SEGES INNOVATION

MEDDELELSE

No. 1248

Published 4 March 2022

EFFECT OF ROVABIO EXCEL AND ROVABIO ADVANCE ON EDOMi

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Brief

The addition of two enzyme products increased in vitro ileal digestibility of organic matter by 0.6-0.9% and this increased measured energy level by 0.7-1.0%. The effect was dose dependent and tended to be higher for Rovabio Advance compared to Rovabio Excel.

Summary

Two experiments were conducted to investigate the effect of two different enzyme products from Adisseo supplemented at three levels (no addition = control, standard dose, and 2 x standard dose = double dose) on the in vitro ileal digestibility of organic matter using the EDOMi method (EDOMi = enzyme digestible organic matter at ileal level). The EDOMi method is used in the Danish energy evaluation system for pig feed.

Supplementation of Rovabio Excel or Rovabio Advance, both at standard and double doses, increased EDOMi significantly compared to control. The effect of the standard dose was about 77% of the effect of the double dose for both enzyme products.

There was tendency ($P<0.07$) for a higher effect of Rovabio Advance compared to Rovabio Excel.

The effect measured on EDOMi would improve “controllable” Danish feed units for growing pigs in the interval 0.65-1.01% depending on enzyme product and dose.

Introduction

SEGES apply two in vitro analyses to simulate the potential digestibility of organic matter at the ileal level (EDOMi) and at the fecal level (EDOMf). Previous experiments (not published) showed a positive effect of xylanases on EDOMi, but not on EDOMf, as the EDOMf analysis includes fiber degrading enzymes.

The Danish energy evaluation system includes both EDOMi and EDOMf. An increase in the EDOMi will increase the energy value of the tested feed ingredient or complete diet – also if EDOMf is unchanged.

The measured increase in EDOMi is the “controllable” effect of the enzymes. In comparison, the effect measured in vivo could differ due to variations in milling, holding time and pH. A previous experiment comparing the effect of increasing pH from pH 2 (standard procedure) to pH 3 in step 1 of the EDOMi analysis simulating the “stomach” (for details see Appendix 2) revealed that the effect of supplemented xylanase from DSM and Danisco/Dupont on EDOMi was lower at pH 2 compared to pH 3, in particular for the xylanase from DSM [1]. The same experiment showed a negative effect of added phytase on the effect of xylanase on EDOMi using pH 3 (step 1 simulating the stomach), while phytase did not influence the effect of xylanase using pH 2. Phytase did not affect EDOMi in feed without xylanase.

In the following, the effect of two different NSP enzyme products (Rovabio Excel and Rovabio Advance) on EDOMi was investigated using the standard set up of the in vitro measurements at pH 2.0 (step 1) used in Danish energy evaluation of pig feed.

Materials and methods

Two experiments were conducted with two different enzyme products from Adisseo supplemented at three levels (no addition = control, standard dose, and 2 x standard dose = double dose) to test the effect on the in vitro ileal digestibility of organic matter using the EDOMi method (EDOMi = enzyme digestible organic matter at the ileal level).

Each experiment comprised three treatments and 20 replicates/treatment (Table 1). This corresponds to EDOMi analyses of 20 samples per treatment, in total 60 EDOMi analyses per product. Each experiment of each product was designed to identify a difference between treatments of 0.5% unit with a standard deviation of 0.5. One replicate / “one” analysis is in fact a double analysis of EDOMi, which is the standard analytical procedure normally used to measure EDOMi, ie. 60 replicates per product include 120 single EDOMi analyses.

In experiment 1, control (no enzyme) was compared with two doses of the commercial product Rovabio Excel, approved by EFSA for its content of betaglucanase and xylanase.

In experiment 2, control (no enzyme) was compared with two doses of Rovabio Advance.

Both enzyme products were tested in doses of 100 mL and 200 mL per ton of feed.

The feed represented a regular diet for Danish growing-finishing pigs, with a slightly higher inclusion of fibrous ingredients than average, but still within the normal interval of Danish feed for growing-finishing pigs. Details about feed composition are provided in Appendix 1. The feed was pelleted and with no added phytase to eliminate the risk of interference between phytase and the tested enzymes.

Feed from the same production was used for both experiments. A sample of one kg of feed was split into 4 batches of 250 gram. Each batch was milled separately, and one batch was used for one experimental day. The effect of batch represents the effect of a subsample at a specific experimental day.

Appendix 2 provides details of the in vitro methods, including levels and concentrations of the enzyme product added.

Table 1. Number of samples in the EDOMi analysis.

Experiment 1, Rovabio Excel Multi-NSP			
Dose	Control: 0 mL/ton	Standard: 100 mL/ton	Double: 200 mL/ton
Batch 1	10	10	10
Batch 2	10	10	10
Experiment 2, Rovabio Advance			
Dose	Control: 0 mL/ton	Standard: 100 mL/ton	Double: 200 mL/ton
Batch 3	10	10	10
Batch 4	10	10	10

Results

Table 2. Effect of two enzyme products in standard and doubled dose on EDOMi, LS-means.

EDOMi, Experiment 1, Rovabio Excel			
Dose	Control: 0 mL/ton	Standard: 100 mL/ton	Double: 200 mL/ton
Batch 1, %	79.07	79.63	79.96
Batch 2, %	79.37	79.94	80.00
Average, %	79.22	79.79	79.98
Difference from control, %-units	0	0.57	0.76
Relative to double dose	0	0.75	1.00
EDOMi, Experiment 2, Rovabio Advance			
Dose	Control: 0 mL/ton	Standard: 100 mL/ton	Double: 200 mL/ton
Batch 3, %	79.50	80.18	80.46
Batch 4, %	79.67	80.39	80.50
Average, %	79.59	80.29	80.48
Difference from control, %-units	0	0.70	0.89
Relative to double dose	0	0.79	1.00

In this balanced design within each experiment, the “raw” mean and LS-means were identical. Results showed effect of both enzyme products and an increased effect when the dose was increased. Rovabio Advance was not significantly different from Rovabio Excel, but it may tend to have a better effect than Rovabio Excel at the same dose.

Statistical analyses

Experiment 1: Rovabio Excel

1. The increment of 0.57% unit by a standard dose was statistically different from control (P< 0.0001)
2. The double dose was also statistically different from the control (P<0.0001)
3. The difference (0.195% unit) between standard dose and double dose was not statistically significant (P=0.10).

Experiment 2: Rovabio Advance

1. The increment of 0.70% unit by a standard dose was statistically different from control (P< 0.0001)

2. The double dose was also statistically different from the control (P<0.0001)
3. The difference (0.195% unit) between standard dose and double dose was not statistically significant (P=0.07).

The statistical analyses of both experiments analyzed together revealed no significant difference between the two products, but there may be a tendency (P< 0.07) for a better effect of Rovabio Advance compared to Rovabio Excel. The response curve was largely the same and there was no statistical interaction between enzyme product and dose.

For both enzymes, the effect of the standard dose on EDOMi is about 77% of the double dose (75% and 79% in Table 2).

Effect of the enzyme products on the energy content of the feed

Average values from four analyses of the 1 kg sample of feed for water, crude protein, crude ash and EDOMf (enzyme digestible organic matter at the fecal level) were applied to calculate the effect of the enzyme products on analyzed energy.

The analyses for calculation of energy in Danish feed units for growing pigs are shown in Table 3.

Table 3. Analyses and analyzed energy content in feed.

	Rovabio Excel			Rovabio Advance		
Batch	1+2	1+2	1+2	3+4	3+4	3+4
Enzyme dose, mL/ton	0	100	200	0	100	200
Dry matter, %	87.6	87.6	87.6	87.6	87.6	87.6
Crude protein, %	14.7	14.7	14.7	14.7	14.7	14.7
Crude fat, %	3.7	3.7	3.7	3.7	3.7	3.7
Crude ash, %	4.3	4.3	4.3	4.3	4.3	4.3
EDOMf, %	85.85	85.85	85.85	85.85	85.85	85.85
EDOMi, %	79.22	79.79	79.98	79.59	80.29	80.48
Feed units per 100 kg	106.01	106.70	106.93	106.45	107.30	107.53
Enzyme effect, feed units per 100 kg	0	0.69	0.92	0	0.85	1.08
Enzyme effect relative to double dose	0	0.75	1.00	0	0.79	1.00

Table 3 shows an increase of 0.69-1.08 in measured feed units per 100 kg, which is a 0.65-1.01% improvement depending on product and dose.

Discussion

In previous experiments, SEGES investigated the effect of Danisco xylanase on EDOMi in the most important ingredients [2]. The effects used in the Danish feed evaluation system for xylanase are equal to the effect of 4000 units from Porzyme 9300. This experiment also demonstrated an effect of dose, as 3 times the standard dose (12000 units) of Porzyme 9300 (from Danisco) increased EDOMi by 0.3% in wheat.

In an experiment with a wheat-based diet (71% wheat) conducted in 2015, the effect of 4000 units of Danisco xylanase was 0.7%-units higher EDOMi compared with no xylanase using the same standard in vitro method as the current experiment [1].

By using the Danish feed calculation system for effect of xylanase on the feed composition in this current experiment, feed units would increase by 0.62 per 100 kg compared to a calculation without xylanase (the effect of xylanase on EDOMi is 0.9% in wheat and wheat bran, 0.7% in rye and 0.5% in barley and no effect on soybean meal, sunflower seed meal or rapeseed meal [2]). This is calculated by official Danish table values of feed ingredients without xylanase followed by a calculation in which wheat, barley, rye and wheat bran without xylanase are replaced by the same ingredients with xylanase, eg. 'Wheat with xylanase'.

It seems as if the broader spectrum of enzyme activities, including betaglucanase and other enzymes, generates a slightly higher effect on EDOMi at the standard dose in this diet than the calculated effect of Danisco xylanase in standard dose for Rovabio Excel, but in particular for Rovabio Advance.

Based on the present in vitro results of EDOMi, it is not possible to conclude whether the enzyme products at the tested doses will have the same effect - around 77% effect of single dose compared to double dose - on the in vivo ileal digestibility where pH, milling and holding time in different compartments of the gastrointestinal system differ from the idealized conditions in the in vitro system.

For the feed industry in Denmark, the effect of measurable/controllable energy on a complete diet is an important factor in defining the value of a product.

Conclusion

The two enzyme products improved the in vitro digestibility of organic matter at the ileal level (EDOMi). For both enzyme products, the standard dose generated an effect of approx. 77% compared to the double dose. For both enzyme products, the effect of the standard dose resulted in a statistically significant higher EDOMi than control.

There was a tendency ($P<0.07$) for better effect of Rovabio Advance compared to Rovabio Excel, but this difference was not significant on a 5% level given the present data.

The recorded energy content in feed units for growing pigs per kg feed improved by 0.65-1.01% depending on enzyme product and dose.

References

[1] Kjeldsen, N.J & D.K. Rasmussen. Enzymet xylanase har positive effekt på EFOSi i svinefoder. Meddelelse nr. 1045. SEGES, Videncenter for Svineproduktion, 2015

[2] Tybirk, P & N. J. Kjeldsen. Værdisætning af xylanase ud fra hensyn til enzymets effekt på de kontrollerbare foderenheder. Notat nr. 0422. Landsudvalget for svin, Dansk landbrugsrådgivning, Landscentret, Svin, 2004

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Appendix 1: Feed composition

Experimental diet for investigating the effect of Adisseo enzyme products on EDOMi. The diet is a regular Danish diet for growing-finishing pigs with a fibre level in the high end of normal practice.

Ingredient	%	
Barley	30.00	
Wheat	32.77	
Rye	12.00	
Wheat bran	4.00	
Soybean meal	5.01	
Sunflower seed meal	6.00	
Rapeseed meal	6.00	
Palm oil mix	1.06	
Limestone	1.23	
Monocalcium phosphate	0.26	
Salt	0.53	
Vitamin premix	0.20	
Magnesium oxide	0.10	
Lysine sulphate, 70%	0.64	
Methionine 99	0.03	
Threonine 98,5	0.15	
Tryptophane	0.01	
Valine	0.02	
Calculated nutrient by official Danish table values for 2020 feed		
	Per kg	Per FUgp (or per EW)*
Danish feed units, growing pigs (FUgp)	1.05	1.00
Dry matter, g	872	
Total crude protein, g	145	138
Dig. crude protein, g	118	113
Dig. lysine, g	8.2	7.8
Dig. methionine, g	2.4	2.3
Dig. met + cys, g	4,8	4.5
Dig. threonine, g	5.3	5.1
Dig. tryptophane, g	1.6	1.52
Dig. valine, g	5.50	5.2
Dig. leucine, g	7,8	7,5
Calcium, Total, g	6.0	5.7
Phosphorus Total, g	4.6	4.4
EDOMf (fecal), %	87.0	
EDOMi (ileal), %	79.7	
Crude fibre (old methods), g	49	47
Soluble fibre, g	36	34
Insoluble fibre, g	149	142
Fermentable fibre, Danish equation, g	83	79

*For practical comparisons FUgp is very close to Dutch EW in a complete diet.

Appendix 2: In vitro method and added enzyme dilutions

The EDOMi method is an in vitro method that forms the basis for a predetermination of the “potential” ileal digestibility of organic matter in pigs in feed ingredients and complete diets. The measuring range is 0-100%. “Potential” means the maximum digestibility attainable by the pig’s own enzymes under ideal pH conditions and fine milling at a 1 mm screen.

Principle: A feed sample (0.5 g) is incubated with pepsin at pH 2.0 for 75 minutes (step 1 simulating the stomach) and subsequently with pancreatin at pH 6.8 for about 18 hours (step 2 simulating the small intestine). Solubilized, but incompletely degraded protein is precipitated with sulphosalicylic acid. Insolubilized and precipitated materials are collected after filtration, dried and finally ashed. Enzyme digestibility of dry matter and organic matter is calculated based on the analyses of dry matter and ash in the sample and residue.

In the present experiment, a buffer solution was used alone or in combination with the relevant enzyme at concentrations equal to 100 g (standard dose) or 200 g (double dose) per ton. In this experiment we used 2 mL of buffer solution to 0.5 gram of sample – and we calculated the dilutions to ensure that 2 mL of added buffer with the relevant enzyme product would give the same dose as 100 or 200 g enzyme premix per ton. All buffer solutions were provided by Adisseo ready to be added with 2 mL to 0.5 g feed sample.

Thus, a dilution for standard dose of 1:40,000 was used – see calculation:
2 mL of 1:40,000 per 0.5 g = 4 mL per g = 4,000 mL per kg = 4,000,000 mL pr ton
Control: Dilution 1:40,000 means 4,000,000 mL per ton x 1/40,000 mL/ton dilution = 100 mL per ton.

Dilution of the double dose was 1:20,000.

Practical design for both enzyme products

Treatment	1	2	3
	No addition of enzyme product	Standard dose (100 g per ton)	2 x standard dose (200 g per ton)
Additions to step 1	2 mL buffer added to 0.5 g feed sample	2 mL buffer Dilution 1:40,000 enzyme* added to 0.5 g feed sample	2 mL buffer Dilution 1:20,000 enzyme* added to 0.5 g feed sample

* Rovabio Excel in experiment 1 and Rovabio Advance in experiment 2.

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