USING ALGAL EXTRACTS TO IMPROVE WEANER GROWTH PERFORMANCE AND DIGESTIBILITY

Innovation Project 5A-107

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By

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Executive Summary

This experiment examined the effects of two algal seaweed extract products being developed in Australia to similar to products developed in Ireland. Reduced growth performance is commonly observed in new weaners, which can be associated with intestinal inflammation and disruptions to intestinal microbial populations. Seaweed extracts have been investigated for their bioactive components and their effects on the anti-inflammatory responses in the gut as well as their ability to enhance gut microbial populations and improve responses to bacterial challenges.

A 4 x 2 factorial experiment with two algal extracts (fucoidan and laminarin, or Ulva extracts) in four combinations and two diet formulations, a conventional diet or a high pro-inflammatory diet (higher soybean meal and n-6-n-3 PUFA ratio), was conducted. Growth performance was evaluated during 0-7 d and 0-21 d after weaning. Faecal scores were assessed on the 7th day and 20th day after weaning. Pigs from each treatment were sacrificed and intestinal samples were taken for possible subsequent analysis of inflammatory biomarkers.

The results showed that the pro-inflammatory diets slightly increased scour score, from a low level, but did not statistically affect the growth performance of the pigs. None of the algal products statistically affected growth performance or scour score. Overall, pigs achieved a good growth performance compared with industry standard. The lack of any significant performance improvement suggested that any differences in gut microbial populations or the product ion of inflammatory compounds were likely to be small, and thus the analysis of intestinal biomarkers was not carried out as per the agreed Stop:Go point.

The recommendations of this experiment are that the use of the studied algal extracts under ideal conditions may have slight differences in microbiota but do not have any effect on performance or health of the pig. Further examination of these products should be on larger groups of pigs under more challenging conditions.

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1. Introduction

The weaning stage involves many stressful factors which can lead to reduced performance and lowered gut health status. The sudden change in diet results in disruptions to gut microbial populations and can lead to inflammation, diarrhea and lower weight gain in pigs. During the first week after weaning, metabolisable energy intake may decrease by 60-70% as well as negatively impact gut structure and function (Campbell et al. 2013). When combined, these alterations to gut structure and function during this time can have short- and long-term effects on the growth and performance of pigs. Significant amounts of resources are dedicated to weaner pigs in order to optimize their post-weaning health and performance. Weaner nutrition is a significant component of this process and with the desire to use less medications, functional feed additives are likely to increase their role.

Seaweed extracts have been investigated for their bioactive components and their effects on the anti-inflammatory responses in the gut as well as their ability to enhance gut microbial populations and responses to bacterial challenges. Seaweed extracts are complex due to the large number of species and extracts available. Brown macro algae contains three unique polysaccharides, fucoidan, laminarin and alginate. Fucoidans are currently used in human health for their anti-inflammatory, antibacterial, antiviral and anti-tumour properties (Fitton et al. 2015). Déléris et al. (2016) reported that laminarin contributes to the dietary fibre intake and plays a role in cancer prevention and that laminarin has also been proposed for its antitumor effect. The challenge for commercial use of these extracts in animal production is finding a sustainable and efficient production method for high purity products.

Work using algae extracts in pigs has generally shown positive results. For example, Reilly et al. (2008), Gardiner et al. (2008) and O'Doherty et al. (2010) reported positive changes in the gut microbial populations when feeding various macro brown algae extracts to pigs. These findings led to suggestions that the use of algae extracts may be able to reduce some negative health issues caused by gut bacteria especially during times of health challenges brought on by environmental factors including stress, disease exposure and diet changes such as those experienced by pigs during the weaning process.

Working with a world-leading algal extract company in Australia, a novel extract from macro algae has been developed in conjunction with previous poultry work. This project looks to build on previous research from the University College Dublin of Professor John O'Doherty, who have developed a product sold by Bioatlantis to use as the base for developing a new product for Australian pigs using the novel extract. These previous works (as mentioned above) found changes in gut microbial populations when feeding various macro brown algae extracts to pigs, specifically a significant reduction in microbial populations in the large intestine. Within the caecum and colon, bifidobacteria and lactobacilli populations were reduced. Similarly, the enterobacteria population was significantly reduced in the caecum and colon. In addition, seaweed extract was also reported to reduce the colonic cytokines associated with inflammation in the pigs challenged with Salmonella

(Bouwhuis et al. 2017). These effects, combined with an increase in butyrate production, are suggested to help with the reduction of diarrhoea in weaning piglets.

To investigate the impacts on pig performance, gut microbial populations and inflammatory markers, the efficacy of a number of algal extracts was evaluated in post-weaning diets. Pigs were fed either a standard weaner diet or one designed to be pro-inflammatory (based on higher amounts of soybean meal and a higher n-6-n-3 PUFA ratio), with or without algal extracts. The products of algal extracts may provide a solution to improve post-weaning growth performance and reduce the severity of post-weaning diarrhoea.

2. Methodology

The experiment was approved by the Rivalea (Australia) Animal Ethics Committee (19N050C).

A total of 96 male pigs, weaned at approximately 28 days of age, was randomly allocated into individual weaner pens and assigned to 1 of 8 dietary treatments in a 4 x 2 factorial design. The experiment studied the effects of algal extracts (A, B, A+B vs control) on growth performance, post-weaning diarrhoea, and potentially markers of gut health (microbiome, inflammatory markers) of weaner pigs fed on a conventional diet or a high pro-inflammatory diet (higher soybean meal and n-6-n-3 PUFA ratio).

The treatments were designated as:

Treatment	Diet Designation	Description
1	A	Conventional Diet (including ZnO)
2	E	Pro-inflammatory Diet (including ZnO)
3	B	Conventional + Algae Extract A
4	F	Pro-inflammatory + Algae Extract A
5	C	Conventional + Algae Extract B
6	G	Pro-inflammatory + Algae Extract B
7	D	Conventional + Algae Extract A+B
8	H	Pro-inflammatory + Algae Extract A+B

Algal Extract A = Combination Laminarin and Fucoidan at the concentration of 300 ppm
Algal Extract B = Ulva at the concentration of 300 ppm
Algal Extract A+B = Combination Laminarin Fucoidan Extract and Ulva at the concentration of 300 ppm each.

Details of each of the experimental diets are displayed in Appendix 1. The Conventional diet was formulated along standard commercial specifications for a pig of 7.5-15 kg in bodyweight. It included all additives designed to maximise performance and minimise digestive issues. The diet contained a commercial level (30%) of cooked cereals with more digestible protein meals as protein sources [e.g., Nupro yeast, fishmeal, soy protein concentrate (SelectaTM soy)) and a higher level of lactose (8% versus 6%)]. The proinflammatory diet used less steam-flaked wheat and protein sources of vegetable origin such as canola meal and a high level of soyabean meal (20% v 8%). Enzymes and emulsifiers were removed from the inflammatory diet and 1.5% tallow was replaced with 3% safflower oil to maximise the omega 6 content. This resulted in the ratio of omega 6 to omega 3 being greater than the maximum level of 10:1 (12.2:1). All diets contained 1 kg/t of Zinc Plus (JEFO) and 4 kg/t of benzoic acid acidifier "Vevovital".

The study was carried out at the Weaner Discovery Centre, Rivalea, throughout the 21-day experiment. Pigs were individually housed and had ad libtitum access to feed and water. Weekly growth performance including day 0, 7, 14 and 21 day weights, and feed intake from day 0-7, 0-21, was recorded. The severity of scours was categorised based on a faecal consistency score developed by Pedersen and Toft (2011) with slight modifications; 0= normal consistency, 1=pasty consistency (mild), 2=sloppy consistency (moderate), 3=watery consistency (severe) (Appendix 2).

Statistical analysis

Differences in growth performance due to the main effects of basal diet type and algae inclusion were analysed using an analysis of variance for a factorial design. The experimental unit for the analyses was the pig. Differences in mortalities and removals and scour score were analysed using Chi-Square analyses. All analyses were performed using SPSS (version 25-SPSS Inc., Chicago, Illinois, USA). Statistical significance was accepted at P<0.05.

3. Outcomes

The average daily feed intake, average daily gain and feed efficiency evaluated over the first 7 days and over the entire 21-day experimental periods were not significantly affected by the inclusion of any of the algal products or by the diet designed to produce an inflammatory response. There was no significant interaction between diet type and algal type and thus only main effects were shown in Table 1. The overall growth performance during the first 7-day experimental period was as expected at approximately 200 g/day and achieved over 400 g/day for the entire 21-day period (Table 1).

The faecal score results from the experiment (Table 2) did show that there was a significant increase in the score (P<0.05) when piglets were fed the diets designed to increase the gut inflammatory response (pro-inflammatory diet) (Table 2.). The faecal score was not affected by any algal additives. The faecal scores in all cases were low and no significant diarrhoea was recorded on any treatment.

Table 1 Growth performance of pigs after weaning fed two different basal diets in combination with different algal extracts.

Variables	Basal diet type (B)		SEM	Algal*				SEM	*P* values		
	Conventional	Pro-inflammatory		Control	FU/LA	ULVA	Combo		Basal diet	Algal	Interaction
Body weight, d 0	8.0	8.0	0.09	8.0	8.0	8.0	8.0	0.13	1.00	1.00	1.00
Body weight, d 7	9.4	9.3	0.14	9.4	9.3	9.3	9.3	0.15	0.37	0.99	0.35
Body weight, d 21	15.7	16.0	0.30	16.2	15.9	15.8	15.7	0.43	0.44	0.84	0.33
0-7 d											
ADI, g/d	231	219	11.6	235	224	223	215	16.4	0.47	0.87	0.56
ADG, g/d	198	179	14.9	193	189	187	185	21.1	0.37	0.99	0.35
FCR	1.35	1.49	0.136	1.30	1.24	1.63	1.51	0.190	0.46	0.46	0.29
0-21 d											
ADI, g/d	425	431	15.0	445	422	422	423	21.2	0.76	0.84	0.31
ADG, g/d	368	383	14.3	390	375	371	366	20.2	0.44	0.84	0.33
FCR	1.17	1.15	0.019	1.17	1.13	1.16	1.17	0.027	0.40	0.81	0.56

Entry body weight=8.00 kg was used as a covariate when analysis all variables except for body weight (0 d).
* FU/LA – Fucoidan/Laminarin Algal extract 300 ppm, ULVA – Ulva algal extract 300 ppm, Combo – 300 ppm of each algal extract.

Table 2 Faecal Score of pigs after weaning fed two different basal diets in combination with different algal extracts (data analysed within each day point). Faecal score was assessed on a scale of 0 to 3.

Variables	Basal diet type (B)		SEM	Algal				SEM	*P* values		
	Conventional	Pro-inflammatory		Control	FULA	ULVA	Combo		Basal diet	Algal	Interaction
Day 10	0.31	0.67	0.102	0.50	0.50	0.33	0.63	0.144	**0.02**	0.56	0.86
Day 20	0.08	0.56	0.079	0.29	0.21	0.42	0.38	0.111	**<0.001**	0.56	0.56

4. Application of Research

The hypothesis of this project was that the inclusion of algal extracts laminarin/fucoidan combination and/or ulva at 300 ppm into the diet would improve growth performance and reduce scouring of weaner pigs. Data from the experiment did not support the hypothesis. Growth performance of weaned piglets during the 0-7 d or 0-21 d period was not affected by any supplementation of the algal extracts. The use of a pro-inflammatory diet to increase the challenge on the young pigs' gut did not result in a performance difference over the 21 day experimental period. The design of this diet, aiming at similar nutrient specifications in terms of amino acid ratios although different in expected digestible and net energy contents, was not sufficient to produce a performance difference, at least under the individually-housed conditions of this experiment. The ideal housing conditions tended to override the differences expected in diet nutrient digestibility and changes in performance.

Analysis of the faecal score results from this experiment indicated that the use of the proinflammatory diet significantly increased the softness of the faeces at both time points (day 10 and 20) following weaning, which might be due to an inflammatory response. It must be noted that this while this difference was significant it was not at a high level considering the average score across all treatments was less than 0.5 on a scale of 0 to 3. Thus, the level of scour was generally less than mild implying that there was not a significant amount of bacterial scour and probably related to the inflammation related to the diet. Therefore, the diet possibly had the intended response of creating an inflammatory response, but clearly did not affect the animals significantly in terms of growth performance. If the animals had been a more stressful environment with a significantly higher bacterial challenge then it is possible that a more significant scour response would have occurred and the growth performance would have been more impacted. The lack of changes in growth performance and the low level of scours seen in the experiment suggest that the impact on gut microbiome, biomarkers of the inflammatory response, and total-tract digestibility, are unlikely to be impacted significantly. As such, this analysis was not conducted.

The expectation from the literature was that the inclusion of the laminarin/fucoidan algal product extracted from brown algae would improve growth performance of the weaner pigs by at least a difference of 1 kg in liveweight after 21 days. It has also been shown by the University College Dublin group over the last 10 years that improvements in some gut microbiota populations can also occur. This experiment possibly suggests that when pigs are kept in a low stress/low challenge environment there are less major fluctuations in microbiota even with a more challenging diet and that the resulting growth rate is unaffected. The use of the algal extract from ulva species has not been extensively researched in pigs and is more commonly targeted for feeding fish. It is, though, easier to obtain that the brown seaweed extracts and thus maybe utilised at a more economical price. The ulva algal extract similarly did not show any improvements in growth rate and while it produced a better faecal score it was not significantly different from the control or the other algal extract.

We thus can conclude there was no benefit to the utilisation of this algae in the context of this experiment. The combination of the algal extracts did not show any difference in performance or scour score to the individual algal extracts or the control and thus there is likely no interaction between them.

5. Conclusion

In conclusion, the current experiment suggested that the use of the algal extracts from brown seaweed or ulva species did not improve growth performance or had any effect on scour score, and thus any major disturbances in the gut were insufficient under the ideal growing conditions of this experiment. The level of inclusion at 300 g/t of the algal products is relatively low and there is potential to look at the level of algal extract inclusion that is required to influence gut microflora and potentially growth performance under a wider range of environmental conditions.

6. Limitations/Risks

The limitations of this research were related to the experimental conditions where pigs were housed individually and the level of environmental pathogens the pigs were exposed to was likely reduced. The pigs were able to cope with the changes in gut microflora and this resulted in no performance difference.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

1. The use of algal extracts is not recommended in pigs under individually housed environments.
2. Experiments involving changes in gut microbiota may need to be larger to examine changes in performance.

8. References

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Appendix 1: Experimental Diets

Experiment Basal Diets

RM code	Name	BASAL CONTROL DIET	BASAL INFLAMATION DIET
1	WHEAT	29.8	37.95
7	STEAM FLAKED WHEAT	30	10
12	BARLEY	5	5
300	CANOLA MEAL 36%	0	8
325	SOYABEANMEAL-46%	8	20
384	NUPRO ALLTECH	2.5	0
400	MEATMEAL 58%	5	4
410	FISHMEAL 60%	2.5	0
420	BLOODMEAL	1.5	1.5
433	SELECTA SOY 600 (SOYCOMIL)	2.5	0
460	LACTOSE	8	6
500	WATER	1	1
520	TALLOW-MIXER	1.5	0
530	SAFFLOWER OIL (LINOLEIC)	0	3
551	SALT BIN ADD	0.5	0.5
576	DICALPHOS BIN ADD	0	1.4
605	DL-METHIONINE	0.16	0.12
627	VALINE H/A	0.06	0.06
774	MOULD ZAP CITRUS	0.05	0.05
950	RED MICRO-GRITS	0.1	0
951	BLUE MICRO-GRITS	0	0.1
1536	VEVOVITALL MICRO	0.4	0.4
1540	ROVABIO ADVANCE T/FLEX 25%	0.02	0
1541	QUANTUM BLUE 5G PHYTASE	0.02	0
1551	LYSINE MICRO	0.55	0.41
1553	THREONINE MICRO	0.235	0.165
1556	ZINCO PLUS MICRO	0.1	0.1
1574	ENDOX MICRO	0.02	0.02
1580	ISOLEUCINE MICRO	0.16	0
1583	TRYPTOPHAN MICRO	0.05	0.025
1584	LYSOFORTE BOOSTER MICRO	0.075	0
1599	RIVALEA PIG STARTER PREMIX MICRO	0.2	0.2
	DE_PIG MJ/Kg	15.39	14.77
	NE PIG	11.65	10.48
	PROTEIN	21.57	22.73
	FAT	3.22	4.71

STARCH	43.01	36.14
FIBRE	2.37	3.32
#ALY/DE_	0.086	0.087
#MET/LYS	0.331	0.304
#M+C/LYS	0.547	0.549
#THR/LYS	0.689	0.660
#ISO/LYS	0.655	0.573
#TRY/LYS	0.215	0.208
#VAL/LYS	0.696	0.769
ABC	457.44	652.06
N:D:F:	8.74	10.17
LINOLEIC	0.86	2.98
A:D:F:	3.13	4.98
W6 FA	0.80	1.77
W3 FA	0.14	0.14
W6:W3	5.67	12.20
SAT FA	1.12	0.61
MONO FA	1.04	1.70
POLY FA	0.94	1.64

Standard Operating Procedure for Scour Score

Aim:
To record the incidence and severity of scours and to ensure that the technique used to record severity of scours is consistent amongst observers.

Application / location

This work instruction will apply to all personnel required to score scouring piglets during the course of a research trial.

Work instruction

The scour scores are as follows (consistency of faeces):

Score 0: NORMAL

Score 1: Pasty; MILD

Score 2: Sloppy; MODERATE

Score 3: Watery; SEVERE