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Dietary supplementation of finishing pigs with the docosahexaenoic acid-rich microalgae, *Aurantiochytrium limacinum*: effects on performance, carcass characteristics and tissue fatty acid profile

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Objective: The aim of this experiment was to evaluate the effect of dietary supplementation with the docosahexaenoic acid (DHA)-rich microalgae, *Aurantiochytrium limacinum* (AURA) on pig performance, carcass traits, and the fatty acid composition of pork *Longissimus lumborum* (LL) and backfat.
Methods: A total of 144 Pig Improvement Company (PIC)×Goland finishing pigs (72 females and 72 castrated males) of mean weight 117.1 (±13.1) kg were blocked by sex and body weight and provided with 0% or 1% AURA in isonutritive and isocaloric diets. A total of 24 pens provided 12 replicates per treatment. Animals were weighed on day 0 and 28 with feed and water intake recorded per pen. After 31 days supplementation (28 days of study and 3 days until the slaughtering date) three animals per pen (n = 72) were slaughtered and the LL and backfat thickness, lean meat content and dressing percentage were recorded for the carcasses. The fatty acid (FA) profile of the LL and backfat was established by direct FA methyl ester synthesis.
Results: No differences were observed for any performance parameters or carcass traits. Supplementation with AURA resulted in significant changes to the FA profiles of both the LL and backfat with male and female pigs responding differently to supplementation in terms of particular FAs. Overall, pork LL samples had significantly higher eicosapentaenoic acid ($p<0.001$) and DHA concentrations ($p<0.001$), and higher omega-3 (n-3) FAs ($p<0.001$), as well as an increased omega3:omega6 (n-3:n-6) ratio ($p = 0.001$). For backfat, supplementation resulted in significantly higher amounts of DHA ($p<0.001$) and n-3 FAs ($p<0.001$).
Conclusion: These results indicate that dietary supplementation with 1% AURA over a 31 day period can increase the FA composition of pork LL and backfat, specifically the DHA, with no major impact on growth performance and carcass traits.

Keywords: Docosahexaenoic Acid; Algae; Polyunsaturated Fatty Acids; Enrichment; Pigs

INTRODUCTION

Humans and other animals are incapable of synthesising essential fatty acids (FA) endogenously and as such, must obtain them from their diet [1]. The essential FA are split into two categories, omega 6 (n-6) and omega 3 (n-3) FA, for which linoleic acid (LA) and α-linolenic (ALA) are the respective parent compounds [2]. The n-3 FA, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to have a positive impact on a variety of human health problems including cardiovascular disease and depressive disorders [3].

Oily fish are considered the best dietary source of polyunsaturated FA (PUFA), however

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the consumption of oily fish is low in western style diets [4]. In addition, the conversion of ALA into EPA and DHA is limited in the human body, and as such the direct consumption of EPA and DHA rich products is considered necessary to meet the recommended daily requirements of each [3]. The enrichment of commonly consumed products with n-3 FA represents a way to increase their consumption without requiring any drastic dietary changes. Plant and marine (algae and fish) oils have been added to animal feed in order to increase the n-3 FA content of animal products. The tissues of monogastric animals, such as pigs, are susceptible to FA alteration through dietary modification [5], making feed supplementation a viable option to increase n-3 FA concentrations in their products.

The enrichment of pork with PUFA following dietary supplementation with plant and marine (fish and algae) oils has been successfully demonstrated by a number of authors [6-11]. Vossen et al [11], recently showed that supplementation with ALA rich linseed did not increase the concentration of DHA and EPA in pork. As in humans, the conversion of ALA to long chain PUFA is limited in pigs [12]. As such, direct supplementation with DHA and EPA rich sources is required. Despite fish being considered the richest source of EPA and DHA, productivity limitations and the issue of overfishing mean that the n-3 FA requirements of the population cannot be met using fish products alone [13]. As such marine algae represent a rich source of DHA that can be produced at scale in a sustainable manner.

Supplementation with the marine algae *Schizochythrum* sp. has been shown to increase the levels of EPA and DHA in meat without negatively impacting the productivity of the animals [5,10,14,15]. Little information is available regarding dietary supplementation of pigs with other sources of algae with the potential to be heterotrophically grown, especially in low sodium conditions. Recently, the microalga, *Aurantiochytrium limacinum*, was fed at low inclusion concentrations, 0.25% and 0.5%, to pigs for the entire grower-finisher phase, 121 days resulting in *Longissimus lumborum* (LL) and backfat enrichment with DHA [16,17]. However, the commercial application of algae for a prolonged feeding period may not be practical for most farms. Therefore, the aim of this study was to investigate the effect of providing a moderate level of algae supplementation for the final month prior to slaughter on the potential to enrich DHA in the LL and backfat without influencing the productivity of finishing pigs.

MATERIALS AND METHODS

Animals, experimental design and diets

The study was carried out at the CERZOO S.r.L. Research Centre (Piacenza, Italy) in compliance with G.L.P guidelines (Directives 2004/9/EC and 2004/10/EC) for the collection, handling and documentation of data. The research protocol and animal care were carried out in accordance with European guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU). A total of 144 Pig Improvement Company (PIC)×Goland finishing pigs (72 female and 72 castrated males) of mean weight 117.1 (±13.1) kg were recruited for the study. The animals were housed in four growing-finishing rooms, with a total of 24 pens providing 12 replicates (6 female and 6 castrated male) for each of the two treatments. The growing-finishing rooms were equipped with a dynamic ventilation system with heating provided by water aerotherms and positive pressure ventilation achieved by variable speed fans linked to temperature sensors which automatically responded to maintain an appropriate temperature based on the age and temperature of the pigs. Pigs were fed *ad libitum* via a steel feeder present in each pen and were provided with free access to water. On arrival at the facility, the pigs were allowed to acclimatise for a period of seven days after which they were weighed, sorted by sex and weight and allocated to pens such that each pen contained small medium and heavy, female or castrated male pigs. The pigs were then fed the experimental diets for a period of 31 days (28 days study duration followed by three days until the slaughtering date).

Animals were assigned to 1 of 2 isonitrogenous and isoenergetic diets (Table 1): control diet or treatment diet supplemented with 1% heterotrophically grown unextracted *A. limacinum* (AURA; CCAP 4087/2) biomass, provided by Alltech Inc. (ALL-G-RICH, Nicholasville, KY, USA). The analytical composition of the microalgae was determined prior to the start of the study (MVTL, New Ulm, MN, USA) in compliance with current Good Laboratory Practices (GLP) guidelines: crude protein (AOAC 990.03), crude fat (AOAC 954.02), FA composition (AOAC 996.06), moisture (AOAC 930.15), and ash (AOAC 942.05). The nutrient composition of the experimental diets was established using the following standardised methods: dry matter (DM)/moisture (ISO 6496); crude protein (ISO 5983-1); crude fat (ISO 6492); crude fibre (ISO 6865); crude ash (NEN 3329; ISO 5984-2002); starch (ISO 10520); the digestible (DE) and net energies (NE) of the diets were calculated according to Noblet and Perez [18] and Noblet et al [19] respectively. DHA was determined after direct FA methyl ester synthesis according to the procedure as described by O'Fallon et al [20].

Performance parameters and carcass measurements

Pigs were individually weighed on d 0 and d 28 of the study. Feed and water intake was recorded for each pen from d 0 to d 28. Average daily gain (ADG) and gain:feed (G:F) ratio was calculated per pen. Three pigs per pen were selected based on the mean weight for the treatment group, providing a total of 72 animals for slaughter (18 female and 18 castrated males for each experimental diet). LL and backfat thicknesses were measured, where lean meat content was determined using a

Table 1. Ingredient composition (%) and calculated analysis of the control and docosahexaenoic acid-rich microalgae (AURA) diets

Item	Control	1% AURA[1)]
Ingredient composition		
Corn meal (%)	55.00	54.95
Soybean meal 48%	13.40	13.10
Barley meal (%)	14.00	14.00
Wheat bran (%)	13.00	13.00
Hydrogenated fat of palm oil (%)	2.00	1.35
Dicalcium phosphate (%)	0.40	0.40
Calcium carbonate (%)	1.20	1.20
L-lysine HCl 99%	0.40	0.40
Sodium chloride (%)	0.25	0.25
Premix grower/finisher[2)] (%)	0.20	0.20
L-threonine (%)	0.12	0.12
DL-methionine 99%	0.03	0.03
AURA (%)	0	1.00
Analytical characteristics		
Dry matter (%)	89.92	89.93
Crude protein (%)	16.63	16.60
Crude fibre (%)	4.38	4.36
Crude fat (%)	5.93	5.91
Starch (%)	53.13	53.07
Total lysine (%)	1.08	1.07
Total methionine (%)	0.30	0.30
Total threonine (%)	0.72	0.71
Total tryptophan (%)	0.18	0.18
Calcium (%)	0.72	0.72
Total phosphorus (%)	0.58	0.57
Sodium (%)	0.13	0.13
Digestible energy[3)] (kcal/kg)	3,713	3,708
Net energy[3)] (kcal/kg)	2,703	2,705

DE, digestible energy; CP, crude protein; EE, ether extract; CF, crude fibre; NE, net energy; ST, starch.

[1)] AURA, unextracted *Aurantiochytrium limacinum* algae containing 18.0 g DHA/100 g.

[2)] Content of vitamins and oligo minerals/kg feed: vit A, 15,000 IU; vit D_3, 1,500 IU; vit E, 20 mg; vit B_1, 2 mg; vit B_2, 0.38 mg; vit B_6, 2 mg; vit B_{12}, 0.015 mg; vit H, 40 mg; vit K, 2 mg; vit PP, 25 mg; vit H, 0.10 mg; D-pantothenic acid, 10 mg; choline chloride, 375 mg; manganese oxide, 60 mg; iron carbonate, 200 mg; copper sulphate, 20 mg; zinc oxide, 75 mg; Co, 0.75 mg; potassium iodide, 2.4 mg; sodium selenite, 0.30 mg.

[3)] The digestible energies of the diets were calculated according to Noblet and Perez [18] using the following equation: DE = 4,151–(122 × %ash)+(2 3.2 × %CP)+(38.2 × %EE)–(64.2 × %CF). The net energies of the diets were calculated according to Noblet et al [19] using the following equation: NE = 2,796+4.15 × EE+0.81 × ST–7.07 × ash–5.38 × CF.

Fat-O-Meter (SFK Technology, Herlev, Denmark) optical probe positioned 8 cm to the side of the central line of the carcass between the third and fourth last ribs, in accordance with the Commission Implementing Decision of 24 January 2014 on authorizing methods for grading pig carcasses in Italy (2014/38/EU). Carcasses were classified as per the SEUROP carcass classification system (Reg. 2013/1308/EU). LL muscle and backfat Samples were removed from the right side of each animal, within 15 min post-slaughter, between the third and fourth last ribs, placed in vacuum bags and immediately frozen and stored at –18°C until FA analysis.

The FA profiles of carcass samples were determined at the Institute of Food Science and Nutrition (Faculty of Agricultural Sciences, Food and Environment, Catholic University of Sacred Heart, Piacenza, Italy). Samples were thawed, ground and homogenized for subsequent FA profile analysis after direct FA methyl ester synthesis according to the procedure as described by O'Fallon et al [20]. The complete FA profiles included short-chain FAs (C4.0 through C8.0), medium-chain FAs (C10.0 through C15.1), long-chain FAs (C16.0 through C22:6n3), and very long-chain FA (C24:0 and C24:1). The total omega-3 FA composition was calculated as the ∑(α-linolenic acid [C18:3n3]+*cis*-11,14,17-eicosatrienoic acid [C20:3n3]+EPA [C20:5n3]+DHA [C22:6n3]). The total omega-6 FA composition was calculated as the ∑(linolelaidic acid [C18:2n6*trans*]+linoleic acid [C18:2n6cis]+γ-linolenic acid [C18:3n6]+*cis*-8,11,14-eicosatrienoic acid [C20:3n6]+arachidonic acid [C20:4n6]).

Statistical analysis

Data were analysed by the general linear model (GLM) procedure of SAS (2010, release 9.3) using analysis of variance (ANOVA) as the main statistical test. For the performance parameters the pen was the experimental unit with 24 replicates blocked by sex (12 castrated male pens and 12 female pens). For each parameter the GLM was fitted with the following terms: Treatment (Control, AURA); Sex (Castrated male, female); plus the treatment×sex interaction. For the carcass characteristics and the FA profile the pig was the experimental unit with a total of 72 replicates (36 castrated male, 36 female). When a significant treatment×sex interaction was detected, the sexes were analysed separately by GLM to investigate the effect of treatment on either castrated male or female pigs. Students "t" and Tukey tests were used to compare the means of each group. For the ANOVA model, $p \leq 0.05$ indicated a significant difference while $0.05 < p \leq 0.10$ indicated a trend.

RESULTS

Diet analysis, performance and carcass characteristics

The test article, AURA, used in the study primarily consisted of 70.2 g crude fat/100 g DM biomass composed of a significant level of palmitic acid and DHA, 34.5 g and 18.0 g/100 g DM biomass respectively. Additionally, AURA contained 15.8% crude protein, 3.9% ash, and 2.3% moisture. The EPA accounted for only 0.23 g/100 g DM biomass. The chemical composition, energy value and DHA content of the experimental diets are show in Table 2. The pigs maintained good health throughout the study with no animals being removed or culled for welfare reasons. Treatment with AURA had no effect on ADG, average daily feed intake, average daily water

Table 2. Analytical characteristics of the finishing pig experimental diets on a dry matter basis

Item	Control	1% AURA[1)]
Dry matter (%)	89.22 ± 0.18	89.37 ± 0.16
Crude protein (%)	16.39 ± 0.13	16.53 ± 0.08
Crude fibre (%)	4.22 ± 0.09	4.11 ± 0.02
Crude fat (%)	5.77 ± 0.13	5.78 ± 0.04
Starch (%)	51.99 ± 0.21	52.46 ± 0.03
Digestible energy[2)] (kcal/kg)	2,978 ± 20	3,024 ± 21
Net energy[2)] (kcal/kg)	2,503 ± 5	2,510 ± 4
DHA (g/kg)	0	1.62 ± 0.08

DHA, docosahexaenoic acid; DE, digestible energy; CP, crude protein; EE, ether extract; CF, crude fibre; NE, net energy; ST, starch.
[1)] AURA, unextracted *Aurantiochytrium limacinum* algae containing 18.0 g DHA/100 g.
[2)] The digestible energies of the diets were calculated according to Noblet and Perez [18] using the following equation: DE = 4,151–(122 × %ash)+(23.2 × %CP)+(38.2 × %EE)–(64.2 × %CF). The net energies of the diets were calculated according to Noblet et al [19] using the following equation: NE = 2,796+4.15 × EE+0.81 × ST–7.07 × Ash–5.38 × CF.

intake, or G:F ratio (Table 3). No differences were observed between the groups in terms of LL thickness, backfat thickness, lean meat content or dressing out (Table 3). Carcass evaluation according to the SEUROP system is provided in Table 4 and indicates the expected difference in lean meat percentage between the sexes.

Fatty acid profile

Supplementation with AURA resulted in significantly higher levels of the following FAs in the pork LL samples: C8:0, C10:0, C14:0, C15:0, C16:0, C18:0, C18:1n9 cis, C18:2n6c, C18:3n3, C20:2, C22:1n9, C24:0, EPA, and DHA (Table 5). Animals supplemented with AURA had higher PUFA, n-3, and n-6 FAs, in addition to an increased n-3:n-6 ratio. The GLM results indicated an interaction between sex and treatment for some FAs and as such the male and female pigs were analysed separately to explore the sex specific effects. The FA profile of the

Table 4. Effect of supplementation of a finisher pig diet for 31 days with a docosahexaenoic acid rich microalgae (AURA) at 1% on EUROP carcass classification[1)]

EUROP score	Lean meat content (%)	Control		1% AURA[2)]	
		n	%	n	%
All animals (n = 36)					
S	≥60	0	0	0	0
E	55-60	5	14	7	20
U	50-55	18	50	18	50
R	45-50	11	30	8	22
O	40-45	1	3	3	8
P	<40	1	3	0	0
Females (n = 18)					
S	≥60	0	0	0	0
E	55-60	5	28	5	28
U	50-55	8	44	8	44
R	45-50	5	28	3	17
O	40-45	0	0	2	11
P	<40	0	0	0	0
Males (n = 18)					
S	≥60	0	0	0	0
E	55-60	0	0	2	11
U	50-55	10	55	10	55
R	45-50	6	33	5	28
O	40-45	1	6	1	6
P	<40	1	6	0	0

[1)] EUROP classification, Regulation 2013/1308/EU.
[2)] AURA, unextracted *Aurantiochytrium limacinum* algae containing 18.0 g DHA/100 g.

Table 3. Effect of supplementation with a docosahexaenoic-rich microalgae (AURA) at 1% of diet on performance parameters and carcass characteristics of finishing pigs

Item	Control	1% AURA[1)]	Standard error	p-value		
				Treatment effect[2)]	Sex effect[2)]	T×S interaction[2)]
Performance parameters[3)]						
Weight d 0 (kg)	117.7	116.5	2.89	0.69	0.08	0.90
Weight d 28 (kg)	141.2	140.3	2.89	0.75	0.11	0.82
Average daily gain (g)	838.3	847.7	40.5	0.82	0.73	0.37
Daily feed intake (kg)	3.405	3.392	0.02	0.51	0.56	0.70
Daily water intake (kg)	19.58	20.48	0.92	0.34	0.87	0.50
G:F ratio	4.10	4.05	0.01	0.74	0.66	0.48
Carcass characteristics[4)]						
Backfat thickness (mm)	30.31	30.33	1.53	0.99	0.09	0.57
Longissimus lumborum thickness (mm)	51.86	50.14	2.42	0.48	0.33	0.27
Lean meat content (%)	51.00	50.80	0.91	0.82	0.06	0.40
Dressing (%)	76.92	76.59	0.43	0.59	0.17	0.08

[1)] AURA, unextracted *Aurantiochytrium limacinum* algae containing 18.0 g DHA/100 g.
[2)] Effect of treatment, sex and the treatment × sex interaction reported from the general linear model investigating the effect of treatment on performance parameters and carcass characteristics.
[3)] Performance parameters analysed on a per pen basis (12 replicates, 6 castrated males, and 6 females).
[4)] Carcass characteristics analysed for 3 pigs per pen (72 replicates, 36 castrated males, and 36 females).

Table 5. Pork *Longissimus lumborum* (LL) fatty acid (FA) profile (mg/100 g tissue) for control and AURA[1] supplemented pigs

Fatty acid[2]	All pigs					Female pigs				Male pigs			
	Control	AURA	SEM	Treat[3]	T×S[3]	Control	AURA	SEM	Treat	Control	AURA	SEM	Treat
C8:0	1.0	1.3	0.1	0.003	0.094	0.9	1.3	0.1	0.019	1.2	1.4	0.1	0.065
C10:0	10.3	12.8	0.6	0.005	0.079	9.2	12.0	0.8	0.021	11.4	13.5	0.9	0.092
C12:0	13.3	15.0	0.7	0.113	0.091	11.7	14.7	1.0	0.040	14.9	15.3	1.1	0.813
C14:0	156.1	183.3	7.9	0.017	0.071	138.2	178.5	11.0	0.014	174.0	188.2	11.3	0.382
C14:1	1.9	2.2	0.1	0.092	0.032	1.6	2.0	0.2	0.083	2.2	2.3	0.1	0.564
C15:0	6.0	7.0	0.3	0.033	0.602	5.7	7.3	0.5	0.040	6.2	6.8	0.5	0.384
C16:0	2,380.4	2,765.3	99.9	0.008	0.038	2,131.9	2,683.1	131.3	0.006	2,628.9	2,847.5	150.6	0.312
C16:1	216.7	245.7	11.7	0.083	0.043	190.3	231.4	16.7	0.092	243.0	260.1	16.3	0.463
C17:0	28.1	32.3	1.5	0.054	0.963	27.7	32.2	2.1	0.148	28.5	32.4	2.1	0.205
C18:0	1078	1,244.8	43.1	0.008	0.197	1005	1,215.2	55.4	0.011	1151	1,274.3	66.1	0.196
C18:1 trans	19.7	18.3	0.6	0.086	0.798	20.1	18.1	0.8	0.098	19.4	18.5	0.8	0.445
C18:1n9 cis	3,592.8	4,234.4	152.4	0.004	0.063	3,249.1	4,109.4	188.0	0.003	3,936.5	4,359.4	239.8	0.221
C18:1 cis11	269.5	285.3	9.9	0.267	0.068	248.0	276.0	12.5	0.122	291.1	294.5	15.4	0.878
C18:2n6c	1,131.6	1,314.8	48.9	0.010	0.185	1,058.8	1,369.5	68.7	0.003	1,204.3	1,260.1	69.7	0.575
C18:3n3 (ALA)	49.6	59.3	2.5	0.007	0.182	45.1	60.5	3.5	0.004	54.1	58.2	3.5	0.415
C18:3n6	3.0	3.3	0.1	0.203	0.364	3.1	3.5	0.2	0.182	3.0	3.1	0.2	0.782
C20:0	8.4	9.4	0.4	0.065	0.015	7.4	9.1	0.4	0.010	9.5	9.7	0.6	0.764
C20:1	59.5	64.7	2.6	0.164	0.010	51.6	62.5	2.7	0.008	67.3	66.8	4.4	0.930
C20:2	42.8	48.0	1.9	0.050	0.125	39.3	49.6	2.2	0.002	46.3	46.5	3.0	0.963
C20:3n3	59.9	58.9	0.8	0.360	0.000	62.1	61.6	1.1	0.761	57.7	56.2	1.0	0.314
C20:3n6	13.7	14.3	0.4	0.254	0.534	13.4	14.7	0.5	0.092	14.0	14.0	0.6	0.985
C20:4n6	10.0	11.3	0.5	0.050	0.054	8.8	11.4	0.6	0.003	11.1	11.2	0.8	0.955
C20:5n3 (EPA)	2.1	3.4	0.1	<0.001	0.144	2.0	3.2	0.1	<0.001	2.2	3.5	0.1	<0.001
C22:1n9	1.2	1.6	0.1	0.000	0.153	1.1	1.6	0.1	0.002	1.4	1.0	0.1	0.056
C22:6n3 (DHA)	9.0	31.2	0.9	<0.001	0.803	9.2	31.7	1.4	<0.001	8.8	30.6	1.1	<0.001
C24:0	12.9	11.7	0.3	0.015	0.276	12.9	12.2	0.4	0.324	12.9	11.1	0.5	0.020
ΣShort chain[4]	1.0	1.3	0.1	0.003	0.094	0.9	1.3	0.1	0.019	1.2	1.4	0.1	0.065
ΣMedium chain[4]	187.6	220.3	9.5	0.018	0.079	166.5	214.5	13.3	0.015	208.7	226.1	13.6	0.375
ΣLong chain[4]	8,989.1	10,458.1	353.9	0.005	0.072	8187	10,256.6	449.0	0.003	9,791.2	10,659.5	547.1	0.270
ΣSaturated FA	3,694.6	4,282.9	149.6	0.007	0.059	3,350.8	4,165.5	194.0	0.005	4,038.5	4,400.2	227.8	0.269
ΣUnsaturated FA	5,483.1	6,396.8	219.9	0.005	0.093	5,003.7	6,306.9	280.1	0.002	5,962.5	6,486.7	339.0	0.282
ΣMonounsaturated FA	4,161.4	4,852.2	173.5	0.006	0.055	3,761.9	4,701.1	216.6	0.004	4,560.9	5,003.2	271.2	0.257
ΣPolyunsaturated FA	1,321.7	1,544.6	54.8	0.005	0.193	1,241.8	1,605.7	76.3	0.002	1,401.6	1,483.5	78.7	0.467
Omega 3[5]	120.7	152.8	3.5	<0.001	0.395	118.5	157.1	4.8	<0.001	122.8	148.5	5.1	0.001
Omega 6[6]	1,158.3	1,343.8	49.7	0.010	0.185	1,084.1	1,399.1	69.7	0.003	1,232.5	1,288.4	71.0	0.581
Omega 3/Omega 6	0.106	0.117	0.0023	0.001	0.094	0.111	0.116	0.003	0.358	0.102	0.119	0.003	<0.001

SEM, standard Error of the mean; ALA, α-linolenic acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FA, fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids.

[1] AURA, unextracted *Aurantiochytrium limacinum* algae containing 18.0 g DHA/100 g.

[2] FA below the level of detection are not reported and include the following: C4:0, C6:0, C11:0, C13:0, C:15:1, C17:1, C18:2n6t, C18:2 c9,t11–CLA, C19:0, C21:0, C22:0, C22:C24:1.

[3] Effect of treatment and the treatment × sex interaction reported from the general linear model investigating the effect of treatment on fatty acid profile.

[4] Short-chain fatty acids: C4.0 to C8.0; medium-chain fatty acids: C10.0 to C15.1; long-chain fatty acids: C16.0 to C22:6n3.

[5] The total omega-3 fatty acid composition was calculated as the ∑(α-linolenic acid [C18:3n3]+*cis*-11,14,17-eicosatrienoic acid [C20:3n3]+EPA [C20:5n3]+DHA [C22:6n3]).

[6] The total omega-6 fatty-acid composition was calculated as the ∑(linolelaidic acid [C18:2n6*trans*]+linoleic acid [C18:2n6*cis*]+γ-linolenic acid [C18:3n6]+*cis*-8,11,14-eicosatrienoic acid [C20:3n6]+arachidonic acid [C20:4n6]).

AURA supplemented female pigs showed increased concentrations of the following FAs: C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C18:1n9 cis, C18:2n6c, C18:3n3, C20:0, C20:1, C20:2, C20:4n6, C22:1n9, EPA, and DHA. In contrast, the FA profile of the AURA supplemented male pigs differed only in terms of a higher EPA and DHA content and a lower C24:0 content in the LL meat. Both male and female pigs had significantly higher n-3 FA levels following AURA supplementation. Female pigs had a greater n-6 content while male pigs did not. As such, the n-3:n-6 ratio for the female pigs did not differ between control and supplemented groups while and increase in the ratio was observed for the male pigs (Table 6).

Table 6. Pork backfat fatty acid profile (mg/100 g tissue) for control and AURA supplemented pigs

Fatty acid[1]	All pigs					Female				Male			
	Control	AURA[2]	SEM	Treat[3]	T×S[3]	Control	AURA	SEM	Treat	Control	AURA	SEM	Treat
C8:0	4.4	4.5	0.1	0.848	0.350	4.4	4.3	0.1	0.646	4.4	4.6	0.1	0.452
C10:0	45.8	47.5	0.8	0.163	0.018	45.3	45.1	1.3	0.916	46.4	49.9	1.1	0.032
C12:0	109.4	105.6	3.0	0.373	0.648	112.2	105.8	3.9	0.253	106.6	105.3	4.6	0.848
C14:0	1,074.9	1,075.5	18.2	0.981	0.911	1,081.1	1,070.7	28.5	0.797	1,068.6	1,080.3	22.7	0.718
C14:1	15.0	15.2	0.4	0.771	0.024	14.4	14.3	0.5	0.987	15.7	16.0	0.6	0.681
C15:0	57.5	61.0	2.0	0.211	0.069	62.1	62.1	3.4	0.990	52.9	60.0	2.1	0.024
C16:0	16,285.9	16,422.6	225.7	0.670	0.970	16,274.5	16,367.6	349.3	0.852	16,297.2	16,477.5	286.1	0.659
C16:1	1,376.8	1,374.3	35.4	0.960	0.276	1,338.8	1,331.3	48.6	0.914	1,414.9	1,417.4	51.6	0.973
C17:0	297.0	306.1	11.4	0.576	0.028	328.3	306.1	19.1	0.418	265.7	306.1	12.5	0.028
C18:0	7,993.5	8,150.9	181.8	0.542	0.634	8,158.5	8,206.8	254.3	0.894	7,828.4	8,095.1	259.9	0.473
C18:1 trans	188.5	169.9	4.2	0.003	0.001	202.9	178.8	6.4	0.012	174.1	160.9	5.4	0.097
C18:1n9 cis	27,616.9	27,488.3	352.8	0.798	0.568	27,955.3	27,654.4	513.4	0.681	27,278.5	27,322.3	484.2	0.949
C18:1 cis11	1,868.1	1,851.4	29.8	0.693	0.895	1,859.4	1,840.4	42.3	0.753	1,876.9	1862.4	42.0	0.809
C18:2n6c	10,505.8	10,471.5	174.2	0.890	0.040	10,771.9	10,838.3	255.7	0.855	10,239.7	10,104.7	236.6	0.689
C18:3n3 (ALA)	483.0	485.4	8.3	0.839	0.577	488.4	492.2	12.2	0.823	477.6	478.5	11.3	0.955
C18:3n6	20.9	19.9	0.5	0.166	0.052	22.0	20.7	0.7	0.228	19.9	19.1	0.8	0.446
C20:0	94.3	100.4	2.7	0.119	0.810	92.7	101.3	3.9	0.129	95.8	99.4	3.8	0.504
C20:1	513.1	506.1	11.3	0.661	0.783	505.5	508.2	12.1	0.874	520.7	503.9	19.1	0.538
C20:2	422.7	428.5	12.3	0.739	0.304	417.0	446.9	19.5	0.287	428.3	410.2	15.1	0.400
C20:3n3	151.2	146.9	3.0	0.311	<0.001	163.3	155.5	4.7	0.243	139.2	138.4	3.8	0.877
C20:3n6	76.8	73.4	1.7	0.163	0.023	79.8	77.2	2.3	0.413	73.8	69.6	2.5	0.527
C20:4n6	97.8	95.8	2.0	0.489	0.314	97.5	98.8	2.5	0.706	98.0	92.8	3.1	0.240
C20:5n3 (EPA)	0.0	0.0	0.0	n/a	n/a	0.0	0.0	n/a	n/a	0.0	0.0	n/a	n/a
C22:1n9	11.3	13.4	0.4	0.002	0.692	11.6	13.1	0.7	0.116	11.1	13.6	0.6	0.003
C22:6n3 (DHA)	61.6	192.4	6.6	<0.001	0.986	62.6	191.8	10.9	<0.001	60.6	192.9	7.3	<0.001
C24:0	57.6	53.6	1.4	0.043	0.023	60.7	56.0	1.8	0.067	54.6	51.1	2.2	0.271
ΣShort chain[4]	4.4	4.5	0.1	0.848	0.350	4.4	4.3	0.1	0.646	4.4	4.6	0.1	0.452
ΣMedium chain[4]	1,302.6	1,304.8	22.4	0.949	0.820	1,315	1,298	34.9	0.733	1290.1	1,311.5	28.0	0.593
ΣLong chain[4]	68,122.8	68,350.7	783.0	0.838	0.493	68,890.8	68,885.5	1141.1	0.997	67,354.8	67,816	1,072.4	0.763
ΣSaturated FA	26,020.2	26,327.6	400.1	0.589	0.883	26,219.9	26,325.8	609.4	0.903	25,820.5	26,329.4	518.5	0.492
ΣUnsaturated FA	43,409.6	43,332.4	491.3	0.912	0.285	43,990.4	43,862	685.7	0.896	42,828.9	42,802.7	703.9	0.979
ΣMUFA	31,589.8	31,418.6	399.1	0.763	0.723	31,887.8	31,540.7	582.5	0.676	31,291.8	31,296.5	545.8	0.995
ΣPUFA	11,819.8	11,913.8	195.8	0.736	0.047	12,102.5	12,321.4	286.4	0.593	11,537.1	11,506.1	267.1	0.935
Omega 3[5]	695.8	824.6	14.9	<0.001	0.291	714.2	839.5	23.6	0.001	677.4	809.8	18.2	<0.001
Omega 6[6]	10,701.3	10,660.6	177.2	0.871	0.039	10,971.3	11,035	259.4	0.863	10,431.4	10,286.2	241.3	0.673
Omega 3/Omega 6	0.007	0.077	0.001	<0.001	0.102	0.065	0.076	0.0009	<0.001	0.065	0.079	0.001	<0.001

SEM, standard error of the mean; ALA, α-linolenic acids, EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FA, fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids.

[1] FA below the level of detection are not reported and include the following: C4:0, C6:0, C11:0, C13:0, C:15:1, C17:1, C18:2n6t, C18:2 c9,t11–CLA, C19:0, C21:0, C22:0, C22: C24:1.

[2] AURA, unextracted *Aurantiochytrium limacinum* algae containing 18.0 g DHA/100 g.

[3] Effect of treatment and the treatment × sex interaction reported from the general linear model investigating the effect of treatment on fatty acid profile.

[4] Short-chain fatty acids: C4.0 to C8.0; medium-chain fatty acids: C10.0 to C15.1; long-chain fatty acids: C16.0 to C22:6n3.

[5] The total omega-3 fatty acid composition was calculated as the ∑(α-linolenic acid [C18:3n3]+*cis*-11,14,17-eicosatrienoic acid [C20:3n3]+EPA [C20:5n3]+DHA [C22:6n3]).

[6] The total omega-6 fatty-acid composition was calculated as the ∑(linolelaidic acid [C18:2n6*trans*]+linoleic acid [C18:2n6*cis*]+γ-linolenic acid [C18:3n6]+*cis*-8,11,14-eicosatrienoic acid [C20:3n6]+arachidonic acid [C20:4n6]).

The FA profile of the backfat indicated significantly higher levels of C22:1n9 and DHA and lower levels of C18:1 trans, and C24:0 in the AURA supplemented animals. Overall a significantly higher n-3 content was found in the backfat in addition to an increased n-3:n-6 ratio. Again, the analysis indicated an interaction between sex and treatment for some FAs and as such each sex was analysed separately. Female pigs had significantly lower C18:1 trans and higher DHA. Male pigs had greater levels of C:10, C15:0, C17:0, C22:1n9, and DHA than the control group. Both male and female pigs had an increased n-3 concentration and a higher n-3:n-6 ratio.

DISCUSSION

Supplementation with AURA had no effect on the production parameters or on the measured carcass characteristics, which is generally in agreement with other studies that have fed microalgae [5,10,16,17,21], plant oils [22,23] or fish oils [6,24] to manipulate the FA profile of pork products. Incorporation of 1% AURA into the diet of pigs with a mean initial weight of 117.1 (±13.1) kg, for a period of 31 days resulted in significant changes to the FA profile of pork LL and backfat. Pork LL samples from the AURA-fed pigs differed significantly in terms of their EPA and DHA content ($p<0.0001$), with treated animals having 62% and 247% greater EPA and DHA respectively, than the control group. For the backfat samples, AURA treatment had a significant effect on the level of DHA (with an increase of 218% compared to controls) but did not lead to an increase in EPA concentration. Overall, pork LL and backfat were enriched to a level of 31.2 and 192.4 mg of DHA/100 g respectively. The effect of dietary supplementation of pigs with AURA had previously been investigated by Moran et al [17,18] over longer periods (117 and 121 days), using animals with lower initial weights (approximately 20 to 30 kg) than the current study. In both previous trials, similar increases in LL DHA content (approximately 3X the control) were observed for pigs supplemented with 0.25% AURA. In the current study, a similar level of enrichment was achieved after 31 days of supplementation with 1% AURA. Vossen et al [12], who investigated the effects of dietary supplementation with *Schizochytrium* (0.3% to 2.2% algae) for a period of 45 days found similar levels of enrichment to the current study, reporting 10 to 20 mg of DHA/100 g of pork *longissimus thoracis*. Sardi et al [11] also used *Schizochytrium* to supplement the diet of pigs and demonstrated increases in the DHA content of LL from 100% to 250%. Supplementation with 2.5% algae for a period of 56 or 28 days led to similar levels of LL enrichment (50 mg and 40 mg DHA/100 g LL). A significantly higher LL DHA content was achieved with supplementation at a level of 5% for 28 days (70 mg/100 g LL). These studies indicate that supplementing a higher concentration for a shorter period of time can achieve similar or increased levels of enrichment than longer term supplementation with lower concentrations.

In contrast to the current study, Sardi et al [11] found no increase in the EPA content of LL or backfat. In a seperate study, in which pigs were supplemented with a large dose of 9,400 mg DHA/d, the authors showed a significant increase in both EPA and DHA content of both the LL and backfat [21]. The differences in the EPA content of LL and backfat observed in the current study could be influenced by a number of factors. Firstly, the concentration of ALA, the parent compound of EPA and DHA, differed significantly between the control and treatment LL samples but did not differ in the backfat samples. Smink et al [13] demonstrated that higher concentrations of ALA significantly increased EPA levels in intramuscular fat, but had no effect in the backfat, which may explain the increased concentration of EPA in pork LL, but its absence from the backfat in the current study. In addition, following dietary DHA supplementation of pigs, the expression of FA synthesis genes has been shown to increase significantly, in the muscle and liver tissue but not in fat [21]. Secondly, the supplemental levels of ALA and EPA detected in AURA were relatively low (0.02 and 0.23 g/100 g DM, respectively). As n-3 FAs are preferentially incorporated into phospholipids over triacylglycerol, the low level of supplementation with ALA and EPA might help explain their relative increases in the phospholipid rich muscle as opposed to the triacylglycerol rich backfat [25]. However, it is unlikely that these are the only causes for the increased concentration of EPA in the LL. Vossen et al [12], also observed an increase in the concentration of EPA that could not have come directly from the diet. The authors suggested that higher EPA concentrations in algae supplemented pigs could be attributed to DHA retro-conversion, a minor metabolic pathway which involves one cycle of β-oxidation [26].

Both previous studies by Moran and colleagues [17,18], indicated that higher levels of AURA supplementation (0.5%) may affect the sexes differently. While the LL DHA content of both males and females significantly increased relative to the controls, the LL of the males was enriched to a higher degree (5× vs 4.5× and 4× vs 2.9×; [17], [18], respectively). In contrast, no treatment×sex interaction was detected for the pork LL or backfat DHA content in the current study, indicating that the sexes responded similarly under these conditions. However, treatment×sex interactions were observed for a number of other FAs. In the LL samples of female pigs, the FA composition was significantly different from the control for 17 FAs, while only three significant differences in the male FA profile were observed. In the backfat of female pigs, the only changes detected involved c18:1 trans and DHA, while in male pigs the level of five different FAs differed from the control animals. The FA composition of pigs is mainly influenced by nutrition, however, other factors such as genotype, sex, age and the total amount of carcass fat also impact the individual FA composition [25]. In humans and mice, sex hormones are thought to play a role in the metabolism of n-3 PUFAs [27]. Higher DHA concentrations in the tissues and plasma of women have been attributed to greater levels of DHA synthesis [28]. These studies indicate that the sex hormones may influence FA metabolism and could explain the difference in response to supplementation observed in this study. Overall, EPA and DHA both increased to similar degrees in male and female pigs. In pork LL, an increase in the n-3:n-6 ratio was not observed for the female pigs, likely due to the increase in the level of n-6 FA observed in this group. For the backfat however, no significant increase in n-6 FAs led to similar n-3:n-6 ratios for the both males and females.

CONCLUSION

These results indicate that dietary supplementation with 1% AURA over a 31-day period can increase the FA composition of pork LL and backfat, specifically the DHA, with no major impact on growth performance and carcass traits. Both female and castrated male pigs responded similarly to AURA supplementation in terms of the DHA content of the LL and backfat but differed in terms of a number of other FAs. These results support the use of AURA as an effective, sustainable method by which to increase the n-3 content of pork.

CONFLICT OF INTEREST

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