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Short communication

Effects of a starch binding agent on in vitro rumen degradability of maize and sorghum starch

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Abstract

The objective of the study was to quantify the potential of a starch binding agent (BioProtect™) to reduce in vitro rumen starch degradation of maize and sorghum particles that varied in size. Maize and sorghum grain samples were ground through 2-mm sieves with a Wiley mill and subsequently sieved to obtain these sizes: less than 250, 250 - 500, 500 - 1180, and 1180 - 2000 µm (i.e., very fine to coarse). All fractions were analysed separately for starch content. Samples were treated 24 hours before fermentation by spraying BioProtect onto the substrate. Both treated and untreated samples were fermented in vitro for 0, 6, 12 and 24 hours to quantify starch degradability. Rates of degradability (k_d) were calculated with a first-order decay model. BioProtect was effective in decreasing starch degradability and rates of degradability for both grains (P <0.0001). The product was more effective with smaller particle size, by reducing starch degradability 17% for the smallest particles as opposed to 7% for the largest particles. A time interaction was observed (P <0.0001), which showed that the highest impact of BioProtect occurred after 12 hours of fermentation for both grains. The starch binding agent resulted in an effective decrease of in vitro starch degradation, but results were affected by particle size and fermentation time. Starch digestion could possibly be shifted to the small intestine with BioProtect.

Keywords: BioProtect, rates of degradation, starch digestibility
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Although the optimal site of starch digestion is debatable (Reynolds, 2006; Owens *et al.*, 2016), strategies that partially shift starch digestion and absorption post ruminally continue to evolve with the aim of increasing the efficiency of nutrient utilization, hence improving the performance of dairy cows. This is because excessive ruminal fermentability, may cause a severe drop in ruminal pH, resulting in ruminal acidosis, decreased fibre digestibility and lower efficiency of microbial protein production (Firkins *et al.*, 2001). It also results in reduced feed intake (Allen, 2000), altered rumen biohydrogenation (Bauman & Griinari, 2001), and ultimately decreased milk production. Starch that flows to the small intestine can be digested enzymatically, resulting in the production of glucose, fermented in the hindgut to volatile fatty acids (VFA) or excreted. Shifting the site of starch digestion to the small intestine could overcome problems encountered with excessive ruminal fermentability. Moreover, other potential benefits include higher energy efficiency (30% to 42% higher) (Owens *et al.*, 1986), leading to higher metabolizable energy utilization, increased supply of glucogenic substrates, and increased milk protein production. This releases amino acids from being used for gluconeogenesis in the liver (Huhtanen & Sveinbjörnsson, 2006).

Traditionally, the processing of maize and sorghum focused on increasing rumen digestibility to meet the energy demands of high-producing dairy cows to optimize milk income over feed costs and efficiency. However, during certain physiological stages, decreasing rumen digestion and shifting the site of digestion may be useful for these reasons. There have been several studies on the potential of shifting the site of starch digestion to the small intestine by using various starch sources or by processing or chemical treatments (e.g. sodium hydroxide (NaOH) and formaldehyde (HCHO)). However, strategies aimed at

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shifting the site of starch digestion from the rumen to the small intestine will be successful only if it is digested extensively there (Bird *et al.*, 1999).

Recently, there has been interest in a product named BioProtect (Realistic Agri, Ironbridge, UK), a starch binding agent. The active ingredient in BioProtect is a stable non-volatile organic salt that complexes with the hydroxyl groups of starch at neutral or slightly acidic conditions (pH 6 to 7), as observed in the rumen. These complexes decompose under more acidic (pH 2 to 3) conditions, as in the abomasum and duodenum, making the starch available for enzymatic digestion (Dunshea *et al.*, 2012). BioProtect has been claimed to decrease the rate of rumen fermentation of highly digestible starch and to increase the rate of starch utilization post ruminally (Dunshea *et al.*, 2012, 2013; Gonzalez *et al.*, 2014). However, Gonzalez *et al.* (2014) recommended further in vitro and in vivo studies to confirm these claims. More recently, Van Zyl (2017) reported a positive effect of BioProtect on in vitro starch disappearance of coarsely ground (4-mm screen) low vitreous maize, but none on finely ground (1-mm) low vitreous maize. In the in vivo trial, Van Zyl (2017) reported that BioProtect decreased total tract starch digestibility of maize, contrary to Gonzalez *et al.* (2014), who recorded that BioProtect did not reduce whole tract starch digestibility of wheat in sheep. Although these authors used different starch sources, the effectiveness of BioProtect is still not clear. Moreover, these studies focused on processing grains simply by grinding them through various sieves and therefore reducing particle size, but also obtaining a variable distribution of particles, which could affect starch digestibility (Firkins *et al.*, 2001). Within grain type, various fragment sizes (sieve fractions) possess different surface area characteristics, which affect the magnitude of starch digestion (Al-Rabadi *et al.*, 2012). The potential of BioProtect on maize and sorghum of different particle sizes needs to be investigated. Therefore, the objective of this study was to quantify the potential of a starch-binding agent (BioProtect) to reduce in vitro rumen starch degradation of maize and sorghum that varied in particle size.

Maize and sorghum grains were ground through a 2-mm sieve using a Wiley mill and subsequently sieved to obtain these particle sizes: <250, 250 - 500, 500 - 1180, and 1180 - 2000 µm (i.e., from very fine to coarse). All fractions were analysed separately for starch (Hall, 2009). Samples were treated 24 hours before fermentation by spraying them with BioProtect according to the product guidelines (8 L/tonne of cereal grain). Treated and untreated maize and sorghum samples were fermented in vitro (Goering & Van Soest, 1970) for 0, 6, 12, and 24 hours to quantify starch degradability. Rumen fluid was collected from two ruminally cannulated Holstein dairy cows before morning feeding and mixed in pre-warmed insulated thermos flasks at Welgevallen Experimental Farm of Stellenbosch University. Cows were fed a total mixed ration consisting of roughage (40%) and a concentrate mixture (60%). Rumen fluid was filtered through two layers of cheesecloth, glass wool, and a double layer of 200 µm filter paper before injecting 10 ml into a 125-mL Erlenmeyer flask with 0.25 g of substrate and 40 ml of Van Soest buffer for incubation at 39.5 °C under CO_2 (Goering & Van Soest.,1970). Blank flasks without substrate were used to correct for possible starch from the rumen fluid. The residual starch was determined using the entire incubated content of samples in flasks (that is, residue plus medium) and therefore soluble starch was included in the resulting starch content. Three separate runs were conducted for each cereal in duplicates of each sample for each incubation time.

Rates of digestion (k_d) were computed using a first-order decay model according to this equation:

$$S_{(t)} = S_{(0)}\, e^{-kd(t-L)} + uS;$$

where: $S_{(t)}$ is residual starch at time t,
$S_{(0)}$ is starch at time 0 of the soluble and insoluble starch,
k_d is the fractional rate of starch digestion,
L is the lag, and
uS is the estimated indigestible starch.

Simultaneous estimates of the parameters were obtained using PROC NLIN (version 9.3, SAS Institute, Inc., Cary, North Carolina, USA) and the Marquardt algorithm. Resulting parameters and starch degradability were analysed according to a randomized complete block design with a factorial arrangement of particle size, cereal, BioProtect treatment, time (for starch degradability only) and interactions by the GLIMMIX procedure. The main effects were grain, particle size, product, time (for starch degradability only) and their interactions. Fermentation run (n = 3) was considered a random effect. Differences between means were declared significant at $P \leq 0.05$ using the LSMEANS and the Tukey adjustment.

Starch degradability and rate of starch degradation (K_d) increased linearly with decreased particle size ($P < 0.0001$) for both types of grain. Starch degradability and K_d, when time points were pooled, increased from 41.75% and 58.20% and from 10.80% to 26.40 %/h, respectively, for maize. The correspondent values for sorghum increased similarly from 30.45 %to 53.78% for starch degradability and from 11.20% to 24.40 %/h for K_d (Table 1). In agreement with previous studies, starch degradation was inversely related to particle

size (Ferraretto *et al.*, 2013). Decreasing the particle size increased the surface area available for bacteria attachment or enzymatic digestion (Huntington, 1997; Ferraretto *et al.*, 2013; Gallo *et al.*, 2016), resulting in an increased rate and extent of starch degradation (Nocek & Tamminga, 1991). In addition to the surface area effects, starch in coarse or large particles is harboured in granules in the endosperm, still protected by the pericarp, which is the foremost barrier, limiting microbial accessibility to starch granules (Huntington, 1997), hence reducing starch degradability.

Overall, BioProtect was effective (P <0.0001) in decreasing starch degrading for maize and sorghum grains (Table 2). When time and size were pooled, the extent of starch degradation decreased from 55.65% to 45.75% in maize, and from 48.44% to 37.47% in sorghum. When particle size was pooled, treatment decreased K_d from 25.21% to 14.40%/h and from 18.80% to 13.50 %/h for maize and sorghum, respectively. Interestingly, Van Zyl (2017) reported that BioProtect was not effective in decreasing in vitro starch fermentation when maize was milled through a 1-mm screen, irrespective of vitreousness, but was effective on low vitreous maize milled with a 4-mm screen. In the same study, BioProtect decreased the K_d of 4-mm ground low vitreous maize by 4 percentage units.

Table 1 Effects of particle size on the rate and extent of degradability of maize and sorghum starch

		Particle size					
		Very fine	Fine	Medium	Coarse	SE	*P*-value
Maize	Starch degradability, %	58.20[a]	53.55[b]	49.29[c]	41.75[d]	0.045	<0.0001
	$K_{d,}$ %/h	26.40[a]	23.00[b]	19.00[c]	10.80[d]	0.021	<0.0001
Sorghum	Starch degradability, %	53.78[a]	47.08[b]	40.52[c]	30.45[d]	0.046	<0.0001
	$K_{d,}$ %/h	24.40[a]	14.10[b]	14.80[b]	11.20[c]	0.041	<0.0001

[a-d] Within a row, means with a common superscript were not different at P =0.05; K_d: rate of degradation

The variability in response in these studies could be explained by differences in techniques, methodology, grains, degree of processing of grains, and the amount and process of BioProtect incorporation. BioProtect decreased the rate of ruminal starch fermentation of wheat in a dose-dependent manner (0, 4, 8, and 16 ml/kg), with response maximized at 8 ml/kg, hence the product guideline of application rate of 8 L per tonne (Dunshea *et al.,* 2013).

Table 2 Effects of a starch binding agent on the rate and degradability of maize and sorghum starch

		Untreated	BioProtect -treated	SEM	*P*-value
Maize	Starch degradability, %	55.65[a]	45.75[b]	0.044	<0.0001
	K_d, %/h	25.20[a]	14.40[b]	0.021	<0.0001
Sorghum	Starch degradability, %	48.44[a]	37.47[b]	0.046	<0.0001
	K_d, %/h	18.80[a]	13.50[b]	0.041	<0.0001

[a-b] Within a row, means with a common superscript were not different at P =0.05; K_d: rate of degradation

The effects of BioProtect on maize and sorghum starch degradation of varying particle sizes are presented in Figure 1. When the results were compared, the product was more effective with smaller particle size, reducing starch degradability by 17 percentage units for the smallest particles as opposed to 7 percentage units for the largest particles. This result implied that the binding agent was more efficient with finer particles where starch was highly degradable, and with a disrupted protein matrix and disorganized starch granules. It could also be due to the accessibility of starch and its interaction with the product (BioProtect). Starch granules in particles larger than 500 µm will predominantly be within intact cell walls, whereas particles smaller than 250 µm would be expected to have many broken cells with exposed intra-cellular content (Al-Rabadi *et al.,* 2009). Besides, BioProtect had more access to starch in finer particles,

possibly because of the surface area available for reaction between BioProtect and starch, making starch more exposed for BioProtect contact. The product was therefore able to bind more efficiently to starch in finer particles inhibiting its fermentation.

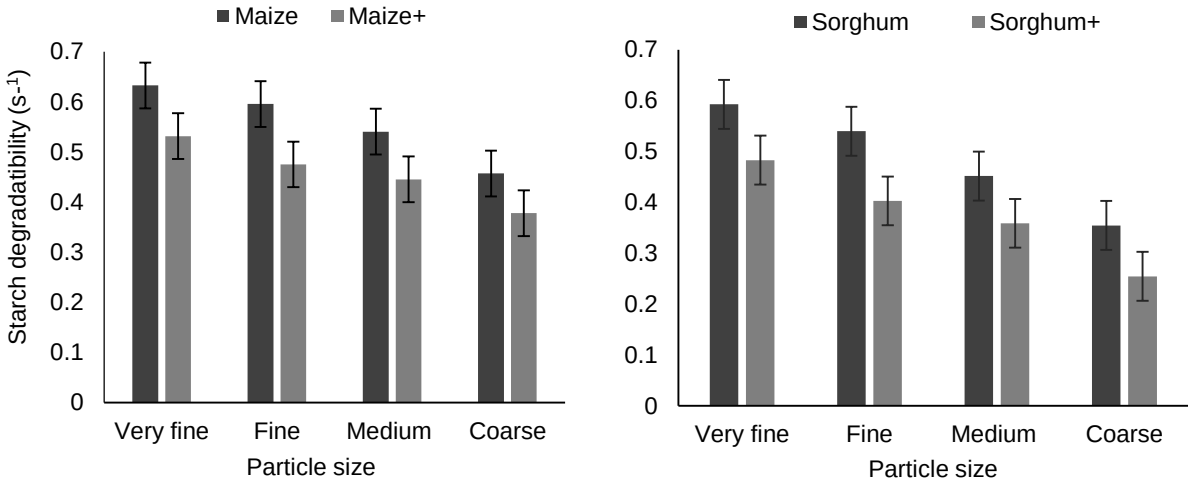

Figure 1 Effect of a starch binding agent on maize and sorghum starch degradation of varying particle sizes

Overall, untreated grains showed higher degradability throughout the incubation period with most of the starch having disappeared by 24 hours of incubation (Figure 2).

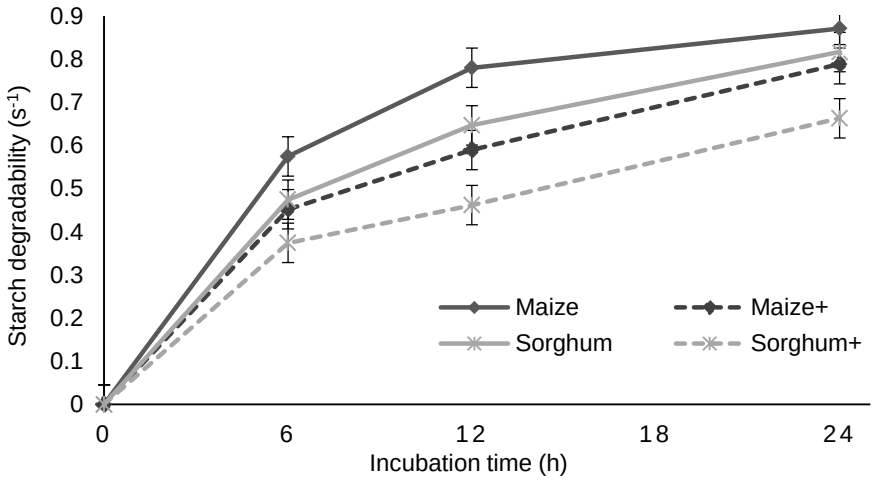

Figure 2 Effect of a starch binding agent on maize and sorghum starch degradation at 0, 6, 12 and 24 hours of incubation

A time interaction was observed ($P < 0.0001$), showing that the highest impact of BioProtect occurred after 12 hours of fermentation for both grains, with 19 and 18 percentage unit decreases in starch degradability of BioProtect-treated maize and sorghum, respectively. However, in ruminants, the extent to which starch is fermented in the rumen is dependent on the retention time of the feed in the rumen, and that is more important when considering the proportion of ruminal starch digestion. A reasonable mean retention time of concentrate in the rumen of lactating dairy cows is seven hours (Allen & Piantoni, 2014; Gallo *et al.*, 2016). After seven hours of rumen retention, starch that is not fermented in the rumen flows to the small intestine, where enzymatic starch digestion occurs. This unfermented starch is not considered for the ruminal starch pool. In this study, the starch degradability for untreated grains reached 57% and 47% after 6 hours of incubation in maize and sorghum, which was reduced to 45% and 37% in BioProtect-treated maize and sorghum, respectively. At six hours, BioProtect decreased starch degradability by 12% and 10%-units in maize and sorghum, respectively. Thus, more starch could possibly be shifted to the small intestines with

BioProtect treatment. In conclusion, BioProtect, a starch binding agent, reduced in vitro rumen starch degradation of maize and sorghum grains, giving an opportunity to increase the amount of starch shifted to the small intestine, but results would be affected by particle size and fermentation time.

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Authors' Contributions

All authors participated in the planning of the experiments, data analysis and reviewed the manuscript. MNT was responsible for the in vitro laboratory analysis and wrote the manuscript.

Conflict of Interest Declaration

Authors declare that there is no conflict of interest for this work.

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