients where absorption efficiency is greater, reduce fermentative loss, and contribute to overall improved efficiency of energy utilization. However, reduction in digesta viscosity is usually far greater than improvement in energy utilization. In Macleod et al. (2008), a small increase (<5%) in metabolizable energy of naked oats was associated with a substantial decrease (>250%) in jejunal digesta viscosity. Similarly, in Adeola and Bedford (2004), the decrease in jejunal viscosity (70%) was associated with only a small increase in metabolizable energy (4%).

Since non starch polysaccharides may reduce the capacity for absorption by reducing enzyme accessibility to substrate, it is reasonable that there are observations of increased digestibility of energy-yielding nutrients after carbohydrase supplementation. For example, Adeola and Bedford (2004) reported improved starch and fat digestibility in wheat after xylanase supplementation. Juanpere et al. (2005) and Vahjen et al. (2007) also noted improved fat and starch digestibility in response to supplementation of xylanase and  $\beta$ -glucanase. The improvement in fat digestibility is especially noteworthy because non starch polysaccharides are known to increase hydrolysis of bile salts (Mathlouthi et al., 2002) and hence reduce fat utilization. Meng and Slominski (2005) suggested that hydrolysis of encapsulating cell walls may be responsible for enhanced energy utilization in a corn diet but that disruption of cell matrix resulting in a release of structural protein may be responsible for

improved energy utilization in a soybean meal-based diet.

#### ***Effect of carbohydrases on nitrogen and amino acids***

Nitrogen digestibility reduction by neutral detergent fiber (NDF) and acid detergent fiber (ADF) has been ascribed to increased loss of endogenous and microbial N, low availability of N in the fibers themselves, or increased excretion of N trapped in the fibers or the digesta (Stanogias and Pearce, 1985). The increased N loss with feeding of fibrous feedstuffs resulted from endogenous (59%) and exogenous (41%) nitrogen sources. Loss of pancreatic enzymes and bile, as well as sloughed mucosa, will result in endogenous amino acid and nitrogen losses (Schulze et al., 1995). Consequently, reduction of the endogenous and exogenous losses and increased hydrolysis of dietary protein are two possible modes of action of carbohydrases in improving nitrogen and amino acid utilization. However, observations regarding carbohydrases reducing endogenous protein or amino acid have been inconsistent. Yin et al. (2000) reported a modest decrease in endogenous amino acid loss after carbohydrase supplementation, whereas Rutherford et al. (2007) did not observe such effects. These may be diet-, ingredient-, or enzyme-specific (that is, presence or absence of certain antinutrients, difference enzymes and others). Tahir et al. (2008) noted that combination of enzymes were only effective in the presence of hemicellulases, which is capable of cleaving the resistant galacturonic acid and rhamnose bonds. This was corroborated by the observation that, for soybean meal, there was a strong correlation between the amount of galacturonic acid and crude protein digestibility, indicating that the hydrolysis of pectic substances in the cell wall enhanced protein digestibility. Another indirect effect of carbohydrases on nitrogen and amino acid utilization was recently demonstrated in the Yin et al. (2010) study, in which carbohydrases enhanced starch digestibility indirectly and improved nitrogen and amino acid digestibility and absorption. The authors observed that starches with increased amylose:amylopectin are more resistant to digestion and have decreased digestibility, especially at the proximal part of the small intestine, in the overall length of the intestine, and the decreased digestibility is closely associated with decreased AA digestibility and plasma AA concentration.

#### ***Effect of carbohydrases on mineral availability***

Similar to other nutrients, Nortey et al. (2008) observed reduced digestibility of minerals in diets, in which different by-products of wheat milling were added to a wheat-soybean meal basal diet. In several studies, supplementation of xylanase (Nortey et al., 2007) or xylanase, amylase,

and protease (Olukosi et al., 2007b) resulted in improved P digestibility. One possible explanation is provided by an observation of the relationship between phytic acid and NSP in plants. In cereal grains and legumes, most of the P is bound in phytic acid. Frølich (1990) indicated phytic acid and NSP are both found in the aleurone layer of wheat. In many cereals, grains or fractions thereof are sequestered with phytic acid. Consequently, when carbohydrases hydrolyze their substrates, phytic acid and other minerals may be exposed to digestive enzymes. As Parkkonen et al. (1997) observed, carbohydrases may increase permeability of the aleurone layer and consequently increase the release of otherwise unavailable minerals. Therefore, the increase in mineral availability as a result of carbohydrase supplementation is an indirect response to the carbohydrase effect.

### **Effect of carbohydrases on gut health**

Increased digesta viscosity encourages slower diffusion rate, accumulation of particulate matter for microbial adhesion, and greater flow of solids rather than liquid. These factors encourage slower shedding of microorganism and encourage the proliferation of harmful microorganisms (Vahjen et al., 1998). In newly weaned swine receiving diets that promote increased digesta viscosity, McDonald et al. (2001) observed increased shedding of enterotoxigenic *Escherichia coli*. Poorer gut health in high-NSP diets may also arise from alteration of the morphology of the digestive surfaces in animals receiving a high-NSP diet.

Similar observations have been made in swine and poultry. Teirlynck et al. (2009) observed greater evidence and markers of gut damage, apoptosis, increased mounting of immune defense and microbial invasion of intestinal tissues in broilers receiving wheat-rye diets when compared with those on corn-based diets. The observation by Langhout et al. (2000) that the negative effects of NSP were less pronounced in germ-free birds indicates that microorganisms play a critical role in mediating the negative effects of NSP.

Carbohydrase supplementation reverses these negative effects by increasing the proportion of lactic and organic acids, reducing ammonia production (Kiarie et al., 2007), and increasing VFA concentration (Hübener et al., 2002), which is indicative of hydrolysis fragmentation of NSP and supporting growth of beneficial bacteria. Increased proportions of lactic acid promote gut health by suppressing the growth of presumptive pathogens (Pluske et al., 2001). Hillman et al. (1995) showed that certain strains of *Lactobacillus* inhibit the growth of coliforms such as pathogenic *E. coli*. Increased colonization of the gut with *Lactobacilli* has been associated with xylanase supplementation of a wheat diet and reduction in digesta viscosity (Vahjen et al., 1998). In addition, xylose (possible product of exogenous and endo-

genous carbohydrase activity) has been shown to be important in preferentially enhancing the growth of beneficial bifidobacteria (He et al., 2010). There were reports of improvement in the health of poultry as a result of carbohydrase supplementation (Hampson et al., 2002). In layers, Hampson et al. (2002) observed a reduced excretion of *Brachyspira intermedia* in hens receiving 265 mg/kg of an enzyme product containing xylanase and protease activities, which are indicative of infection reduction as a result of improved intestinal microbial population. Consequently, there is a basis for improved gut and overall health of the animal as a result of carbohydrase supplementation.

### **Proteases**

Proteases have been added to poultry and swine diets routinely for many years as part of enzyme admixtures containing xylanases, pectinases, glucanases, amylases and other activities (Cowieson and Adeola, 2005). In recent years, proteases have grown in profile, there are currently several stand-alone proteases available, and new mechanisms of action have been proposed. Early research on the usefulness of proteases as supplemental feed enzymes is equivocal. For example, Caine et al. (1997) treated soybean meal with *Bacillus subtilis* in an attempt to reduce the adverse effects of proteinaceous antinutrients in soy when fed to weaner piglets. However, the authors observed a protease-induced decrease in AA digestibility from 68.7 to 63.9%. Ghazi et al. (1997) and Rooke et al. (1998) supplemented broiler and piglet diets respectively with either an acid fungal (*Aspergillus*) or alkaline bacterial (*Bacillus*) protease. In both studies, the acid fungal protease proved effective in improving body weight gain and feed conversion, whereas the bacterial protease resulted in depressed growth and poor feed conversion. For example, Blazek (2008) observed that some proteases are capable of coagulating soy protein and the extent of this reaction is dependent both on the characteristics of the soy protein and the nature of the protease.

Gelation of soy protein, as could occur *in situ* in the gut of poultry and swine, may be one explanation for some of the variable responses that have been observed in the literature. These effects have been previously reported where treatment of soy protein with 3 different proteases resulted in substantial, though transient, gelation of the protein (Hrckova et al., 2002). Interestingly, incubation with the different proteases resulted in different quantities and types of free AA production, with 1 protease producing mainly His (30%), Leu (24%) and Tyr (19%), and another Arg (22%), Leu (11%), and Phe (13%). The importance of these product profiles is not clear, but generation of free AA may interact with feed intake or absorption or both.

Contrary to some observed negative responses to

exogenous protease, there have been several reports where beneficial effects were reported. Mahagna et al. (1995) found positive effects of protease (and amylase) supplementation of sorghum-based diets for broiler chicks, and this was associated with a reduction in chymotrypsin secretion by the pancreas. This apparent feedback mechanism may explain why feed conversion efficiency was improved because synthesis of endogenous protein is energetically expensive for animals. Of interest is that Mahagna et al. (1995) observed no effect of supplemental enzymes on digestibility coefficients, indicating that the mechanisms involved in feed efficiency improvement may be "net." Odetallah et al. (2003) observed improved performance of broiler starters when a corn/soy-based diet was supplemented with a keratinase from *Bacillus licheniformis*. Furthermore, the beneficial effects of protease did not persist to market weight, an observation that was later confirmed (Odetallah et al., 2005). O'Doherty and Forde (1999) found that supplementation of barley/wheat/soy-based diets for swine with a neutral protease resulted in an improvement in feed efficiency. There is potential for protease in the diets of swine and chickens.

### Phytase

Phytase dephosphorylates insoluble phytic acid in grains and oilseeds into orthophosphate and inositol phosphates. In broad terms, phytases are classified as 3- and 6- phytase on the basis of the site on the phytic acid molecule of initial dephosphorylation (McDonald et al., 2010). Phytate is found in most vegetable feed ingredients at concentrations from 5 to 25 g/kg, contributing between 1.4 and 7 g/kg of phytate-P. Phytate is found in virtually all seeds at concentrations from around 5 g/kg up to well over 20 g/kg and, therefore, typical pig, poultry and fish diets will contain between 8 and 12 g of phytic acid/kg (Selle et al., 2003b).

A further contribution to the elucidation of this mechanism is Cowieson et al. (2011) where the effect of phytate, phytase, and Na on ileal endogenous flow was reported. The antinutritive effects of phytate were confirmed as were the ameliorative effects of microbial phytase. However, several interactions were detected between Na (0.15 or 0.25%) such that greater Na concentrations were associated with a reduced antinutritive effect of phytate and a less obvious effect of phytase. As mentioned previously, it has been shown that Na is capable of partially disrupting the detrimental effect phytate has on protein solubility, and consequently greater dietary Na concentrations may reduce the antinutritive effect of phytate and reduce nutrient digestibility response to phytase. These effects were complex and AA specific. Thus, in a phytate-free diet, the associated Ca requirement is only about 0.6%. However, it is also important to consider that not all esters of phytate are

similarly malignant and it is not necessary to reduce all phytate to *myo*-inositol and free phosphate to remove the antinutritive effects.

### CONCLUSION

Exogenous enzymes are added to an animal's food to supplement its own digestive enzymes and to break down anti-nutritive fractions in foods. Current worldwide feed enzyme utilization in diets of swine and poultry is substantially greater than originally anticipated. The non-ruminant feed enzyme market that includes phytases, carbohydrases and proteases has generated a lot of interest in recent years. Appropriate use of exogenous enzymes in feeds requires strategic reductions in dietary energy and nutrient content, as well as careful choice of feed ingredients to capture economic benefits of the various enzymes. The efficacy of enzymes will vary depending on ingredients because nutrient and energy release caused by enzyme supplementation will depend on the structure of the feedstuff itself. It is important to continue the effort to understand the use and limitations of matrix values of enzymes, which, if inappropriately applied, will result in depressed performance because of inadequacy of diets or will lead to wastage of resources. Regardless, the use of exogenous enzymes in diets of non-ruminants continues to be promising for a variety of reasons that hinge on sustainability, economics and the environment. Future research will increase our understanding of feed enzymes and how they can be more beneficially used to further improve the efficiency of non-ruminant animal production.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

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