Can feeding of prefermented rape seed and seaweed to weaners substitute medicinal zinc?

Without compromising piglet health and growth performance (pilot study)

Mette Olaf Nielsen, Gizaw Dabessa Satessa, & Rajan Dhakal Department of Veterinary and Animal Sciences

mette.olaf.nielsen@sund.ku.dk

UNIVERSITY OF COPENHAGEN





Background

- Use of medicinal zinc oxide in piglet diets must be phased out by 2020
- Creates a need to find alternative and effective strategies to promote gut health of weaner piglets in the future
- Lactic acid producing bacteria are used as probiotic supplements to promote gut health
 - Results not always convincing
 - It has been proposed that additional gut health benefits for weaner piglets may be achieved if lactic acid bacteria are used to preferment certain feeds
- Macroalgae, particularly brown algae species, contain different bioactive compounds, of which some appear to possess antimicrobial properties





Hypotheses of this pilot study:

- Inclusion of lactobacillus prefermented rape seed cake in piglet diets from 10 days before weaning until 91 days after weaning (33 kg liveweight):
 - Can substitute 2500 ppm zinc in diarrhea prevention
 - Without negative impact on feed intake and growth rate of pigs neither by 91 days of age (exit from weaner unit; appr. 33 kg) nor when slaughtered
- The preventive effect on diarrhea is more pronounced if the supplement in addition to prefermented rape seed cake also contains certain **brown macroalgae species**

Design of the pilot experiment:

- Conducted at private, commercial farm in Poland (120 km from Lublin)
 - 1350 sows (LY Danbred C22 line)
 - 33.5 weaned piglets ((LY)x(DP)) per sow annually (weaning: 28 days)
 - 440g/d average daily gain in weaner unit (exit weight: ~33 kg)
- Feeding trial with 9 different dietary treatment groups (TG; mash feed)
 - Litters standardized to 14 piglets per sow; fixed from day 14 post-partum
 - Differential feeding start: 10 days before weaning (25.09.2017)
 - end: at exit from weaner unit (at 91 days of age)
 - Will be followed until slaughter (fixed date mid-March ~ 115 kg)
- No systemic or individual antibiotics treatment of experimental pigs
 - Piglets needing treatment (condition deteriorated): eliminated from the experiment (treatment elsewhere)
- D15 after weaning, 6 piglets/TG were slaughtered (blood, intestines)







Dietary treatments

9 different treatment groups (TG; 1 pen per treatment, 50 piglets per pen):

| TG1: No supplementation | 0Zn | (negative control) |
|--|-----------------|--------------------|
| TG2: 2500 ppm zinc oxide | 2500Zn | (positive control) |
| TG3: 8% of DM as prefermented rape seed: EP100i | 8%EP100 | |
| TG4: 10% of DM as prefermented rape seed: EP100i | 10%EP100 | |
| TG5: - As TG4 + 0.6 % of DM brown algae product: EP900 | | +0.6%BA |
| TG6: - As TG4 + 1.0% of DM brown algae product: EP900 | | +1.0%BA |
| TG7: 12% of DM as prefermented rape seed: EP100i | 12%EP100 | |
| TG8: 15% of DM as prefermented rape seed: EP100i | 15%EP100 | |
| TG9: 25% of DM as prefermented rape seed: EP100i | 25%EP100 | |

- Supplements were supplied by Fermentation Experts, Denmark, and mixed into the standard pre-weaner and starter diets
- Statistical analyses: all models included TG, gender and their interactions, start weight (linear and quadratic)

Results: Growth performance around weaning* for individual piglets (<50 observations per TG)



| Parameter | Parameter 0Zn 2500Zn 8%EP100 10%EP100 12%EP100 15%EP100 25 | 2500Zn | 8%FP100 | 10%FP100 | 12%FP100 | 15%FP100 | 25%FP100 | 10%FP100 | | 10%EP100 |
|---|--|---------------------|--|--------------------|---------------------|--------------------|--------------------|--------------------|---------------------|--------------------|
| | | | 20/021 200 | +0.6%BA | 1.0%BA | | | | | |
| BW age: D18 | 5.83 | 5.28 | 5.62 | 4.92 | 4.92 | 4.92 | 5.22 | 4.92 | 5.22 | 5.21 |
| kg ± SEM | ± 0.88 | ± 0.78 | ± 0.83 | ± 0.87 | ± 0.83 | ± 0.91 | ± 0.75 | ± 0.87 | ± 0.99 | ± 1.11 |
| 10 days before weaning until weaning (18 to 28 days of age) (corrected for BW D | | | | | | | D18 = star | weight) | | |
| ADG, g* | 144 ^a | 197 ^{bcd} | 174 ^{ab} | 180 ^{bc} | 198 ^{bcd} | 224 ^d | 225 ^d | 180 ^{bc} | 206 ^{bcd} | 214 ^{cd} |
| BW D28, kg | 6.67ª | 7.22 ^{bcd} | 6.99 ^{abc} | 6.94 ^{ab} | 7.22 ^{bcd} | 7.48 ^d | 7.50 ^d | 6.94 ^{ab} | 7.29 ^{bcd} | 7.36 ^{cd} |
| From weaning | until 14 | days after | weaning (28-41 days of age) (corrected for start weight) | | | | | | | |
| ADG, g* | 108 ^{ab} | 98 ^a | 153 ^b | 103 ^{ab} | 148 ^{ab} | 99 ^a | 116 ^{ab} | 103 ^{ab} | 124 ^{ab} | 123 ^{ab} |
| BW D41, kg | 7.78 ^a | 8.34 ^{ab} | 8.96 ^{bc} | 8.31 ^{ab} | 9.18 ^c | 8.80 ^{bc} | 9.01 ^{bc} | 8.31 ^{ab} | 8.91 ^{bc} | 8.96 ^{bc} |
| From 10 days | before w | veaning un | l 14 days af | ter weaning | g (18-41 da | corrected f | r start weight) | | | |
| ADG, g* | 110 ^a | 135 ^{ab} | 162 ^{bc} | 134 ^{ab} | 171 ^c | 154 ^{bc} | 164 ^{bc} | 134 ^{ab} | 159 ^{bc} | 162 ^{bc} |

^{*}ADG: Average Daily Weight Gain; BW: Body weight Piglets eliminated included until time of elimination; P<0.0001 (ADG;W -> D14: 0.0032)

<0Zn and =2500Zn

<2500Zn

Results: Growth performance around weaning* for individual piglets (<50 observations per TG)



| | r | | | | | | | | | |
|---|-------------------|--------------------|--------------------|--------------------|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Parameter | 0Zn | 2500Zn | 8%EP100 | 10%EP100 | 12%EP100 | 15%EP100 | 25%EP100 | 10%FP100 | | 10%EP100 1.0%BA |
| BW d18 | 5.83 | 5.28 | 5.62 | 4.92 | 4.92 | 4.92 | 5.22 | 4 .92 | 5.22 | 5.21 |
| kg ± SEM - | EP100i | increased | ADG pre- | weaning to | o the same | e extent (a | at least) th | nan 2500Z | n | |
| 10 days be | Dro-wo: | ning ADG | and weig | ht at woa | ning incre | acad with | increasing | ~ ED100i . | ın to 15% | in DM |
| BW d28 kg | | | | | | | increasing | g EP1001 t | ih (0 13% | III DIVI |
| ADG, g*** | Slight f | urther inc | rease in w | eaning we | eight with | BA ८८७ | <u></u> | | 200 | <u></u> |
| From weaning | until 14 | days after | weaning (28 | 8-41 days of | f age) (corre | ected for sta | art weight) | | | |
| BW d41 kg | 7.78 ^a | 8.34 ^{ab} | 8.96 ^{bc} | 8.31 ^{ab} | 9.18 ^c | 8.80 ^{bc} | 9.01 ^{bc} | 3.31 ^{ab} | 8.91 ^{bc} | 8.96 ^{bc} |
| ADG, g | 108 ^{ab} | 98ª | 153 ^b | 103 ^{ab} | 148 ^{ab} | 99 ^a | 116 ^{ab} | 103 ^{ab} | 124 ^{ab} | 123 ^{ab} |
| From weaning until 14 days after weaning (28-41 days of age) (corrected for start weight) | | | | | | | | | | |
| ADG, g | 110 ^a | 135 ^{ab} | 162 ^{bc} | 134 ^{ab} | 171 ^c | 154 ^{bc} | 164 ^{bc} | 134 ^{ab} | 159 ^{bc} | 162 ^{bc} |

^{*}Piglets eliminated included until time of elimination Treatment effect: P<0.0001 (ADG; W -> D14: 0.0032)



Results: Feed Conversion Rate (kg feed/kg body weight gain) (pen level; 1 observation per TG; raw data)

| | | - | | | | | | | | |
|-----------------------|----------|--------------------------|-----------------|--------------|------------|-------------|------------|-----------|---------|--------------|
| Parameter: | 0Zn | 2500Zn | 8%EP | 10%EP | 12%EP | 15%EP | 25%EP | 10%EP | +0.6%BA | +1.0%BA |
| Average daily weightl | gain a | fter wean | ng (from | n average | piglet wei | ights at pe | eriod star | t and end | d): | ! |
| D15-30 | 226 | 230 | 316 | 329 | 293 | 291 | 294 | 329 | 281 | 298 |
| D31-64 | 625 | 633 | 594 | 651 | 558 | 647 | 612 | 651 | 540 | 599 |
| Feed conversion rate | after w | ı eaning _ا | kg fee | d/kg live | weight g | ain): | | ! | | |
| D1-14 | 2.10 | 2.30 | 1.55 | 1.80 | 1.55 | 1.77 | 1.72 | 1.80 | 1.60 | 1 .73 |
| D15-37 | 1.67 | 1.62 | 1.62 | 1.54 | 1.57 | 1.54 | 1.52 | 1.54 | 1.60 | 1.59 |
| D38-65 | 1.55 | 1.45 | 1.64 | 1.56 | 1.56 | 1.53 | 1.56 | 1.56 | 1.61 | 1.58 |
| Whole weaner unit p | eriod ([| 01-64): ¦ | I | | | | | 1 | | 1 |
| Exit D64 post-weaning | 33.6 | 33.8 | 34.7 | 35.4 | 32.4 | 35.0 | 34.5 | 35.4 | 31.7 | 34.1 |
| Daily weight gain | 408 | 414 | 425 | 450 | 400 | 437 | 422 | 450 | 382 | 418 |
| FCR | 1.60 | 1.54 | 1.63 | 1.56 | 1.56 | 1.54 | 1.56 | 1.56 | 1.61 | 1.59 |

Better than 2500Zn

As good as 2500Zn

Results: Feed Conversion Rate (kg feed/kg body weight gain) (pen level; 1 observation per TG; raw data)

| | ı | | | | | | | | | | |
|---|--|-------|-----------|-----------|--------------|----------|----------|----------|--------|---------|---------|
| Parameter: | ı | 0Zn | 2500Zn | 18%EP | 10%EP | 12%EP | 15%EP | 25%EP | 10%EP | +0.6%BA | +1.0%BA |
| Average daily <mark>y</mark> | | | | | | | | | | | |
| D15-30 | Data f | rom 1 | pen onl | y, but if | anythin | g – EP10 | 00i comp | pared to | 2500Zı | 281 | 298 |
| D31-64 | | | | | | | | | | 540 | 599 |
| Feed conversion | - Increased weight at exit from weaner unit (91 days of age)~1 kg onversional one of the control | | | | | | | | | | |
| D1-14 | - Improved FCR in weaner unit: | | | | | | | | 1.60 | 1.73 | |
| D15-37 | • • • • • • • • • • • • • • • • • • • | | II from E | | | | | | | 1.60 | 1.59 |
| D38-65 | - Same overall FCR from insertion to exit from weaner unit | | | | | | | | 1.61 | 1.58 | |
| Whole weaner | | | | | | | | | | | ı |
| Exit D64 post-v No additional beneficial effect of BA | | | | | | | | 31.7 | 34.1 | | |
| Daily weight ga | n : | 408 | 414 | 425 | 45U | 400 | 437 | 422 | 45U | 382 | 418 |
| FCR | | 1.60 | 1.54 | 1.63 | 1.56 | 1.56 | 1.54 | 1.56 | 1.56 | 1.61 | 1.59 |

Better than 2500Zn

As good as 2500Zn

Results: Piglet diarrhea after weaning (pen level; 1 observation per TG)

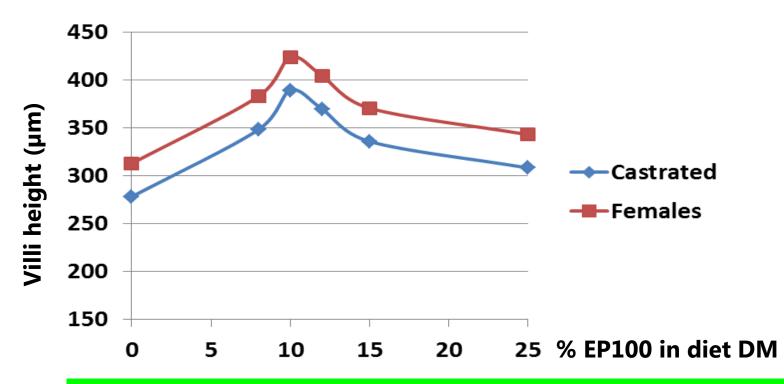
| No. of diarrhea cases: | 0Zn | 2500Zn | 8%EP | 10%EP | 12%EP | 15%EP | 25%EP | 10%EP | +0.6%BA | +1.0%BA |
|--------------------------|-------------|--------|------|-------|-------|-------|-------|-------|---------|---------|
| Until D14 | 6 | 1 | 0 | 4 | 0 | 2 | 2 | 4 | 2 | 0 |
| D15-64 | 6 | 2 | 12 | 15 | 6 | 10 | 4 | 15 | 6 | 10 |
| Duration per case (avg.) | 1 1.41 | 1.0 | 1.83 | 1.55 | 1.33 | 2.08 | 1.33 | 1.55 | 1.69 | 1.77 |
| Total diarrhea days | 17 I | 3 | 22 | 31 | 8 | 25 | 8 | 31 | 22 | 39 |
| Piglets completing (%) | 77 | 91 | 93 | 84 | 89 | 81 | 89 | 84 | 90 | 83 |

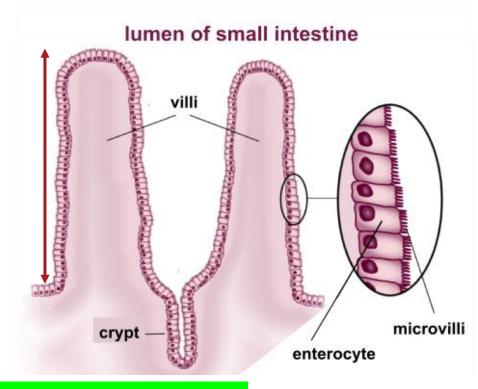
EP100:

- Ensured piglet completion rates ~ 2500Zn
- Without preventing diarrhea symptoms
- Ie. diarrhea was less detrimental (survival, growth)
 - Reduced intestinal damage and immune cell infiltration
 - Feed induced osmotic diarrhea?



Results: Small intestinal villi height (6 slaughtered piglets D11 after weaning per TG)



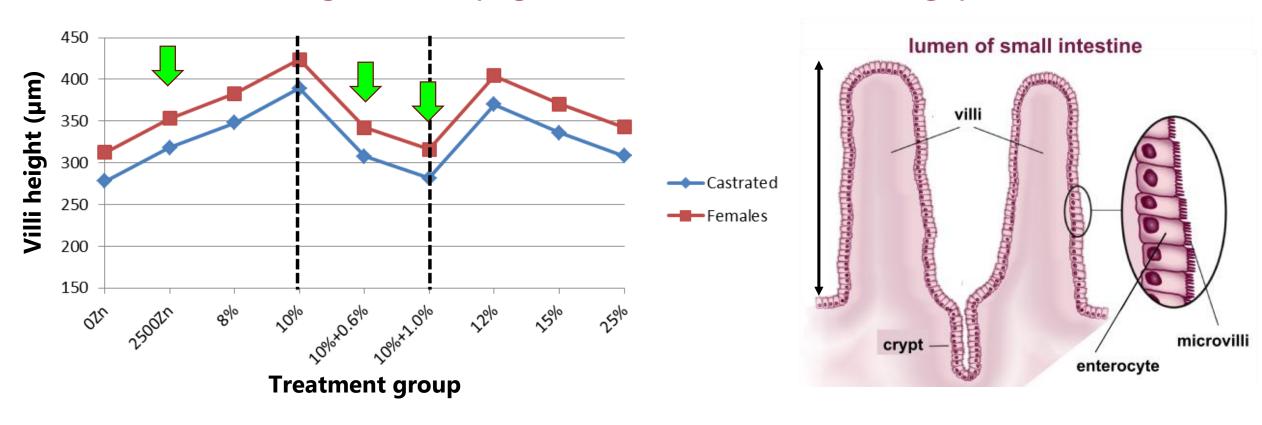


EP100:

- Increased small intestinal villi height <35% (maximal effect: 10% EP100i in diet)
- Stimulated extensive folding of villi $=> \uparrow \uparrow \uparrow$ increase in surface area
- Immune cell infiltration and enterocyte morphological changes absent
 - Also in colon

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Results: Small intestinal villi height (6 slaughtered piglets D11 after weaning per TG)



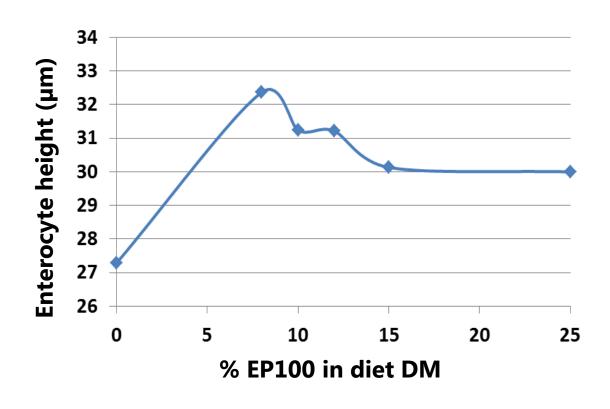
2500Zn: No significant impact on villi height

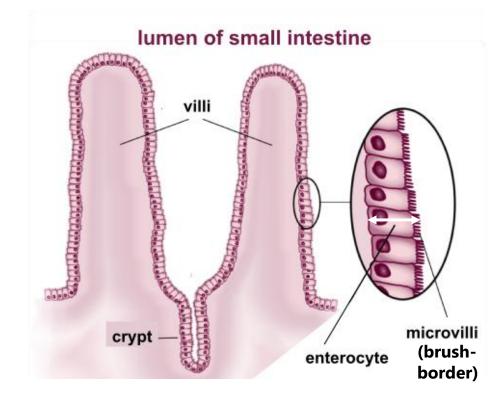
Did not prevent immune cell infiltration or enterocyte morphology changes

BA: Counteracted the effect of EP100i on villi height

But not on barrier function traits

Results: Small intestinal enterocyte height (6 slaughtered piglets D11 after weaning per TG)

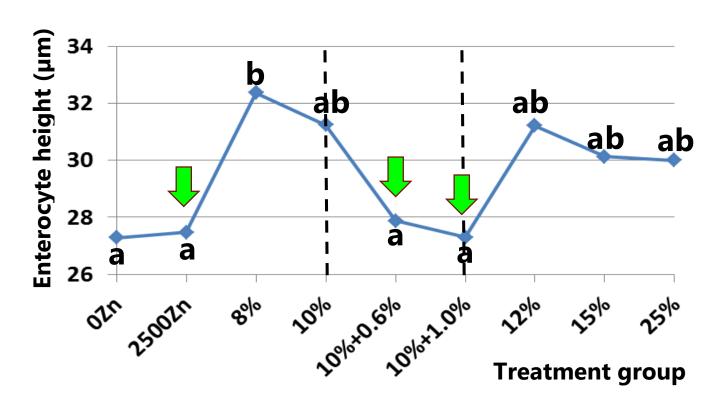


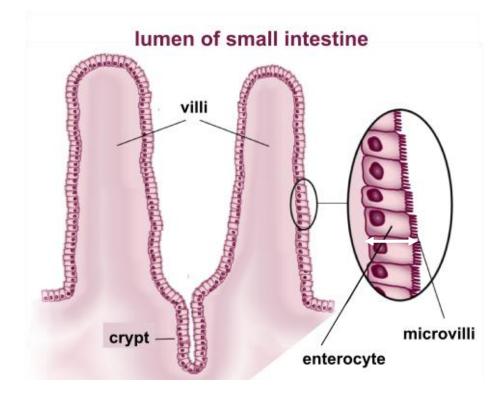


EP100i addition:

- Increased enterocyte height <20% (diminishing effect >8% in DM)
- Clear and intact brush border

Results: Small intestinal enterocyte height (6 slaughtered piglets D11 after weaning per TG)





2500Zn: No impact on enterocyte height

Did not block immune cell infiltration in small and large intestinal tissues

Some improvement in brush border integrity (compared to 0Zn)

Abolished effect of EP100i on enterocyte height (but not on brush border integrity) BA:

Conclusion 1 – Effect of EP100i (prefermented rape seed)

- EP100i compared to 0Zn:
 - Increased ADG (dose-response) from 10 days before weaning until exit from weaner unit => increased weaning and exit weights
 - Stimulated villi and enterocyte development (maximum effects at 10 and 8% DM in diet)
 - Improved small and large intestinal barrier function and small intestinal surface area
 - Increased absorptive capacity and improved resistance to bacterial infections
 - Reduced no. of lost and eliminated piglets without reducing diarrhea incidence
 - Feed induced osmotic rather than pathogen induced diarrhea
- EP100i compared to 2500Zn:
 - Higher ADG and FCR (D10 pre- to D30 post-weaning)
 - => heavier piglets at weaning and exit
 - Prevented mortality and need for antibiotics treatment to the same extent

Conclusion 2 – Effect of brown algae (BA) product: EP900 (when added to 10% EP100i diet)

- Increased piglet weight at weaning
- Decreased ADG from D31-64 after weaning and hence exit weight
- Blocked stimulating effect of EP100i on villi length and enterocyte height
 - But not the effect of EP100i on intestinal barrier function
- Beneficial in susceptible period pre-weaning?
 - Antimicrobial effect?
 - Complementing barrier function effect of EP100i?

Acknowledgements

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