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Exogenous Polysaccharidases for Young Ruminants: A Review Interfacing Nutrition, Economic and Health

Review Article

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ABSTRACT

Neonate ruminants possess little cell-wall and starch degrading enzyme activity. Importantly, early establishment of fibrolytic, amylolytic, and proteolytic capacities is influential for the early expansion of the reticulorumen epithelia. Such an early development in reticulorumen fermentation will enable a timely hepatic adaptation to volatile fatty acids assimilation. The early nutrient release in the reticulorumen can thus facilitate early weaning, reduce labor costs, save milk, and lessen health issues associated with late weaning. The principal objectives of this review are to delineate roles of exogenous, polysaccharidases (EP) in stimulating fermentation development of the reticulorumen in young ruminants, to discuss the literature on nutrient digestibility and calf performance response to dietary EPs, and to provide insights into future possibilities for using dietary EP for young ruminants. Evidence has been increasing that cow milk may reduce the risk of cancer development and cardiovascular diseases. Therefore, nutritional implications for humans of supplementing calf diets with EP and its potential for milk savings is also discussed. Dietary incentives leading to savings in milk would aid in meeting the rising human demands for well-distributed milk products. Applying EP to both pre- and post-weaning starters would need to be evaluated before EP could be commercially expected for young ruminants on a large scale. Any benefits of nutritional strategies to the animal industry must also consider their implications for human health. As such, dietary use of EP for young ruminants may be considered as an interface of animal nutrition, farm economics, and animal-human health.

KEY WORDS exogenous polysaccharidases, growth, milk, starter, weaning, young ruminant.

INTRODUCTION

Despite their multifaceted mechanisms of action, difficulties in detection and distribution in feed, and variable animal response, exogenous polysaccharidases (EP) have received much interest in ruminant nutrition (Beauchemin *et al*. 2004; Colombatto *et al*. 2003a; Colombatto *et al*. 2003b; Hristov *et al*. 2000; Rode *et al*. 1999; Schingoethe *et al*. 1999; Wallace and Hartnell, 2001).Dietary use of EP has, for instance, improved milk yield (Beauchemin *et al*. 1998) and body weight gain (Beauchemin *et al*. 1995).

No, slight, or moderate inclusion of forage-fiber in preweaning diets has, apparently, not magnetized much consideration into the use of EP for young ruminants. With respect to the reticulorumen development, nonetheless, limited dietary forage does not justify overlooking needs for early and more efficient uses of fiber and non-fiber carbohydrates by young ruminants (Baldwin *et al*. 2004). The early supply of volatile fatty acids (VFA), particularly propionate and butyrate, is crucial for rumen papilla growth and hepatic adaptation to VFA metabolism (Baldwin *et al*.

Abbreviations: ADF, acid detergent fiber; ADG, average daily gain; BW, body weight; ENP, exogenous non-starch polysaccharidases; EP, exogenous polysaccharidases; NDF, neutral detergent fiber; VFA, volatile fatty acids.

2004; Heinrichs and Lesmeister, 2005; Williams and Frost, 1992). The potential exists that EP can hasten these effects. Universally, dairy calves are usually weaned between 5 to 12 weeks of age, depending on growth rate, body size, and milk price. Milk price, however, does not play a major role at the expense of a desirable calf growth. Hence, if EPs can stimulate an earlier use of starter polysaccharides by the young ruminant, they will in turn hasten rumen maturity and reduce weaning age, labor costs, and will avoid milk overfeeding (NRC, 2001). Such an early nutrient use will be most beneficial where commercial weaning occurs late (>5-7 weeks) and consumer demands for dairy products are high. This review provides insights into: (1) potential roles of EP in young ruminants production, (2) the literature on performance and weaning criteria in EP-fed calves, (3) the animal-human health implications of feeding EP to young ruminants, and (4) the research outlooks of feeding young ruminants EPs. This paper does not aim to review the literature on adult cattle with fully-grown reticulorumen (Beauchemin *et al*. 2003; Beauchemin *et al*. 2004).

Transition in gut physiology of the young ruminant: A stimulatory role for EP

The activity of fiber degrading enzymes is negligible in young ruminants (Toullec and Guilloteau, 1989; Van Soest, 1994). The size of the reticulorumen relative to the whole digestive tract rises from 25-35% in the infant calf to 62-80% in the adult ruminant (Van Soest, 1994). Such a transitory enlargement of the reticulorumen depends heavily on adequate fibrous feed intake and microbial inoculation (Anderson *et al*. 1987). Timely dry feed intake is a prerequisite for timely microbial settlement. Volatile fatty acids supplied from microbial fermentation of dry feed enable rumen epithelial cells to proliferate effectively (Sander *et al*. 1959). Consequently, rumen papilla growth will accelerate. The early extension of the reticuloruminal VFA release is, in addition, mandatory for the timely adaptation in hepatic VFA metabolism (Baldwin *et al*. 2004; Williams and Frost, 1992). As such, any strategy that can trigger substrate release from fibrous dry feed could contribute to a timely accommodation of microbial population. As a result, a potential would be offered for the neaonate rumen with limited fibrolytic capacity (Williams and Frost, 1992) to develop into a capacious fermentor in an early and sizable manner. Early reticulorumen development will enable the early weaning. Early transition from liquid to dry feed will in turn reduce feed and labor costs but improve the calf health (NRC, 2001; Ghorbani *et al*. 2007b). Also, the same opportunity would exist for non-fibrous potion of dry feed. The mixtures of fiber- and non-fiber degrading EP are expected to have cumulative positive impacts on ruminal nutrient digestibility. Altogether, the EPs could thus be an appropriate candidate for hastening the above cascades and improving the weaning economics.

It has been a concern that the EP's action may be weakened by proteolytic microbes already existing in the reticulorumen (Hristov *et al*. 1998; Beauchemin *et al*. 2004). However, prefeeding enzyme application appears to overcome this concern (Beauchemin *et al*. 2003). The solution is thought to be partly due to the formation of stable enzyme-substrate bindings which increase EP resistance against rumen proteolysis. Also, such enzyme-substrate bindings may stimulate bacterial colonization on feed particles in the rumen (Beauchemin *et al*. 2003). In the neonate ruminant, the reticulorumen initially does not possess a major proteolytic activity (Terosky *et al*. 1997; Van Soest, 1994). Thus, the EP application to calf starter feed is not expected to encounter such an inhibitory effect of rumen proteolysis on the EP's action, which is more likely to occur in adult ruminants. This may ease establishing the EP's activities of interest in the reticulorumen. Consequently, the rumen accommodation of microbial population (e.g. fibrolytics) will occur earlier. Early weaning is undoubtedly a determining factor in reducing the health issues and calf raising costs (NRC, 2001).

Literature dietary use of EP for young calves

Documented research on the dietary use of EP for young ruminants is not vast. This may suggest that the EP have not been considered in relation to earlier rumen development and earlier weaning. Also, the recent use of enzyme additives in diets for lactating and growing ruminants has mainly focused on the ruminant industries with earlier weaning ages (e.g. North American and Western European countries; Beauchemin *et al*. 1998; Beauchemin *et al*. 2003; Beauchemin *et al*. 2004; Morgavi *et al*. 2000; Rode *et al*. 1999; Schingoethe *et al*. 1999). Such inter-continent differences in feeding exogenous enzymes would reflect differences in government legislations, animal productivity level, and diet types. For instance, human health concerns as to the use of antibiotic products in diets for livestock have stimulated research on EPs (European Union, 2003; Sheppy, 2001). This has further advanced enzyme technology. In many dairy industries around the world, however, the commercial weaning occurs much later than 5-7 weeks of age. The late weaning increases the likelihood of health issues in young calves (NRC, 2001). Thus, when calves are weaned late, there should be a greater potential for the EP to enhance the nutrient availability in the reticulorumen. Recently, Ghorbani *et al*. (2007a) suggested that the addition of EP to the starter concentrate increased total tract NDF and ADF digestibility in 28-d-old dairy calves. These researchers used an enzyme additive with high endo-cellulase, exo-cellulase, and xylanase activities. However, starter intake and growth rate were not significantly affected by the EP (Ghorbani *et al*. 2007a).

Growth rate was additionally estimated via regressing calf body weight against time (Morris, 1999), which again did not show any inter-treatment differences. As a result, weaning criterion or age at a daily consumption of 680 g starter concentrate was comparable among treatments (Ghorbani *et al*. 2007a). In that study, two fibrolytic enzyme additives were used, but only the one with greater xylanase activity enhanced NDF digestibility (Table 1). The enhancing impact of EP on apparent total tract NDF digestibility was not observed at week-8 of age (Table 2).

Table 1 Effects of exogenous, non-starch polysaccharidases (ENP) on apparent total tract nutrient digestibility and calf performance at d-28 (see Ghorbani *et al*. 2007a)

	Treatment[1] (T)				P-value		
Item	C	EA	EB	SEM	C vs. EA	C vs. EB	EA vs. EB
Birth body weight (kg)	47.70	48.50	47.90	1.10	0.62	0.89	0.74
ADG (g/d)	97.90	77.70	122.50	29.80	0.61	0.55	0.30
Starter intake (g/d)	376.00	346.00	375.00	20.30	0.31	0.93	0.37
ADG/starter intake	0.26	0.19	0.33	0.07	0.49	0.57	0.24
DM digestibility	73.40	75.00	75.20	1.40	0.39	0.37	0.93
NDF digestibility	49.00	57.10	47.20	2.60	0.04	0.61	0.02
ADF digestibility	48.60	58.30	52.80	2.40	0.01	0.22	0.12
Ash digestibility	57.50	57.40	64.20	3.80	0.98	0.26	0.27
BW at d-28, kg	50.70	50.10	51.40	0.80	0.61	0.55	0.29

[1] C= control starter (no enzyme), EA= enzyme A-supplemented starter with respective activity of 1437, 788, and 7476 µmol/mL/min for exo-cellulase, end-ocellulase, and xylanase. EB= enzyme B-supplemented starter with respective activity of 1446, 1350, and 5091 µmol/mL/min for exo-cellulase, endo-cellulase, and xylanase.
SEM= standard error of least square means.
ADF, acid detergent fiber; ADG, average daily gain; BW, body weight; DM, Dry matter.

Table 2 Effects of exogenous, non-starch polysaccharidases (ENP) on apparent total tract nutrient digestibility and calf performance at d-56 (see Ghorbani *et al*. 2007a)

	Treatment[1] (T)				P-value		
Item	C	EA	EB	SEM	C vs. EA	C vs. EB	EA vs. EB
Birth body weight (kg)	67.60	67.50	70.30	1.60	0.98	0.30	0.29
ADG (g/d)	606.00	620.20	674.70	39.80	0.80	0.27	0.38
Starter intake (g/d)	1108.00	1097.00	1135.00	28.60	0.79	0.55	0.40
ADG/starter intake	0.54	0.56	0.59	0.030	0.68	0.33	0.54
DM digestibility	85.10	83.60	82.10	1.00	0.33	0.07	0.34
NDF digestibility	73.70	69.60	67.50	2.40	0.21	0.08	0.53
ADF digestibility	69.40	62.40	61.60	2.30	0.06	0.05	0.83
Ash digestibility	63.70	66.70	59.60	3.20	0.51	0.40	0.15

[1] C= control starter (no enzyme), EA= enzyme A-supplemented starter with respective activity of 1437, 788, and 7476 µmol/mL/min for exo-cellulase, end-ocellulase, and xylanase. EB= enzyme B-supplemented starter with respective activity of 1446, 1350, and 5091 µmol/mL/min for exo-cellulase, endo-cellulase, and xylanase.
ADF, acid detergent fiber; ADG, average daily gain; BW, body weight; DM, Dry matter; NDF, neutral detergent fiber;.

Naserian et al. (2005) reported a reduction in dry matter intake of post-weaning calves fed on an enzyme supplemented growing diet. The enzyme additive used by Naserian *et al*. (2005) was a mixture of cellulase, β-glucanase, α-amylase, protease, pectinase, and phytase; whereas, the enzyme additives used in the study of

Ghorbani *et al*. (2007a) contained only cell-wall carbohydrases. Also, dissimilar to Ghorbani *et al*. (2007a) who applied EP to pre-weaning starter diet from birth to 56 d of age, Naserian *et al*. (2005) applied the EP to only the post-weaning diet and monitored calf performance from 60 to 120 d of age. The differences in the enzyme type, calf age, and duration of enzyme application may explain the different inter-study results. Such dissimilar responses to EP would be mediated mainly by different physiological characteristics of the gastrointestinal tract in young calves of different ages (Huber *et al*. 1984).

Naserian *et al*. (2005) also found no effects of exogenous enzymes on post-weaning ADG of calves. The comparable inter-treatment ADG response implies that enzyme specificity and duration of EP application were probably not adequately matched to alter the pre-weaning calf growth. The early increase in fiber digestibility by EP (Ghorbani *et al*. 2007a) may be due to the more transitory state of the reticulorumen growth at wk-4, leading to a sharper reaction to EP, when compared with wk-8. The EP may interact with endogenous rumen enzymes in enhancing or depressing microbial adhesion, colonization, and thus substrate use. For instance, introducing EP into the rumen of sheep as compared to its prefeeding addition to barley silage reduced DM and NDF digestibility (McAllister *et al.* 1999). Depressed fiber digestibility may suggest a contribution from the competitive mcrobial interactions in the rumen. Digestibility measurement techniques may also affect nutrient digestibility results. Ghorbani *et al*. (2007a) took spot fecal samples and used acid insoluble ash as an internal marker to measure nutrient digestibility (Van Keulen and Young, 1977). Spot sampling does not realistically represent the continuous flow of excreta along the gastrointestinal tract. Hence, it is suggested that future studies use other techniques preferably total fecal collection, if it is experimentally feasible.

Health perspectives

Hastening the establishment of mixed microbial populations in the neonate reticulorumen via nutritional strategies will make the early weaning possible (Huber *et al*. 1984). If smooth enough, the early weaning would suggest a great opportunity to save milk. Milk, the most nutritious natural liquid, contains a priceless collection of bioactive substances. Such substances include, but are not limited to, specialized peptides, folic acid, vitamins B_6 and B_{12}, calcium, and polyunsaturated fatty acids (e.g. omega-3 ones) (Cross and Gill, 2000; Parodi, 1997). These nutrients have been reported to contribute to reduced risk of hyperhomocysteinemia, hypertension, atherosclerosis, and tumorigenesis (Cross and Gill, 2000; Ma *et al*. 2001; Pfeuffer and Schrezenmeir, 2000; Tsuda *et al*. 2000). Notably, hyperhomocysteinemia has recently been underlined as a risk factor for cardiovascular diseases. Also, high milk intake has been associated with a decreased risk of ischemic heart diseases (Elwood *et al*. 2004). Evidence has hence been accumulating that milk must not be regarded unfavorable to human health simply because of its cholesterol and saturated fatty acids (Nikkhah, 2011a; Nikkhah, 2011b; Pfeuffer and Schrezenmeir, 2000). Importantly, moreover, a majority of milksaturated fats belongs to short chain fatty acids and stearic acid (C18:1), which have not been shown to trigger the risk factors for heart diseases. Thus, alongside other stimulants, dietary uses of EP help to prevent milk overfeeding to young calves already possessing a well-established reticulorumen. Such an aid would become a viable practice if dietary EP can accelerate the ruminal founding of amylolytic and fibrolytic capacities.

As such, the EP would facilitate early weaning, reduce labor costs, and lead to a saving in milk consumption by the young ruminant. This would imply benefits to animal and human health. In many parts of the world, young calves are weaned about 10-12 weeks of age, while weaning can easily occur as early as 5-7 weeks of age (Ghorbani *et al*. 2005; Ghorbani *et al*. 2007a; Ghorbani *et al*. 2007b). However, among the major challenges is to establish optimum amounts, concentrations, combinations, and duration of using different EP, e.g. fibrolytic and amylolytic preparations. Adding higher than certain levels of cellulase-xylanase has not stimulated gas production rate in vitro (Colombatto *et al*. 2003a).

Importantly, for human health considerations of applying EP to young ruminants diets, complementary nutritional strategies may need to be undertaken. Ghorbani *et al*. (2007b) showed that partial replacement of whole milk with soymilk, which is a cheap liquid with no anti-nutritive impact, can remarkably stimulate the early calf starter intake, thus enabling early weaning, saving more milk, and reducing feed costs. Dietary use of EP along with such feeding strategies could expand EPs' roles in hastening rumen development and promoting early weaning. The resultant savings in milk would support the hypothesis that EPs are interfaces of the young ruminant nutrition, farm economics, and animal-human health.

Research outlooks

Given the limited data on the impact of EP on young calf performance, general suggestions should stimulate the research in a neglected field of study. Applying different types and combinations of EP preparations (amylolytic, fibrolytic, and their mixture) to the calf starter warrants the investigation. Exogenous enzymes possess different modes of action which, although, not fully explored, are known as "feed specificity" (Colombatto *et al*. 2003b). Thus, the opportunity exists that given mixtures of enzymes would lead to an additive impact of EP and rumen enzymes on nutrient availability (Morgavi *et al*. 2000). The daily intake of dry feed is limited during the earlier stages of growth which increases progressively afterwards (e.g. 1% of BW at 3-wk to 3% of BW at 8-wk of age; Davis and Drackley, 1998). For this reason, dietary use of EP is suggested to be considered with other strategies which stimulate starter intake. This could result in a synergistic effect, whereby, the EP would further improve starter nutrient use by rumen microbes. In addition, the duration of the EP application could have a significant influence on the consistency of calf response. Such a response would be more evident in tissue growth which is a continual lasting process. Enzymes with extended courses of action would be needed for such a prolonged growth process. Daily offers of milk and milk replacers decrease following 4 wk of age, and as such, starter intake increases progressively (Davis and Drackley, 1998). From weaning by the time a BW of about 100 kg is achieved, the young ruminant undergoes a critical phase in splanchnic and peripheral tissue growth (NRC, 2001). Thus, continuing EP application to the diet following weaning could thus facilitate overcoming the weaning stress. Such a benefit would stem from a more extensive use of dietary carbohydrates (Colombatto *et al*. 2003a) by the young ruminant. Improved post-weaning nutrient use, milk savings and labor efficiency by early weaning will reduce calf rising costs. The drop in costs would point to the significance of EP as an interface of calf nutrition and farm economics. Stimulated starter intake and early weaning certainly reduce calf raising costs (Ghorbani *et al*. 2007b; NRC, 2001).

Despite these opportunities, the enzyme research has to fulfill several essential requirements before it could yield commercial benefits to the young ruminant industry. Accurate comparisons are to be made among different enzyme preparations. Such information is critical for manufacturers and ruminant producers. Determining the specific site of action and the effective enzyme dose required at a given site would be complementary (Marquardt and Bedford, 2001). Modeling would be an appropriate tool to accurately predict nutrient availability and animal response in the gastrointestinal tract to the use of a specific enzyme additive. However, the present knowledge of the underlying mechanisms of the EP's action must be sufficiently broadened before modeling can generate applicable and meaningful outcomes. Such mechanisms of actions include substrate-specificity, exogenous and endogenous enzymes interactions, enzyme level effects, animal productivity, and the method and time of enzyme provision relative to feeding. Modeling can thus in turn lead to further broadenings of such knowledge.

CONCLUSION

Data on effects of dietary application of exogenous polysaccharidases (EP) for young ruminants are limited. The existing data suggest beneficial but variable impacts on pre-weaning nutrient digestibility. It would be of interest to learn if EPs can hasten the establishment of a capacious mixed microbial fermentation in the rumen. This would enable an earlier transition of the neonate reticulorumen into a functioning fermentor. If so, the EP would enable earlier weaning and reduce labor costs and health issues. The earlier weaning will prevent overfeeding milk to the young ruminant capable to consume adequate dry feed. Savings in milk would consequently help meeting the rising demands for milk products. Evidence has been accumulating that the cow milk possesses bioactive substances which may reduce risks of tumorgenesis and cardiovascular complexities. Future studies may consider applying a mixture of cell-wall and cell-content EP to both pre- and post-weaning calf diets. Such aspects would need to be evaluated for feeding young ruminants before EP can vastly prove to improve animal nutrition, farm economics, and animal-human health.

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