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TRIAL REPORT

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# XYLANASE IN DIFFERENT DOSES AND OF DIFFERENT ORIGIN IN FINISHER FEED

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# Main conclusion

Neither Ronozyme WX in standard or double doses nor Danisco Xylanase in standard dose significantly affected finishers' production value in this trial. The incorporation of xylanase in finisher feed generated a tendency to a lower feed conversion and a lower lean meat percentage.

# Abstract

Results showed no significant effect on the production value, DKK/pig place, of the addition to finisher feed of Ronozyme WX (200 FXU/kg), Ronozyme WX (400 FXU/kg) or Danisco Xylanase (4,000 U/kg). There was no significant effect on mortality or on other production results.

Analyses revealed a tendency to a lower feed conversion per kg gain (P value = 0.06) and a lower lean meat percentage (P value = 0.07) when xylanase was incorporated into the feed.

The trial compared the effect of four experimental treatments: 1) a diet with no xylanase, 2) a diet containing Ronozyme WX (200 FXU/kg), 3) a diet containing Ronozyme WX (400 FXU/kg) and 4) a diet containing Danisco Xylanase (4,000 U/kg). The trial comprised approx. 3,800 finishers in the 32-115 kg period. The pigs were fed pelleted feed that complied with the Danish nutrient standards with an average content of 49.2% wheat and 28.8% barley. Production parameters averaged 1,110 g daily gain, 2.66 FUgp per kg gain and 61% lean meat content.

# Background

It is common practice to add carbohydrate-splitting enzymes to pig feed in Denmark to improve gain and feed conversion. Numerous trials, in Denmark and internationally, with different enzyme products for pigs have all shown highly varying effect. Danish trials have demonstrated a 0-3% improvement in finishers' feed conversion when xylanase was incorporated into the feed.

Two Danish trials revealed that the inclusion of 4,000 units xylanase per kg finisher feed in the form of Porzyme 9300 resulted in +12-15 g daily gain,  $\div$ 0.07-0.09 FUgp per kg gain and  $\div$ 0.4%/+0.5% lean meat percentage, respectively, compared with an identical diet without Porzyme 9300 [1,2]. Three other trials showed no effect on finisher productivity of adding standard doses of Bergazym P,

Ronozyme WX and Econase XT compared with an identical diet without xylanase [3-5]. A dose-response trial investigating three different inclusion rates of Porzyme 9302 (1,000, 2,000 and 4,000 U per kg feed) and two different inclusion rates of Ronozyme WX (200 and 400 FXU per kg feed) found no significant effect on finisher productivity [6].

The aim of this trial was to determine the effect on finisher productivity of incorporating xylanase in different doses and of different origin into the feed. The effect was recorded on daily gain and feed conversion pooled in a calculated production value (PV).

# Materials and methods

## Trial design and practical implementation

The trial included four experimental groups as shown in table 1 below.

Group	1	2	3	4
NSP enzyme	None	Ronozyme WX	Ronozyme WX	Danisco Xylanase
NSP enzyme inclusion	0	200 FXU/kg feed	400 FXU/kg feed	4,000 U/kg feed

The trial was conducted at SEGES Danish Pig Research Centre's Grønhøj trial station in sectioned housing units. Each section included 16-20 pens accommodating 8-9 pigs. All pens were equipped with a single space feeder and one drinking bowl. Mixing of feed and the supply of feed to the pens were operated by a computerised dry feeding system.

A trial unit consisted of one pen with eight pigs, and a replicate consisted of four pens assigned to treatments 1-4 shown in table 1. The pigs weighed roughly 32 kg at trial start, and initial weight and gender distribution were identical in the four pens constituting one replicate. The trial design included 112 replicates to be able to test all groups against each other.

## **Xylanase**

Table 1. Trial design.

Endo-1,4-beta-xylanase are additives that require EU approval and are classified under EC 3.2.1.8.

Ronozyme® WX has ID 4a1 607 and minimum inclusion rate in finisher feed is 200 FXU per kg complete diet. Danisco Xylanase has ID 4a11 and minimum inclusion rate in finisher feed is 2,000 U per kg complete diet [7].

Ronozyme® WX for the trial was supplied by DSM. Danisco Xylanase was purchased commercially.

## Feed

The feed was composed as a regular commercial finisher diet for 30-110 kg pigs. The content of digestible crude protein, digestible amino acids, minerals and vitamins complied with the Danish nutrient standards [8]. The feed was formulated with a safety margin of +5% lysine, methionine and threonine as a precaution against variations in nutrient content. The feed was routinely adjusted according to current ingredient analyses to maintain compliance with the intended nutrient content. The average declared ingredient composition is shown in table 2.

Table 2. Ingredient composition.					
Ingredient	Per cent				
Wheat 2018	49.15				
Barley 2018	28.80				
Soybean meal, dehulled	11.32				
Sunflower meal, dehulled	6.00				
Palm oil	1.47				
Mono calcium phosphate	0.50				
Feed lime	1.35				
Sodium chloride	0.41				
Lysine sulphate 70%	0.58				
Methionine DL 98%	0.04				
Threonine 98%	0.14				
Vitamins-micro minerals-phytase-xylanase	0.23				

The feed consisted of a basic diet mixed with one of three supplementary mixes (green, red and blue). All minerals, free amino acids, phytase etc. were added to the basic diet that constituted 80% of the complete diet in all four groups.

The supplementary mixes consisted of grain, soybean meal and palm oil. Xylanase was added as shown below:

- Green: no xylanase
- Red: Ronozyme WX 2,000 FXU/kg feed
- Blue: Dupont Danisco Xylanase 20,000 U/kg

Table 3 shows the composition of the four diets.

Group	1	2	3	4
Basic diet	80%	80%	80%	80%
Green - Supplementary mix	20%	10%		
Red - Supplementary mix		10%	20%	
Blue - Supplementary mix				20%

#### Table 3. Diet composition.

The feed was produced at a commercial feedmill. Ingredients included in the diets with less than 1 percentage unit were weighed and added manually to improve mixing accuracy. Intermediate mixes without feed additives were produced before production of the trial diets and between production of the red and blue supplementary mixes to minimise the risk of carry-over of feed additives. To ensure that the same ingredient batches were used for all four groups, the three supplementary mixes were always produced on the same day.

All diets were produced as rough pellets by grinding on 5 mm screens. Pelleting temperature was logged and kept below 90 °C out of consideration for enzyme activity.

### Feed analyses

Representative samples were taken at all production rounds according to the TOS principles using SEGES' automatic sampler. Samples were divided into several identical subsamples that were either forwarded for analysis or stored at -18 0 °C as copy samples.

The basic diet was subject to analysis of water, protein, fat, FUgp (energy), phosphorus, lysine, methionine, cysteine, threonine and phytase activity at Eurofins Steins Laboratory.

The supplementary mixes were subject to analysis of water, protein, fat, lysine, methionine, cysteine and threonine at Eurofins Steins Laboratory. The analyses did not include FUgp (energy) as xylanase affects this method [9,10]; instead, declared FUgp values were converted to analysed values of water, protein and fat [11].

The supplementary mixes were subject to analysis of xylanase activity. Analyses of Ronozyme WX were handled by Biopract laboratory in Berlin. Analyses of Danisco Xylanase were handled by Eurofins Steins Laboratory. The amount of Danisco Xylanase incorporated into the feed was also subject to indirect analysis at Biopract laboratory where the recorded activity was compared to the activity in the concentrated product.

Samples of the complete diet were taken from the feeders to determine particle size distribution during wet sieving. Every other week, roughly 100 g were collected from five feeders per group using grab sampling. Every 12 weeks, these samples were pooled and the pooled samples were subject to wet sieving with double determination. Wet sieving is not recognised as an authorised method, and therefore sievings were performed at SEGES Danish Pig Research Centre's trial station according to inhouse procedures.

### Recordings

All recordings were made at pen level. Recordings included initial weight, date and cause of antibiotic treatments and dead and culled pigs. Carcass weight and lean meat percentage were recorded at the slaughterhouse. Final weight was calculated as carcass weight x 1.31.

The consumption of each individual diet at pen level was recorded by the computerised feeding system.

## Statistical analyses

Productivity parameters (gain, feed intake and feed conversion) and economic parameters (production value per pig and per pen) were subject to analysis in SAS using the proc mixed procedure with assumption of normal distribution. Analyses comprised group (treatment) and gender (female/castrate/mixed) as systematic effects and initial weight as co-variate. Batch and housing unit were included as random effects. Analyses concerning weight (initial weight and carcass weight) did not include gender as systematic effect as this was not significant in the models, but initial weight was included as co-variate in the carcass weight model.

Mortality was subject to analysis in a logistic regression model with binominal distribution, solely with group (trial treatment) as systematic effect, and batch and housing unit as random effects.

Data was generally of a good quality. Three pens were excluded from the data material either due to feed overflow or inaccurate number of pigs.

## Economic assumptions

The production value was calculated on the basis of average prices of the last five years (September 1, 2014 – September 1, 2019):

- Weaned pigs 30 kg: DKK 362 per pig, kg regulation of DKK + 5.58 per kg (30-40 kg period)
- Finishers, including bonus payment: DKK 10.45 per kg carcass weight
- Finisher feed: DKK 1.58 per FUgp

The production value (PV), which is the primary effect parameter, was determined on the basis of the production results:

- PV/pig = sales price purchase price feed costs various costs
- PV/pig place per year = PV/pig \* (365 days / feeding days per pig) \* utilization of housing unit

# Results and discussion

#### Feed analyses

Appendix 1 provides an outline of the average declared and analysed nutrient content of the diets. The feed was drier than declared, but, besides this, analyses revealed a good correspondence between declared and analysed nutrient content. The analysed phytase activity was significantly higher than declared, but this is unlikely to affect the conclusion of the trial as the activity level was identical in all four diets.

As shown in appendix 2, particle size distribution was identical in all four groups. Despite the use of 5 mm screens in the grinding process, particle size distribution largely corresponded to that of standard pelleted finisher feed.

### Analyses of xylanase activity

The declared and analysed xylanase activity in the supplementary mixes is shown in table 4.

	Green (control)		Red (Ronozyme)		Blue (Danisco)	
	Declared	Analysis	Declared	Analysis	Declared	Analysis
Ronozyme WX, FXU/kg						
(analysed by Biopract)	0	LOQ 1	2,000	1,475 (74%)		
Danisco Xylanase, U/kg						
(analysed by Eurofins)	0	< 279 <sup>2</sup>			20,000	12,045 (60%)
Danisco Xylanase, g/t <sup>3</sup>						
(analysed by Biopract)	0	21			1,167	1,337 (115%)

Table 4. Declared and analysed xylanase activity in supplementary mixes. Average of 6 samples per diet.

<sup>1</sup>All 6 samples were below the quantification limit LOQ

 $^{\rm 2}\, \rm One\ sample\ was$  < the quantification limit LOQ at 133 U/kg

<sup>3</sup>Biopract indirect method for measuring the amount added, g/t

A small concentration of Danisco Xylanase activity was found unexpectedly in the green supplementary mix. This may be attributed to carry-over at the feedmill where Danisco Xylanase is routinely used for commercial diets. It was attempted to minimise the risk of carry-over by producing intermediate mixes without additives before and in between production of the trial diets. However, analyses ordered by the feedmill and performed at Danisco/Dupont of the green mix did not reveal Danisco Xylanase in any of the mixes. Still, the analysed content in the green mix was very low and is not expected to have affected the outcome of the trial. The analysed xylanase activity in the red supplementary mix averaged 74% of the declared activity. This level is identical to the level found in previous trials with Ronozyme WX revealing an analysed activity of 74-83% of the declared [4,5].

The analysed xylanase activity in the blue supplementary mix averaged 60% of the declared activity. This does not correspond with the indirect analyses made by Biopract that found averagely 115% of the added amount of Danisco Xylanase measured in g per kg. The analyses made by Danisco/Dupont found 93% of the declared content. In two previous trials, analyses found 88-90% of the declared xylanase activity [12,13]. These analyses were also undertaken by Danisco/Dupont.

### **Production results**

The production results for the period from transfer to slaughter for each of the four groups is shown in table 5.

Group	1	2	3	4	P value
Treatment	No	Ronozyme WX	Ronozyme WX	Danisco	
	xylanase	(200 FXU)	(400 FXU)	Xylanase	
				(4,000 U)	
Pens / replicates	116	117	116	116	
Pigs at trial start	943	952	944	943	
Initial weight, kg	31.5	31.5	31.6	31,6	0.77
Carcass weight, kg	88.0	88.2	88.3	88.1	0.45
Dead and culled	-	1			1
Culled, %	3.1	3.2	4.2	3.5	0.49
Of these, dead, %	1.4	1.8	1.6	2.3	0.44
Production results	1	1	I		1
Daily gain, g/day	1,103	1,114	1,108	1,114	0.16
Feed intake, FUgp/day	2.95	2.95	2.94	2.95	0.82
FCR, FUgp/kg gain	2.67	2.65	2.66	2.65	0.06
Lean meat %	61.1	61.0	60.8	61.1	0.07
Production value	1	ı	I		1
Production value, DKK/pig	174	177	175	177	0.50
Production value, DKK/pig	795	813	799	816	0.18
place					
Index, production value,	100	102.2	100.5	102.6	0.18
DKK/pig place <sup>1)</sup>					

#### Table 5. Production results.

<sup>1)</sup> Minimum statistically significant difference in production value index estimated at 3.4 index points

The P values in table 5 illustrate the effect of all pair-wise comparisons of the factor 'group', which in this trial with four groups amounts to six pair-wise comparisons. P values higher than 0.05 indicate no significant effect of treatment and thereby no difference between the treatments.

Table 5 shows that there was no significant effect of the addition of Ronozyme WX (200 FXU/kg), Ronozyme WX (400 FXU/kg or Danisco Xylanase (4,000 U/kg) measured on production value, DKK per pig place. Results showed no significant effect on mortality, daily gain or daily feed intake, but did reveal a tendency to a lower feed conversion, FUgp/kg gain (P value = 0.06) and lean meat percentage (P value = 0.07) when xylanase is incorporated into the feed. The term 'tendency' is applied to P values in the interval 0.05-0.10.

Mean values for treatment frequency are shown in table 6. Treatment frequency did not differ between the groups.

Group	1	2	3	4
Treatment	No	Ronozyme WX	Ronozyme WX	Danisco
	xylanase	(200 FXU)	(400 FXU)	Xylanase
				(4,000 U)
Total treatment frequency (treatments/pig)	0.8	0.7	0.8	0.6
- For diarrhoea	0.5	0.4	0.5	0.4
- For leg problems	0.3	0.3	0.3	0.2

#### Table 6. Treatment frequency – mean values.

# Conclusion

Results showed no significant differences between the four treatments: 1) no xylanase, 2) + Ronozyme WX (200 FXU/kg), 3) + Ronozyme WX (400 FXU/kg) or 4) + Danisco Xylanase (4,000 U/kg) measured on the production value, DKK/pig place.

There were no significant differences in mortality, daily gain or daily feed intake, but there was a tendency to a lower feed conversion, FUgp/kg gain (P value = 0.06) and lean meat percentage (P value = 0.07) when xylanase was incorporated into the feed.

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#### Participants

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# Appendix 1

## Declared and analysed nutrient content

**Table 1.1.** Declared and analysed nutrient content of the basic diet (80% of the complete diet). Average of six samples.

	Basic	: diet
	Declared	Analysis
Water, %	13.5	11.9
Crude protein, %	15.4	15.3
Crude fat, %	3.6	4.0
Ash, %	5.3	4.7
FUgp/100 kg	108.7	110.6
Lysine, g/kg	9.8	9.8
Methionine, g/kg	2.9	2.9
Cystine, g/kg	2.9	2.8
Threonine, g/kg	6.8	6.6
Phytase, FTU/kg	1,900	2,950
Phosphorus, g/kg	4.7	5.0
Calcium, g/kg	8.6	8.4

Table 1.2. Declared and analysed nutrient content of supplementary mixes (20% of the complete diet). Average
of 6 samples per diet.

	Green		Red		Blue	
	Declared	Analysis	Declared	Analysis	Declared	Analysis
Water, %	14.2	12.5	14.0	12.5	14.0	12.8
Crude protein, %	19.7	19.6	19.7	19.4	19.7	19.5
Crude fat, %	3.7	4.3	3.8	4.3	3.8	4.3
Lysine, g/kg	9.8	9.5	9.7	9.,4	9.7	9.4
Methionine, g/kg	2.8	2.8	2.8	2.8	2.8	2.8
Cystine, g/kg	3.5	3.3	3.5	3.3	3.5	3.4
Threonine, g/kg	7.1	7.2	7.1	7.1	7.1	7.1
FUgp/100 kg	113.0		113.2		113.2	
FUgp/100 kg <sup>1</sup>		116.5		116.7		116.1

<sup>1</sup> Declared FUgp corrected for analyses of water, protein and fat.

**Table 1.3.** Digestible lysine, methionine and threonine per FUgp, complete diet. The table shows intended content, calculated on the basis of analysed content, and the third column shows the standards for finishers 30-115 kg [8,14].

	Intended content	Based on analysed con- tent	Standard 30-115 kg
Dig. lysine, g/FUgp	7.95	7.7	7.7
Dig. methionine, g/FUgp	2.4	2.3	2.3
Dig. threonine, g/FUgp	5.3	5.1	5.1

# Appendix 2

## Particle size distribution

 Table 2.1. Sieve profiles - average of 4 samples per group.

	Group 1	Group 2	Group 3	Group 4
Screen, µm	%	%	%	%
>3150	0.1	0.1	0.1	0.1
2000-3150	2.3	2.4	2.6	2.4
1400-2000	7.3	8.4	8.4	8.0
1000-1400	10.8	10.5	11.2	12.1
500-1000	16.0	15.8	16.0	15.7
355-500	5.7	5.2	5.4	5.3
<355	57.6	57.5	56.2	56.3

**Table 2.2.** Sieve profiles, converted to another particle size scale:  $\leq 1 \text{ mm}$ , 1-2 mm, > 2 mm. Average of 4 samples per group.

	Group 1	Group 2	Group 3	Group 4
	%	%	%	%
≤ 1 mm	79.4	78.6	77.7	77.3
1-2 mm	18.1	18.9	19.6	20.2
> 2 mm	2.5	2.6	2.7	2.5



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