

# EFFECT OF NATUGRAIN®TS IN TWO CONCENTRATIONS WITH/WITHOUT NATUPHOS®E ON ENZYME DIGESTIBLE ORGANIC MATTER AT THE ILEAL LEVEL (EDOMI)

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## Main conclusion

There was an improved in vitro digestibility of organic matter at the ileal level (EDOMi) by adding Natugrain®TS to the feed. A numerical positive increase of 19% was seen when a double dose of Natugrain®TS was added to the feed. The effect of Natugrain®TS on EDOMi was reduced by 40-50% when 500 FTU of Natuphos®E per kg was added to the feed.

The in vitro method is not able to rank the effect of the different carbohydrase in relation to the effect in vivo. However, it is presumed that a statistically significant effect found on the enzymes in vitro also will enhance a positive response in vivo. For the time being, the recommendation is to use the same "xylanase calculation system" as already established in Denmark for the tested carbohydrase products from DSM, Danisco, Adisseo, and BASF.

*Tilsætning af produktet Natugrain®TS gav øget fordøjelighed (in vitro) af organisk stof målt ved ileum (EDOMi). Ved at tildele dobbelt dosering af Natugrain®TS øgedes den positive effekt numerisk med 19%. Ved tilsætning af fytase (500 FTU Natuphos®E) pr. kg foder reduceredes effekten af Natugrain®TS med 40-50%.*

*In vitro metoden kan ikke rangere effekten af forskellige kulhydrater i forhold til in vivo. Dog antages det, at enzymer, der viser en statistisk sikker effekt in vitro også vil have en positiv effekt in vivo. Det anbefales at bruge den samme beregningsmetode for xylanase som hidtil i Danmark, ud fra de testede produkter fra DSM, Danisco, Adisseo og BASF i indeværende afprøvning.*

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## Summary

Two in vitro experiments at different pH levels (2 or 3) were conducted to investigate the effect of Natugrain®TS from BASF supplemented at three levels 1) Control: No addition, 2) SD: Standard dose, and 3) DD: Double dose. In addition, each experimental group was further subdivided, and either added 500 FTU of Natuphos®E or not (+/-) resulting in five experimental groups in total tested on each pH level.

The effect was measured on the in vitro digestibility of organic matter, by using the method of EDOMi (Enzyme Digestible Organic Matter at the Ileal level). This method is used in the Danish evaluation system to calculate the energy content in the feed with different diet compositions. The regular method includes two steps, a) incubation with pepsin at pH 2, and b) incubation with pancreatin at pH 6.8. The standard procedure was performed in Exp. 1. In Exp. 2 the pH level was increased to 3 in the “first step”, when pepsin is added to simulate the gut of the pig. Otherwise, the two experiments were alike.

The main results from the two experiments were as follows:

- No statistical interaction between pH and the EDOMi values regarding the different treatments ( $p > 0.10$ ).
- A positive effect on the EDOMi value was seen by adding standard and double doses of Natugrain®TS, compared to the control group ( $p < 0.0001$ ).
- A tendency towards a higher effect when adding a double dose of Natugrain®TS without phytase compared to the standard dose ( $p = 0.10$ ).

The effect of Natugrain®TS measured on EDOMi would improve “controllable” Danish feed units for growing pigs in an interval ranging between 0.45-0.53% depending on dose. If Natuphos®E is present in the feed, the effect on feed units per 100 kg is reduced to 0.19-0.33%.

## Introduction

Two in vitro analyses are applied by SEGES to calculate the potential digestibility of organic matter at the ileal level (EDOMi) and at the faecal level (EDOMf), in Danish termed EFOSi and EFOSf, respectively. Previously unpublished experiments have shown a positive effect of xylanase on EDOMi, but not on EDOMf, as the EDOMf analysis includes fibre degrading enzymes.

The Danish energy evaluation system includes both EDOMi and EDOMf. An increase in EDOMi will increase the energy value of the tested feed ingredient or complete diet – regardless of unchanged EDOMf. 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 the degree of grinding, incubation time, and pH level. Details on the EDOMi analysis and each step in the analysis are provided in Appendix 1.

A study performed by Kjeldsen & Rasmussen (2015) compared the effect on EDOMi on a pH level of 2 and 3, with a supplementation of xylanase from DSM [1]. The results showed that the effect of xylanase supplementation was lower at pH 2 compared to pH 3 measured on EDOMi. In addition, the experiment showed a negative effect by adding phytase (Phyzyme XP), when pH was increased to 3, while the addition of phytase did not affect the EDOMi when pH was 2. Phytase did not affect EDOMi in feed without xylanase [1].

The purpose of the current study was to evaluate the effect of the NSP enzyme product (Natugrain®TS) provided by the company BASF on the EDOMi. The product was tested using the standard in vitro procedure (pH 2 at step 1), and when pH was increased (pH 3 at step 1). The method is applied in the Danish evaluation of energy level in pig feed.

## Material and methods

Two in vitro experiments were conducted with a pH level of 2 or 3 in “step 1” of the EDOMi analysis, respectively. In each experiment, three levels of the enzyme product Natugrain®TS from BASF was tested. Either no addition (control), a standard dose (560 TXU xylanase and 250 TGU glucanase) or a double dose (1120 TXU xylanase and 500 TGU glucanase) were supplied. Furthermore, the groups

receiving a standard or double dose of Natugrain®TS in each experiment were subdivided and added either 0 FTU or 500 FTU of Natuphos®E to test how the presence of Natuphos®E affects the effect of Natugrain®TS. This resulted in five experimental groups in total which were compared in terms of the effect of the in vitro ileal digestibility of organic matter using the EDOMi (Enzyme Digestible Organic Matter at the Ileal level) method.

The experiment was conducted with five treatments with 54 replicates of the control treatment and 42 replicates per experimental group, see Table 1, corresponding to 222 EDOMi analyses with pH 2 and pH 3, respectively. Each experiment at pH 2 or pH 3 in “step 1” was designed to identify a difference between treatments of 0.5%-unit with a standard deviation of 0.5. To qualify each individual analysis, the difference between two analyses within the same group performed next to each other was calculated. If the difference between the analyses was higher than 1.5, both samples were repeated. A normal range of repetitions at Eurofins is around 18% of the samples which corresponds well to the number of necessary repetitions in the current study. The repeated analyses are called batch 7 in experiment 1 and batch 8 in experiment 2.

BASF prepared 5 buffer solutions for the two experiments and was responsible for the correct content of enzymes. Appendix 1 provides details of the in vitro method, including levels and concentrations of the added enzyme product.

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 2. The feed was pelleted without addition of any enzymes and had been stored at -18°C for about a year before this experiment.

Feed from the same production was used for both experiments. A sample of 750 g of feed was split into three batches of 250 g. Each batch was grinded separately, and one batch was used for one batch/experimental day. The effect of batch represents the effect of a subsample at a specific experimental day.

**Table 1. Experimental plan.** Number of samples in the EDOMi analysis

<b>Experiment 1, pH 2 in “step 1.”</b>					
Natugrain®TS	Control: 0	Standard	Double	Standard	Double
Natuphos®E	-	-	-	+	+
Batch 1	18	14	14	14	14
Batch 2	18	14	14	14	14
Batch 3	18	14	14	14	14
Total	54	42	42	42	42
<b>Experiment 2, pH 3 in “step 1.”</b>					
Natugrain®TS	Control: 0	Standard	Double	Standard	Double
Natuphos®E	-	-	-	+	+
Batch 4	18	14	14	14	14
Batch 5	18	14	14	14	14
Batch 6	18	14	14	14	14
Total	54	42	42	42	42

## Results

The “raw” LS-means results from each feed batch on the effect of pH, standard/double doses of Natugrain®TS, and addition of 500 FTU Natuphos®E on the EDOMi are shown in Appendix 3. No significant difference was found between the two pH levels tested. The results of both experiments are therefore combined and shown in Table 2 below.

**Table 2.** Effect of standard and doubled dose of Natugrain®TS +/- Natuphos®E on EDOMi, LS-means.

EDOMi					
Natugrain®TS	Control	Standard	Double	Standard	Double
Natuphos® E	-	-	-	+	+
Average, %	78.709	79.095	79.184	78.881	78.999
Difference from control, %-units	0	0.386	0.475	0.172	0.290
Relative to double dose	0	0.81	1.00	0.59	1.00
Relative to double dose - without phytase	0	0.81	1.00	0.36	0.61

### Results from the statistical analyses

1. The increment of 0.386%-unit by a standard dose of Natugrain®TS without Natuphos®E was statistically different from the control group ( $p < 0.0001$ ).
2. The double dose of Natugrain®TS without Natuphos®E was statistically different from the control group ( $p < 0.0001$ ).
3. The difference between standard and a double dose of Natugrain®TS without Natuphos®E was not statistically significant ( $p > 0.10$ ).
4. A tendency towards a statistical difference ( $p = 0.07$ ) was seen on the increment of 0.172%-unit of a standard dose Natugrain®TS and Natuphos®E compared with the control group.
5. The double dose of Natugrain®TS including Natuphos®E was statistically different from the control group ( $p < 0.0001$ ).
6. No statistical difference was seen between the standard and double doses of Natugrain®TS included with Natuphos®E ( $p > 0.10$ ).

### 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 faecal level) were applied to calculate the effect of the enzyme products on analysed energy.

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

**Table 3.** Analyses and analysed energy content in feed

Natugrain <sup>®</sup> TS	Control: 0	Standard	Double	Standard	Double
Natuphos <sup>®</sup> E	-	-	-	+	+
Dry matter, %	87.6	87.6	87.6	87.6	87.6
Crude protein, %	14.7	14.7	14.7	14.7	14.7
Crude fat, %	3.7	3.7	3.7	3.7	3.7
Crude ash, %	4.3	4.3	4.3	4.3	4.3
EDOMf, %	85.85	85.85	85.85	85.85	85.85
EDOMi, %	78.71	79.10	79.18	78.88	79.00
Feed units per 100 kg	105.56	106.03	106.12	105.76	105.91
Enzyme effect, feed units per 100 kg	0	0.47	0.56	0.20	0.35
Enzyme effect relative to double dose	0	0.84	1.00	0.57	1.00
Relative to double dose - without phytase	0	0.84	1.00	0.36	0.63

Table 3 shows an increase of 0.45-0.53% in measured feed units per 100 kg using Natugrain<sup>®</sup>TS. If Natuphos<sup>®</sup>E is present in the feed, the increase in feed units per 100 kg is reduced to 0.19-0.33%.

## Discussion

In a previous trial, the effect of “Danisco xylanase” on EDOMi in the most important feed ingredients was investigated by SEGES [2]. The effects used in the Danish feed evaluation system for xylanase are equal to the effect of 4,000 units from Porzyme 9300. The trial also demonstrated an effect of dose, as three times the standard dose (12,000 units) of Porzyme 9300 increased EDOMi by 0.3% in wheat.

Another study performed in 2015 tested the effect of 4,000 units of “Danisco xylanase” on a wheat-based diet (71% wheat). The results showed an increase of 0.7%-units in EDOMi compared with no xylanase by using the same standard in vitro method as the current experiment [1].

In the Danish feed calculation system, the effect of xylanase on EDOMi is 0.9% in wheat and wheat bran, 0.7% in rye, and 0.5% in barley. There is no effect of xylanase on EDOMi regarding soybean meal, sunflower seed meal or rapeseed meal [2]. The feed composition in this current study increased the feed units with 0.62% per 100 kg feed compared to a calculation with no xylanase added to the feed. Calculated by Danish standard values of feed ingredients without xylanase, and then followed by a calculation of which xylanase was added to the same ingredients.

Based on the present in vitro results of EDOMi, it is not possible to conclude whether Natugrain<sup>®</sup>TS in the tested doses will have the same effect - around 81% effect of single dose compared to double dose - on the in vivo ileal digestibility due to variations in degree of grinding, incubation time, and pH level which differ from the idealized conditions simulated in the in vitro system.

Overall, the current study showed that the combination of xylanase and glucanase influences EDOMi. Furthermore, a tendency ( $p>0.10$ ) towards a higher effect when providing a double dose was seen. However, the shape of the response curve is like a similar enzyme product [3].

The results also show that the addition of Naturphos<sup>®</sup>E has a significantly negative effect on the effect of xylanase + glucanase. A reduction of 40-50% was seen when phytase was added. In a previous

study performed by SEGES, the same negative effect of phytase was observed [1]. However, it depends on the company's product on the one hand, and the pH level on the other hand, which may be because the activity of some xylanase can be eliminated at a pH level of 2 (DSM's xylanase has no effect after passing through pH 2). In addition, the activity of some phytases is delimited at a pH of 2, and only work probably at a pH of 3, as was seen in 2015 when testing Danisco's Phyzyme product [1]. The explanation of this might be due to the fact that the enzymes are broken down or degraded at a low pH level.

Surprisingly, no interaction was found between pH and Natuphos®E in the current study. The effect of Natuphos®E was the same for each of the pH levels (2 or 3). This suggests that the enzymes in the product (Natugrain®TS) survive whether the pH is 2 or 3 in "the first step" when simulating the gut of the pig.

In conclusion, Natugrain®TS from BASF influences EFOSi at both pH levels. However, the effect is slightly less compared to Danisco's xylanase [1] and Rovabio Excel products [3], which are also xylanase + glucanase based. On the other hand, a less negative effect of phytase was found compared to the pure xylanase enzymes from Danisco and DSM, where phytase largely eliminated the effect of xylanase at a pH level of 3 [1], which is closer to the pH found in the stomach of the pig. Direct comparison of effects between experiments is difficult due to different feed compositions.

A reason for the negative effect found of phytase on the xylanase or xylanase + glucanase is difficult to conclude. Maybe a direct effect of phytase negatively influences xylanase or maybe an effect of liberated phosphate and/or liberated divalent metal ions, resulting in more undigested organic matter due to the precipitation process of organic material. In addition, maybe the negative effect is mainly in relation to xylanase, resulting in a less negative effect of phytase on Natugrain®TS (with glucanase) at a pH level of 3 [1].

## Conclusion

There was an improved in vitro digestibility of organic matter at the ileal level (EDOMi) by adding a normal dose of Natugrain®TS to the feed. Adding double dose of Natugrain®TS increased the effect by 19% but was not statistically different. The effect of Natugrain®TS on EDOMi was reduced by 40-50% when 500 FTU Natuphos®E was present in the feed.

The in vitro method is not able to rank the effect of the different carbohydrase in relation to the effect in vivo. However, it is presumed that a statistically significant effect found on the enzymes in vitro also will enhance a positive response in vivo. For the time being, the recommendation is to use the same "xylanase calculation system" as already established in Denmark for the tested carbohydrase products from DSM, Danisco, Adisseo, and BASF.

## References

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Dyregruppe: Vækstgrise

Fagområde: Foder, In vitro-forsøg

Nøgleord: In vitro, EFOSi, EFOSf, Xylanase, Fytase.

# Appendix 1

## 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 BASF ready to be added with 2 mL to 0.5 g feed sample.

Natugrain®TS

- Standard dose = 560 TXU xylanase and 250 TGU glucanase
- Double dose = 1120 TXU xylanase and 500 TGU glucanase

Natuphos®E

- 500 FTU



## Appendix 2

### Feed composition

Experimental diet for investigating the effect of Natugrain® TS +/- Natuphos®E 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	
<b>Calculated nutrient by official Danish table values for 2020 feed</b>		
	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 (faecal), %	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 are very close to Dutch EW in a complete diet.

## Appendix 3

### Results of the analysis of each batch

Effect of pH 2 and 3 in standard and doubled dose of Natugrain®TS +/- Natuphos®E on EDOMi, LS-means.

Natugrain®TS Natuphos®E	Control = 0			Standard			Double			Standard			Double		
	N	Mean	Std	N	Mean	Std	N	Mean	Std	N	Mean	Std	N	Mean	Std
Batch at pH 2	12	78.66	0.36	12	78.93	0.50	11	79.27	0.40	10	78.70	0.50	12	79.07	0.64
1	18	78.76	0.46	14	79.29	0.74	12	79.33	0.32	14	78.96	0.32	14	79.06	0.26
2	11	77.83	0.35	12	78.45	0.45	9	78.37	0.75	8	78.30	0.85	8	78.44	0.39
3	10	79.22	0.39	2	79.50	0.14	7	79.50	0.32	7	78.97	0.52	5	79.08	0.54
7	51	78.63	0.61	40	78.94	0.66	39	79.12	0.62	39	78.76	0.58	39	78.94	0.52
All	N	Mean	Std	N	Mean	Std	N	Mean	Std	N	Mean	Std	N	Mean	Std
Batch at pH 3	6	78.75	0.46	10	79.00	0.27	8	79.05	0.57	6	78.98	0.17	4	78.50	0.35
4	14	78.76	0.56	12	79.14	0.38	12	79.14	0.50	8	78.90	0.45	10	78.97	0.45
5	16	78.89	0.35	14	79.29	0.35	12	79.55	0.28	14	79.26	0.26	14	79.35	0.43
6	12	79.07	0.33	2	79.15	0.35	5	79.52	0.37	8	79.08	0.42	8	79.36	0.34
8	48	78.88	0.44	38	79.16	0.35	37	79.31	0.48	36	79.09	0.36	36	79.15	0.49
All															



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