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Dietary nucleotides enhance growth performance, feed efficiency and intestinal functional topography in European Seabass (*Dicentrarchus labrax*)

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Abstract

Nucleotides, nucleosides and nucleic acids (NU) have many critical functions in supporting life and increasing evidence suggests that exogenous supply can benefit the health of mammals and fish. For these reasons, a 6-week feeding trial was conducted on juvenile European seabass (*Dicentrarchus labrax*) with diets containing 0%, 0.15% and 0.3% inclusion of a NU mixture (Laltide®) derived from the yeast *Saccharomyces cerevisiae*. At the end of the study no significant differences were found in fish performance, although a tendency towards better performance was indicated in fish fed the Nu0.3 diet. In relation to histological assessment, a significantly greater perimeter ratio; internal to outer (IP/OP) was observed in the posterior intestine of fish fed supplemental NU. Microvilli heights in the posterior intestine were also shown to be significantly promoted in fish fed NU diets (*P* < 0.05). Goblet cell abundance was shown to be unaffected by the inclusion of NU in the diet (*P* > 0.05). Overall, this study indicates that orally administered NU may be effective promoters of gut functional topography with marginal associated improvements to fish performance. Nonetheless, longer exposure and/or commercial scale application, and in diets that were challenging in use of high inclusion levels of plant by-products would potentially amplify improvements in production characteristics, in turn benefiting fish culturists.

KEYWORDS: Aquaculture, Intestine, Microvilli, Morphology, Nucleotides, Nucleosides
1. INTRODUCTION

With an ever-increasing volume of aquaculture activities around the globe comes growing pressure to maximize the efficiency of finfish production whilst at the same time ensuring sustainability. Typically, being the main expenditure in intensive finfish aquaculture, feeds must be formulated to promote fish performance, maintain the health of fish and be economically viable; they must also increasingly conform to the emerging paradigm of extending beyond satisfying basic nutritional requirements (Meng, Ma, Ma, J., Han, D. Xu, Zhang & Mai, 2016; Li and Gatlin, 2004; Hassaan, Mahmoud, Jarmolowicz, El-Haroun, Mohammady & Davies, 2018). However, modern aquaculture continues to face an array of nutritional restrictions which threaten optimal culture. The ever-increasing inclusion of alternative feed ingredients, particularly plant proteins (PP), is associated with amino-acid and fatty-acid deficiencies as well as increases in the presence of anti-nutritional factors (ANF) in diets. PP-derived ANF have been extensively studied in commercial finfish and have often been shown to negatively interact with the digestion process, be it via decreasing nutrient availabilities or impingement upon gastrointestinal physiology and function. Of particular concern is gut enteritis, which results in structural damage to the intestinal absorptive area, caused by saponins in high PP inclusions which have now become common-place (Francis, Makkar, & Becker, 2001). As the site of nutrient assimilation, and to some extent defence against pathogens, damage to the gut can result in marked detriment to the health and production of livestock. Maintaining and promoting gut health is therefore of utmost importance. Several studies on fish confirmed the role of nucleotides on beneficial in growth performance (Ferreira, Pinho, Vieira, & Tavarela, 2010; Jarmołowicz, Rożyński, Kowalska, & Zakęs, 2018; Azeredo, Machado, Kreuz, Wuertz, Oliva-Teles, Enes, & Costas, 2018), physiological response and antioxidant capacity (Tahmasebi-Kohyani, Keyvanshokooh, Nematollahi, Mahmoudi & Pasha-
Zanoosi, 2011), and intestinal health (Cheng, Buentello & Gatlin, 2011). Yeast derived nucleotides have long been acknowledged as valuable agricultural feed ingredients, applied in bovine, poultry and porcine diets; however, their application in aquafeeds remains much less significant (Ferreira et al., 2010; Jarmolowicz et al., 2018; Azeredo, Machado, Kreuz, Wuertz, Oliva-Teles, Enes, Costas, 2018). Although the attention yeasts traditionally received was based upon their attractive protein content, more recently their application as functional feed ingredients have been investigated in endeavours to exceed basic nutritional requirements (Li & Gatlin, 2005). The driving force behind this research appears to be in part due to the numerous nutritionally beneficial compounds present within yeast cells such as mannan-oligosaccharides, β-glucans and nucleotides (NUT) (Li & Gatlin, 2004; Berto, Pereira, Mouriño, Martins, & Fracalossi, 2016). Further investigations into the potential benefits of these individual components are required, in order to understand the nutritive qualities of yeast as an ingredient and to evaluate the feasibility of supplementing yeast-derived products into finfish diets. Nucleotides are present in ingredients of plant or animal origin as well as yeast cells as free nucleotides and nucleic acids (Fegan 2006). Nucleotides participate in many biochemical processes that are indispensable to the support of life. They are crucial in the storage, transfer and expression of genetic information, stand as activated intermediates of energy transport in cells (e.g. ATP), are important components of certain coenzymes (e.g. coenzyme A) and are also fundamental biological regulators (e.g. cyclic AMP) (Cosgrove 1998; Sanchez-Pozo 1998). Because endogenous synthesis occurs, and the NUT contents of dead cells can be recycled through salvage pathways, signs of deficiency are not typically developed in higher vertebrates or fish thus NUT have traditionally been considered to be non-essential nutrients (Grimble, 1996; Sanchez-Pozo, 1998; Li & Gatlin, 2006). However, numerous research publications have successively suggested that dietary supplementation of NUT
may be of significant benefit to consumers under certain conditions, particularly stress (VanBuren and Rudolph, 1997; Hess and Greenberg, 2012).

In aquaculture, NUT were initially recognized as feeding stimulants (Hughes, 1991; Kasumyan and Doving, 2003). Their potential as functional nutritional supplements in fish diets has also since been explored. Supplementation of NUT has been demonstrated to have immunomodulatory effects in numerous species (Low, Wadsworth, Burrells & Secombes, 2003; Lin, Wang, & Shiau, 2009; Cheng et al., 2011; Tahmasebi-Kohyani et al., 2011; Welker, Lim, Yildirim-Aksoy, & Klesius, 2011; Kenari, Mahmoudi, Soltani, & Abediankenari, 2013; Peng, Xu, Ai, Mai, Liufu, & Zhang, 2013), including reports of improved resistance against bacterial, rickettsia, viral and ectoparasitic pathogens (Burrells, Williams, & Forno, 2001a; Li, Lewis, & Gatlin, 2004). Furthermore, modulation of stress responses has also been reported (Kenari et al., 2013; Palermo Cardinaletti, Cocci, Tibaldi, Polzonetti-Magni, & Mosconi, 2013). Effects of NUT supplementation on fish performance have appeared somewhat more variable, although growth promotion has been observed in salmonids (Burrells, Williams, Southgate & Wadsworth, 2001b; Tahmasebi-Kohyani et al., 2011). To date, gut morphological analysis of finfish exposed to supplementary NUT has only been conducted in Atlantic salmon (Salmo salar) (Burrells et al., 2001b), red drum (Sciaenops ocellatus) (Cheng et al., 2011) and turbot (Scophthalmus maximus) (Peng et al., 2013) but benefits to functional gut structure following NUT dietary supplementation have been reported throughout. However, no studies to date have reliably assessed gut structure at the ultrastructural level of microvilli. This is despite their delicate nature and arguably their heightened susceptibility of being impaired compared to gut macrostructure, such as intestinal folds. The potential of supplementary NUT as functional feed additives for improving gut structure warrants further research considering the attractive but sparse research findings in aquaculture to
date. The present study was conducted to identify any effects of orally administered graded levels of a NUT-based product (Laltide®) derived from the yeast Saccharomyces cerevisiae on the performance and intestinal morphology of juvenile European seabass being a very valuable fish farmed in the Mediterranean.

2. MATERIALS AND METHODS

2.1. Diet formulation and proximate analysis

A basal diet (Control) was formulated to satisfy all known nutrient requirements of European seabass (Lim 2003). Two experimental diets were subsequently formulated with the inclusion of the NU ingredient, Laltide®, obtained from Lallemand Animal Nutrition UK; Spring Lane North, Malvern Link, Worcestershire, WR14 1B. Laltide® was added to the basal mix at 0.15% (Nu0.15) and 0.3% (Nu0.3) inclusion. This product contains bioavailable nucleotides (including 5’-nucleotides), as well as nucleosides, nucleic acids and nucleotide containing adducts.

Proximate composition of experimental diets was determined using standard AOAC (2000) procedures: dry matter (105 °C to constant weight), ash (incinerated at 550°C to constant weight), crude protein (Nx6.25) by the Kjeldahl method after an acid digestion (Gerhardt Kjeldatherm KB8 S and Gerhardt Vapodest 50) and crude lipid extracted with hot petroleum-ether (Gerhardt Soxtherm). All analyses were performed in triplicate, bar ash content which was run in duplicate.

2.2. Experimental system and animals

Juvenile European seabass (approximately 50 g) were obtained from Selonda UK ltd and held for a 6-week conditioning period in a salt-water recirculating system at the aquaculture nutrition research facility. During conditioning, fish were fed EWOS Sigma 50 at 1-2% body weight per day as a maintenance diet. After conditioning, the stock fish were graded by size. Selected fish averaging 62.19 g ± 0.42 in weight were stocked into 9 tanks (triplicate groups per treatment) in
groups of 20 individuals, having an average total biomass of 1245g ± 8.37 per tank (15.37 kg/m³ ± 0.1). Adequate water quality was ensured throughout the trial with biological and mechanical filtration, alongside UV sterilization and protein skimming. Water used in the system was natural seawater with additional NaHCO₃ as a buffer. During the trial, pH was 6.16. Air was supplied using a low-pressure side channel blower (Rietschle, UK ltd) via air stones and maintained dissolved oxygen (DO) levels at 7.38 mg/l. Water temperature was controlled by an inline heater (Elecro Titanium) at 21.5 °C ±1.0 throughout the trial. Tanks were illuminated with a photoperiod of 12 h light: 12 h dark with timer-controlled overhead fluorescent light array. Fish were weighed collectively in bulk from each tank at weeks 0, 1, 3, 5, and 6 of the feeding trial. Feeding was performed twice a day at 2% bodyweight for the first 12 days followed by 2.6% for the rest of the trial duration, based upon acceptance of the feed.

2.3. Sampling

Sampling for tissue was performed 6 weeks after the start of the trial, including a 24h starvation period. Fish were randomly selected and anesthetized by immersion in buffered tricaine methane sulphonate (MS-222, 200 mg/l); they were subsequently euthanized by a sharp blow to the head and pithing of the brain. Four fish per tank were sampled for carcass composition and K-factor calculations, a further two fish per tank were sampled for light and electron microscopy.

2.4. Fish performance and condition

Growth performance, feed efficiency and fish condition calculations were performed as follows:

Weight gain = (final weight (g) − initial weight (g)) / 100; Specific growth rate (SGR) = \( \left( \frac{\ln W_2 - \ln W_1}{T} \right) \times 100 \); where \( \ln W_1 \) and \( \ln W_2 \) are the initial and final natural logarithmic weights, respectively, and \( T \) is the number of days in the feeding period; Feed conversion ratio (FCR) = feed...
intake (g) / weight gain (g); Protein efficiency ratio (PER) = weight gain (g) / protein intake (g);
K-factor (K-F) = 100 (weight (g))/(total fish length (cm)³).

2.5. Carcass composition

Carcass chemical analysis of fish was conducted in identical manner to proximate analysis of feed according to standard AOAC (2000) procedures.

2.6. Intestinal morphology assays

2.6.1. Light Microscopy

The gastrointestinal tracts of fish were removed from the body cavity and a section of the posterior intestine was removed, these were fixed in 10% formalin and kept at 4°C for 48 hours then transferred to 70% ethanol at 4°C for storage. After storage, samples were further dehydrated in incremental ethanol concentrations and embedded in paraffin wax according to standard histological techniques. Samples were sectioned at 5μm thickness (Leica RM2235 microtome), dried in an oven overnight and subsequently auto-stained with haematoxylin and eosin (HE) or periodic acid Schiff (PAS) (Leica Autostainer XL). Slides were mounted with cover slips using DPX and left to dry. Micrographs of HE and PAS stained samples were then captured at 1, 4 and 10X magnifications (Leica DMRB microscope and Olympus E410 digital SLR camera).

2.6.2. Transmission electron microscopy (TEM)

Small sections of the distal end of the posterior intestine were excised and placed in gluteraldehyde and stored at 4°C. Samples were immersed twice in 0.12 sodium cacodylate buffer (pH 7.2) at 15minute intervals. The samples were then placed in osmium tetroxide (OsO₄) for 2 hours and subsequently immersed in fresh OsO₄ twice at 15minute intervals. Dehydration of samples was then conducted by immersion in 30, 50, 70, 90 and 100% ethanol at 15minute intervals. Samples were then placed in incremental absolute ethanol: Agar low viscosity resin mixes (70:30, 50:50,
30:70, 0:100) at 24-hour intervals. Samples were subsequently embedded in BEEM® capsules with fresh resin. Sectioning was performed using a Reichert-Jung Ultracut E ultratome and Microstar diamond knife. Sections were stained with saturated uranyl acetate solution and lead citrate. Micrographs of brush borders at 10,000X magnification were captured using a JEOL 1200 ex2 transmission electron microscope with a built in Soft Imaging System Megaview 3 camera.

2.6.3. Microscopic analysis

Image J 1.43 was used for all image analysis in this study. For HE-stained samples, intestinal fold length (FL) was measured from the base to the extremity of folds using an intersecting line. Perimeter ratio (PR) was also calculated from HE-stained samples as follows: \( PR = \frac{IP}{OP} \); where OP is the outer mucosal perimeter of the gut and IP is the inner absorptive surface perimeter. Goblet cell abundances in PAS-stained samples were calculated by measuring the perimeter of folds and subsequently counting the number of goblet cells present within this distance. The abundance measurement calculated and used was number of goblet cells per mm of fold perimeter.

From TEM micrographs, the lengths of 40 visibly complete microvilli were measured per fish, from different locations. All analyses were performed blind.

2.7. Statistical analysis

One-way Analysis of variance (ANOVA) and post hoc Fisher’s LSD was used for the analysis of normally distributed data. Kruskal-Wallis was used for the analysis of non-normally distributed data in association with Mann-Whitney U-tests and Bonferroni correction. Statistical analyses were performed using IBM SPSS Statistics 20 (Chicago, IL, USA).

3. RESULTS

3.1. Fish performance and condition
No statistically significant differences in mean weight gain (WG; \( P = 0.126 \)), specific growth rate (SGR; \( P = 0.267 \)), feed conversion ratio (FCR; \( = 0.42 \)), protein efficiency ratio (PER; \( P = 0.232 \)) or condition factor (K-F; \( P = 0.662 \)) were found among fish fed the experimental diets (Table 2). There was a distinct trend however for seabass fed the Nu1.5 treatment to perform better than the control and Nu3.0 for all parameters measured.

3.2. Carcass Proximate Analysis

No significant difference in mean moisture (\( P = 0.737 \)), crude protein (CP; \( P = 0.498 \)), crude lipid (CL; \( P = 0.927 \)) or ash content (\( P = 0.377 \)) was found among fish fed the experimental diets (Table 3).

3.3. Intestinal morphology assays

3.3.1. Perimeter ratio

Although no significant difference in Perimeter ratio between Nu0.15 and Nu0.3 (PR; \( P = 0.08 \)) was observed (Table 4), significant differences were observed to lie between control diet fed and Nu0.15 (\( P = 0.02 \)) and control diet and Nu0.3 (\( P = 0.04 \)).

3.3.2. Fold length (FL)

No significant difference in FL was observed between the three treatments (\( P = 0.08 \)). Figure 2 displays the Juvenile European seabass posterior intestinal folds. (A, B) fed Control (C) fed Nu0.15 (D) fed Nu0.3. H&E stained. FL = fold length, FB = fold branching. Scale bar: 100\( \mu \)m.

Fold length (\( \mu \)m) was determined as 337.6 \( \pm \) 80.0 (control group) 479.2 \( \pm \) 163.5 (Nu1.5) and 431.0 \( \pm \) 27.6 for seabass receiving Nu0.3.

3.3.3. Goblet cell abundance
No statistically significant difference in mean goblet cell abundance \((P = 0.10)\) was indicated among treatments (Table 4). Figure 3 displays the Juvenile European seabass posterior intestinal folds displaying goblet cells (10X magnification). (A, B) fed control (C) fed Nu0.15 (D) fed Nu0.3. PAS stained. GC = goblet cells. Scale bar: 100µm. Cells ranged in number from 45 to 54 per mucosal fold.

3.3.4. Microvilli height

Statistical analysis of microvilli heights indicated a highly significant difference among the experimental treatments \((P < 0.05)\). Pairwise comparisons revealed significant differences in median microvilli height between Nu0.15 \((P < 0.02)\), Nu0.15 and Nu0.3 \((P < 0.02)\) and Nu0.3 \((P < 0.02)\) (Table 4). Figure 1 shows the posterior intestinal Microvilli of juvenile European seabass (A, B) fed Control (C) fed Nu0.15 (D) fed Nu0.3. MV = microvilli. Scale bar: 5µm. Microvilli height (µm) ranged from 1.71 (control) to 2.04 (Nu0.15) to 1.77 (Nu0.3)

4. DISCUSSION

Since interest in the exogenous supply of nucleotides, nucleosides and nucleic acids (NU) for medical applications and livestock nutrition began, improved growth performance has been reported by several authors. The present results showed that European seabass fed diet supplemented with 0.15% (Nu0.15) and 0.3% (Nu0.3) trended towards improved growth performance, feed utilization (WG, SGR, FCR, PER) and condition factor (Table 2). The current results were marginally consistent with previous findings obtained by (Burrells, Williams, Southgate & Wadsworth, 2001b) who found that inclusion of 0.03% nucleotide (NT) diet fed to Atlantic salmon \((Salmo salar)\) significantly improved growth after just 3 weeks. Similarly, incorporating 0.25% Ascogen® a NT containing dietary supplement, into the diets of rainbow trout \((Oncorhynchus mykiss)\) was reported to significantly improve growth performance (Adamek,
Growth performance enhanced with 0.25% NT in Caspian brown trout (*Salmo trutta caspius*) (Kenari et al., 2013). Furthermore, in grouper (*Epinephelus malabaricus*), 0.15% dietary NT also indicated significant improvements to weight gain (Lin et al., 2009). Recently, growth performance of pikeperch (*Sander lucioperca*) were significantly improved with yeast extract supplemented (Jarmołowicz et al., 2018). In addition, Performance of juvenile red drum (*Sciaenops ocellatus*) also did not significantly improve with diets containing Ascogen® (Li, Burr, Goff, Whiteman, Davis, Vega, Neill & Gatlin, 2005; Li, Gatlin & Neill, 2007a). Later studies in red drum revealed that orally administering 0.5% and 1% Ascogen® had a tendency to improve survival and weight gain of fish but again no significant differences were apparent (Cheng et al., 2011). Despite non-significant findings, this study would seem to suggest a tendency for improved fish performance with dietary inclusion of NU. Interestingly, Cheng et al. (2011) suggested that dietary inclusion of NT may be more beneficial on WG of the fish at 0.5% than 1%. Increasing the inclusion of Ascogen® in the diets of rainbow trout and goldfish (*Carassius auratus*) from 0.25% to 0.5% displayed a similar yet more extreme scenario with a reversal from enhancement to depression of growth (Hamackova, Kouril, Adamek, Vachta & Stibranyiova, 1992). The findings of this investigation may also appear to show that in terms of maximising performance, the optimum inclusion of the supplement is not at its highest tested level. Despite a lack of statistical significance, this indication arises from Nu0.15 fed fish consistently displaying the best performance characteristics; followed by Nu0.3 fed fish, whilst control fish displayed the least desirable production characteristics. Overall, the fish performance results of this study are largely in correspondence with the body of research in this field as improved performance of fish in NU nutrition trials remains rather marginal when considering adult and juvenile fish (Li & Gatlin, 2004; 2006).
No statistically significant differences in moisture, CP, CL or ash of fish carcasses were identified between treatments after the feeding trial, corresponding to Li et al. (2004) whom similarly observed no significant changes to whole body composition of hybrid striped bass fed supplementary NT. The present results were consistent with those reported by Jarmołowicz, Zakęś, Siwicki, Kowalska, Hopko, GŁąbski, & Partyka (2012) who found that juvenile pikeperch Sander lucioperca (L.) fed diets supplemented with brewer's yeast has no significant changes on body chemical composition. Also, no significant differences were found in chemical composition in Nile tilapia (Lunger, Craig, & McLean, 2006; Hassaan et al., 2018) fed died supplemented with nucleotides.

Significantly greater perimeter ratio and Microvilli heights (P ≤ 0.05) were observed in the posterior intestine of seabass fed supplemental NU, while Goblet cell abundance was shown to be unaffected by the inclusion of NU in the diet (Figure 1-4). The present results are consistent with Adjei Morioka, Ameho, Yamauchi, Kulkarni, Al-Mansouri, & Yamamoto, (1996) who found that a nucleotide-nucleoside mixture supplemented into the diets of mice could increase villus height and decrease gut damage. Enhancement of villus height has similarly been described in rats fed 0.8% nucleosides, with increases reported as greatest in the posterior section of the gut (Uauy, Stringel, Thomas & Quan, 1990). Furthermore, research has shown the growth of human and rat intestinal cell lines to benefit greatly from supplementary exogenous provision of DNA-Na+ derived from fish soft roe, RNA derived from yeast and deoxy-mononucleotides from yeast (Holen & Jonsson, 2004). In fish, increases in intestinal fold length in response to dietary NT were first suggested by Burrells et al. (2001a) as the explanation behind enhanced growth of Atlantic salmon. Interestingly, despite continuing work into fish NU nutrition, morphological analyses of the gastrointestinal tract have remained sparse. Nonetheless, evidence of fold length enhancement in
the posterior intestine was shown in red drum fed a NT containing product (Cheng et al., 2011). However, Cheng et al. (2011) did not detect any significant differences in fold height at distal and mid intestinal locations. This study’s analysis did not show any statistically significant difference between posterior intestinal fold lengths of fish fed the experimental diets, but numerical observation would appear that display elongation of folds could be present in NU fed fish. It is of note that there was a rather large variability between individuals, particularly in the Nu0.15 treatment, which could mask effects. Perimeter ratios were significantly increased in both Nu0.15 and Nu0.3 compared to the control group. This suggests that the functional absorbptive inner epithelial layer (perimeter) of the intestine was enlarged following NU supplementation. To the author’s knowledge, this is the first instance that this analysis has been performed in animals and in particular fish fed an exogenous supply of NU. The results of this can nonetheless be treated as comparable to previous discussions of fold length promotion.

Even though NU is increasingly being seen as promising supplementary nutrients for increasing absorption area in the gut by promoting macrostructure (fold/villus length), their potential to enhance absorption area at an ultrastructural level, by promoting microvilli length, seems to have been largely neglected. In red drum (*Sciaenops ocellatus*), microvilli height was reported as significantly increased in the distal, mid and proximal intestine by exogenous supplementation of NT (Cheng et al., 2011). It was also reported that supplemental NT increased microvilli height in Turbot (Peng et al., 2013). Although nucleotides do appear effective promoters of absorptive surface area in the gut, the use of light microscopy in the study of gut micromorphology must be treated with great care due to its low degree of accuracy. The findings of this study give a clear and accurate indication that dietary inclusion of NU can have prominent positive effects on microvilli height, via the use of electron microscopy. It is noteworthy that the results of this
investigation indicated that higher inclusion levels of dietary NU may begin to have slightly more
effective effects on microvilli height. Height and assumed proliferation were greater in Nu0.15
than Nu0.3 fish, although Nu0.3 remained more beneficial to microvilli structure than no
exogenous supply of NU. Interestingly, this is in contrast with findings by Cheng et al. (2011) who
identified microvilli height to be greater in fish fed a 1% inclusion of NT as opposed to 0.5%.
However, non-linear responses in growth, haematological parameters and stress responses have
been reported in Caspian brown trout (*Salmo trutta caspius*) (Kenari et al., 2013).

Goblet cell abundance was not significantly different between treatments, but again numerical
trends may be apparent with reduced average abundances of cells in NU-exposed individuals.
Overall there is a distinct lack of comparable research into how dietary provision of NU may affect
goblet cell abundance and continuing future research into goblet cell responses would be a great
contribution to our knowledge of both mammalian and piscine NU nutrition.

Dietary NUT is partly absorbed in the gut as NS through a variety of mechanisms; they are then
incorporated into body tissues (Hess & Greenberg, 2012), but it has been suggested as particularly
important in development and proliferation of tissues with rapid cell turnover, such as the liver
This explains why inclusion of the commercial supplement resulted in observed improvements to
functional perimeter and microvilli height. Improvements in intestinal morphology are commonly
associated with improvements to animal performance as increases in structural complexity create
a greater surface area for digestion and absorption of nutrients (Burrells et al., 2000a). Despite the
non-significant findings, the fish performance trends in this study appear to be supported closely
by the intestinal functional topography findings of this study.

Conclusion
It is now widely accepted that exogenous supply of nucleotides alongside nucleosides and nucleic acids can be of great benefit to the health of the consumer. These results indicate that dietary provision of combined nucleotides, nucleosides and nucleic acids derived from yeast can positively influence the functional gut topography of European seabass, at both a macro and ultrastructural level. Gut morphology was seen to improve with nucleotide supplementation, increasing absorption surface area for digestion and assimilation of nutrients; tendencies of improved fish performance seemed to reflect this promotion. The results also appeared to show that dosage is an important consideration with lower inclusions outperforming higher ones in certain aspects. This will have important consequences in the use of high inclusion rates of plant proteins in carnivorous fish species such as seabass where the challenges of modern feed formulations may lead to gut enteritis and related lesions. The supplementation of nucleotides could mitigate these effects. The potential of nucleotides in improving gut structural integrity and functional topography in aquaculture remains relatively unexplored and should be investigated further with appropriate modern technologies.

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diets with graded levels of soybean meal (Scophthalmus maximus L.). *Aquaculture*, 392, 51-58.


**TABLE 1** Compositions of experimental diets with graded levels of NU inclusion.

<table>
<thead>
<tr>
<th>Ingredient (% inclusion)</th>
<th>Experimental diets</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Fish meal a</td>
<td>62.93</td>
</tr>
<tr>
<td>Corn Starch b</td>
<td>17.82</td>
</tr>
<tr>
<td>Maize gluten c</td>
<td>10.00</td>
</tr>
<tr>
<td>Fish oil d</td>
<td>7.25</td>
</tr>
<tr>
<td>Vitamin/Mineral Premix e</td>
<td>2.60</td>
</tr>
<tr>
<td>NU f</td>
<td>-</td>
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**Analysed composition (%) of feed**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nu0.15</th>
<th>Nu0.3</th>
</tr>
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<tbody>
<tr>
<td>Crude Protein (CP) N*6.25</td>
<td>49.26 ± 1.09</td>
<td>49.84 ± 0.39</td>
<td>49.73 ± 0.54</td>
</tr>
<tr>
<td>Crude lipid (CL)</td>
<td>13.33 ± 0.95</td>
<td>12.94 ± 0.34</td>
<td>12.47 ± 0.09</td>
</tr>
<tr>
<td>Ash</td>
<td>9.45 ± 0.03</td>
<td>9.61 ± 0.08</td>
<td>9.55 ± 0.16</td>
</tr>
<tr>
<td>Moisture</td>
<td>4.80 ± 0.05</td>
<td>4.83 ± 0.05</td>
<td>4.79 ± 0.04</td>
</tr>
</tbody>
</table>

Control: No inclusion of NU, Nu 0.15: 1.5g kg⁻¹ inclusion of NU, Nu0.3: 3g kg⁻¹ inclusion of NU

* a Herring meal LT94, CC Moore, UK
* b Unmodified starch from maize, Sigma Aldrich, UK
* c Glutalys®, Roquette Frères, Fr
* d Biomar, Dk
* e PNP Fish 2%, Premier Nutrition, UK
* f Laltide®, Lallemant UK (Biotol), UK
**TABLE 2** Fish performance and condition factor of European seabass fed incremental levels of Laltide® over 6 weeks (n=3).

<table>
<thead>
<tr>
<th>Fish Performance &amp; Condition</th>
<th>Experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Weight gain (WG)</td>
<td>28.57 ± 0.26</td>
</tr>
<tr>
<td>Specific Growth rate (SGR)</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>Feed conversion ratio (FCR)</td>
<td>2.19 ± 0.15</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)</td>
<td>1.02 ± 0.02</td>
</tr>
<tr>
<td>K-factor (K-F)</td>
<td>1.13 ± 0.01</td>
</tr>
</tbody>
</table>
TABLE 3 Proximate chemical composition (mean % of total on wet matter basis) of whole carcass of European seabass fed incremental levels of Laltide® for 6 weeks.

<table>
<thead>
<tr>
<th>Carcass</th>
<th>Experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Moisture</td>
<td>64.52 ± 1.25</td>
</tr>
<tr>
<td>Crude protein (CP)</td>
<td>17.28 ± 0.36</td>
</tr>
<tr>
<td>Crude lipid (CL)</td>
<td>13.09 ± 0.49</td>
</tr>
<tr>
<td>Ash</td>
<td>4.02 ± 0.16</td>
</tr>
</tbody>
</table>
Table 4. Morphological characteristics of posterior intestine of European seabass fed incremental levels of Laltide® for 6 weeks.

<table>
<thead>
<tr>
<th>Intestinal parameter</th>
<th>Experimental diet</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nu0.15</td>
<td>Nu0.3</td>
</tr>
<tr>
<td>Fold length (µm)</td>
<td>337.6 ± 80.0</td>
<td>479.2 ± 163.5</td>
<td>431.0 ± 27.6</td>
</tr>
<tr>
<td>Perimeter ratio</td>
<td>3.03 ± 0.19 b</td>
<td>4.17 ± 0.13 a</td>
<td>4.00 ± 0.14 a</td>
</tr>
<tr>
<td>Goblet cell abundance(^1)</td>
<td>54.0 ± 12.0</td>
<td>44.0 ± 6.02</td>
<td>45.0 ± 5.47</td>
</tr>
<tr>
<td>Microvilli height (µm)</td>
<td>1.71 ± 0.32 a</td>
<td>2.04 ± 0.34 c</td>
<td>1.77 ± 0.31 b</td>
</tr>
</tbody>
</table>

\(^a,b,c\) Means possessing different superscripts in the same row are significantly different at P <0.05.

\(^d,e,f\) Medians possessing the same superscript in the same row are not significantly different at P <0.017.

\(^1\) Values expressed as no. cells per mm of fold border.
FIGURE 1 Transversal sections of juvenile European seabass posterior intestine depicting employed measurement techniques. (A) HE stained enteric section at 10X magnification. Arrows indicate example fold length measurements. (B) PAS stained enteric section at 10X magnification. Dashed arrowed line indicates example perimeter measurement and chevrons indicate the goblet cells. (C) Transmission electron micrograph of enteric section at 10,000X magnification. White arrow indicates example microvilli height measurement. (D) Transmission electron micrograph of enteric section at 10,000X magnification. VMV = vertically orientated microvilli (discounted), OMV = overlapping microvilli (discounted), MMV = visibly complete microvilli (measured)
FIGURE 2 Juvenile European seabass posterior intestinal folds (10X magnification). (A, B) fed control diets, (C, D) fed Nu0.15 (E, F) fed Nu0.3 H&E stained. FL = fold length, FB = fold branching, EC = epithelial cells.
FIGURE 3 Juvenile European seabass posterior Nu0.15 (E, F) fed Nu0.3 PAS stained. GC = goblet cells.
FIGURE 4 Posterior intestinal Microvilli (10000X magnification) of juvenile European seabass (A, B) fed C (C, D) fed Nu0.15 (E, F) fed Nu0.3 MV = microvilli. Scale bar = 5µm.