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EFFECT OF DIETARY INCORPORATION OF MICROALGA *(Nannochloropsis oceanica)* ON GROWTH, NUTRIENT DIGESTIBILITY AND MEAT QUALITY IN GROWING RABBITS

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ABSTRACT

This study was designed to test the effect of microalga *(Nannochloropsis oceanica)* inclusion in diets of growing rabbits over production performances, digestibility of nutrients and meat quality. The experiment was performed on forty post-weaned rabbits with an initial live weight of 974 ± 80 g. Animals were fed with two different diets for five weeks: control and microalga (4.45% *Nannochloropsis oceanica* inclusion as a replacement of whole soybean). Each group of 10 rabbits was fed during the whole experimental period with control diet (N0) or microalga diet (N5) and with control and in the last two weeks (N2) or only in the last week of the experiment with microalga diet (N1). Each rabbit was housed individually in a metabolic cage. Faeces were collected to evaluate feed digestibility. At the end of experimental period, all rabbits were slaughtered following standard procedures. Meat samples were collected to determinate colour, chemical composition, lipid oxidation and fatty acid profile. Growth performances were not affected by the different treatments (P>0.05). Digestive utilization coefficients were similar between control and microalga diets for the nutritional fractions studied with exception for crude fat (less than 3.6 percentage units for microalga diet comparing to control diet). The slaughter traits and the meat quality traits assessed were not affected by any treatment. Nevertheless, the inclusion of *Nannochloropsis oceanica* was effective in increasing the n-3 PUFA content in meat.

Key words: *Nannochloropsis oceanica*, Digestibility, Growing rabbits, Meat

INTRODUCTION

Microalgae are characterized by having high protein, carbohydrate and fat contents, in most cases comparable or even higher to conventional feedstuffs, such as soybean meal (Lum *et al.*, 2013). These microalgae often have cellulosic cell wall that might compromise feed digestibility and utilization by monogastric animals (Madeira *et al.*, 2017). The use of microalgae as feedstuff is recent and the studies in this area are scarce. Regarding rabbits, Peiretti and Meineri (2008) studied the incorporation of 5, 10 and 15% of *Spirulina platensis* in 9-week-old rabbit diets and obtained no differences for animal performances. As for *Nannochloropsis oceanica* we did not find studies in rabbit nutrition, albeit being referred as a functional ingredient, because its icosapentaenoic acid (EPA) and crude protein contents are appealing (Alves *et al.*, 2018). Thus, microalgae are useful, towards the nutritional enrichment of meat with n-3 PUFA, which are beneficial for human health.
The aim of this study was to evaluate the effect of dietary inclusion of dried *Nannochloropsis oceanica* biomass on production performances, nutrient digestibility and meat quality of growing rabbits in whole experimental period, and if the inclusion could be restricted to the last 2 weeks or the last 1 week of the feeding period before the slaughter.

MATERIALS AND METHODS

A total of forty 5-weeks-old commercial hybrid rabbits (Hyla®, Eurolap, Gosne, France) were selected from which 4 groups were formed with homogenous initial live weights. Rabbits were housed individually in metabolic cages. Two diets were formulated as shown in Table 1: control and microalga diets (4.45% *Nannochloropsis oceanica* inclusion as a replacement of whole soybean in order to ensure same energy and protein contents of diets). Each experimental group of 10 rabbits were fed with: control diet during 5 weeks (N0), microalga diet during 5 weeks (N5), control diet during 3 weeks and microalga diet during 2 weeks (N2) and control diet during 4 weeks and microalga diet only in last week of the experiment (N1). Rabbits had *ad libitum* access to water and feed. Feed intake was assessed three times per week and rabbits weighed weekly. Faeces were collected for digestibility determinations: total tract apparent digestibility (TTAD) of dry matter, organic matter, crude protein, ether extract, NDF, ADF, hemicellulose, cellulose and gross energy (EGRAN, 2001). At 10 weeks of age, rabbits were slaughtered by electric stunning and exsanguination. Carcasses were dissected according to the World Rabbit Science Association recommendations as described by Blasco and Ouhayoun (1996). Carcass, full digestive tract, liver and scapular and perirenal fats were weighed. After 24 h chilling at 4 °C, meat samples were taken from *Longissimus lumborum* (for colour determination by CIELAB system) and hind leg muscles (kept at -20ºC until lipid oxidation assessment following the method described by Grau *et al.* (2000), or freeze-drying to chemical composition or fatty acid analysis as described by Dalle Zotte *et al*., (2018). Data were analysed to a one-factor analysis of variance by using the Linear Models of the SAS software (Statistical Analysis System, Version 9.3).

Table 1: Ingredients and chemical composition of experimental diets

	Control	Microalga
Ingredient (%)		
Corn	20.0	20.0
Wheat bran	10.0	10.0
Soybean meal, 47.5% CP	16.0	15.0
Sunflower meal	7.50	7.50
Dehydrated alfalfa	25.0	25.0
Grass straw	12.0	12.0
Beat pulp	5.00	5.00
Whole soybean	3.00	0.00
Nannochloropsis oceanica	0.00	4.45
Mineral and vitamin premix[1]	0.20	0.20
Sodium chloride	0.50	0.50
Calcium phosphate	0.50	0.50
DL-methionine	0.03	0.55
L-lysine	-	0.55
Chemical composition		
Dry matter - DM (%)	91.5	90.4
Organic matter (% DM)	91.7	91.3
Crude protein (% DM)	18.5	18.1
Ether extract (% DM)	3.46	3.27
NDF (% DM)	39.3	38.8
ADF (% DM)	22.0	21.6
Hemicellulose (% DM)	17.3	17.2
Cellulose (% DM)	17.7	17.4
Gross energy (kcal/kg DM)	4436	4430

[1] Premix composition (added per kg of feed): vitamin A – 1000 UI; vitamin D3 – 1500 UI; vitamin E – 15 mg; vitamin K3 – 1.5 mg; vitamin B1 – 1 mg; vitamin B2 – 2 mg; vitamin B6 – 1.5 mg; vitamin B12 – 0.01 mg; pantothenic acid – 8 mg; nicotinic acid – 25 mg; biotin – 0.02 mg; betaine – 136.5 mg; robenidine – 50 mg; Co – 0.7 mg; Cu – 5 mg; Fe – 30 mg; I – 1 mg; Mn – 15 mg; Se – 0.2 mg; Zn – 30 mg; ethoxyquin – 12.5 mg.

RESULTS AND DISCUSSION

Growth performances are presented in Table 2. They were not affected by the incorporation and the time span of microalga consumption.

Table 2: Effect of treatment on growth performances of rabbits in the total experimental period (35-71 d)

	N0	N1	N2	N5	SEM	p-value
Live weight at 35 d (g)	964	967	974	992	79.9	0.860
Live weight at 71 d (g)	2751	2725	2663	2641	191.9	0.546
Feed intake (g/d)	167	162	156	156	15.6	0.333
Weight gain (g/d)	51.1	50.2	48.2	47.1	51.3	0.309
Feed conversion ratio	3.27	3.24	3.23	3.30	0.170	0.794

Dietary treatments: N0 - control diet during 5 weeks; N1 - microalga diet in the last week of the experiment; N2 - microalga diet during the last two weeks; N5 - microalga diet during 5 weeks.
SEM – standard error of mean

Table 3 presents the effect of diet on digestibility for the different nutrient fractions studied. Digestive utilization coefficient of crude fat was the only parameter affected by microalga incorporation, with TTAD coefficient less than 3.6 percentage units compared with the control diet. This could originate from the resistant cell wall of the microalga, well reported for this microalga species and others, which impairs the microbial metabolism of fat as previously reported in ruminants (Alves *et al.*, 2018).

Table 3: Effect of diets on TTAD coefficients of rabbits

	Control	Microalga	SEM	p-value
TTAD (%)				
Dry matter	60.8	61.5	0.02	0.462
Organic matter	61.1	61.8	0.02	0.460
Crude protein	76.5	77.0	0.02	0.590
Crude fat	78.8	75.2	0.03	0.001
NDF	28.9	28.5	0.04	0.804
ADF	19.0	17.4	0.04	0.400
Hemicellulose	41.5	42.3	0.04	0.655
Cellulose	21.6	19.3	0.04	0.221
Gross Energy	61.4	61.5	0.02	0.932

Control, Microalga: ingredients and chemical compositions of the diets are shown in Table 1.

Slaughter parameters shown in Table 4 were not affected by any treatment studied.

Table 4: Effect of treatments on the slaughter traits of rabbits

	N0	N1	N2	N5	SEM	p-value
Live weight - LW (g)	2752	2726	2664	2643	192.2	0.550
Hot carcass weight (g)	1602	1582	1513	1539	128.0	0.412
Chilled carcass weight (g)	1512	1504	1424	1457	120.5	0.352
Liver (g)	90.5	91.4	79.9	81.3	3.39	0.520
Liver (g/% chilled carcass)	5.97	6.08	5.54	5.51	0.196	0.620
Scapular fat (g)	9.57	9.70	7.76	6.95	0.505	0.134
Scapular fat (g/% chilled carcass)	0.63	0.64	0.53	0.47	0.031	0.145
Perirenal fat (g)	30.8	30.1	27.3	27.1	1.22	0.637
Perirenal fat (g/% chilled carcass)	2.03	2.01	1.86	1.85	0.08	0.757
Full digestive tract (g)	542	544	561	514	9.9	0.415
Full digestive tract (g/% LW)	19.7	19.9	21.1	19.5	1.94	0.243

N0, N1, N2, N5 dietary treatments see in Table 2.

Meat quality traits assessed are shown in Table 5. Chemical composition, colour and lipid oxidation of meat were not affected by the different treatments. Fatty acid composition was affected by the treatment, as the EPA contained in *Nannochloropsis oceanica* biomass was absorbed by rabbits and

incorporated in meat lipids, mostly as EPA and DPA, and the response was linearly related to the duration of microalgae feeding.

Table 5: Effect of treatments on chemical composition, colour, lipid oxidation and fatty acid (FA) composition of hind leg meat of rabbits

	N0	N1	N2	N5	SEM	p-value
Chemical composition (%)						
Dry matter	25.6	25.5	25.5	25.3	1.29	0.969
Crude protein	20.4	20.5	20.2	20.5	0.80	0.765
Crude fat	2.14	1.92	2.14	2.00	0.547	0.770
Colour						
L*	53.1	53.4	52.5	52.8	1.65	0.626
a*	4.54	4.63	4.90	4.82	0.731	0.671
b*	-1.16	-0.92	-1.25	-0.85	0.601	0.403
TBARS[1]	0.17	0.15	0.18	0.16	0.081	0.815
FA composition (% FA+DMA[2])						
SFA	48.3	46.7	46.8	46.0	3.39	0.462
MUFA	37.0	37.7	36.2	37.0	3.73	0.857
PUFA	14.6	15.7	17.0	17.0	4.42	0.577
n-6 PUFA	14.1	14.9	15.9	15.7	4.02	0.746
n-3 PUFA	0.39^{a}	0.63^{ab}	0.86^{bc}	1.20^{c}	0.412	0.0007
n-6/n-3 ratio	41.4^{a}	26.5^{b}	21.8^{bc}	14.1^{c}	9.31	<0.0001

[1] Thiobarbituric acid reactive substances expressed as mg malondialdehyde/kg meat.
[2] DMA - dimethyl acetals.
N0, N1, N2, N5 dietary treatments see in Table 2.
[a,b,c] - means with different superscripts are significantly different (P<0.05).

CONCLUSIONS

Dietary incorporation of *Nannochloropsis oceanica* did not affect the growth performances, nutritional fraction digestibility, slaughter traits and meat quality traits of rabbits, but increased the n-3 PUFA content of hind leg meat. In the future, considering the reducing costs of microalgae, it would be important to test higher levels of microalgae incorporation to understand if this raw material could be used as an ingredient for rabbit´s diets. Likewise, meat quality traits would also be important to monitor *off-flavour* development.

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