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Impact of nucleotide enriched diets on the production of gilthead seabream, *Sparus aurata* fingerlings by modulation of liver mitochondrial enzyme activity, antioxidant status, immune gene expression, and gut microbial ecology

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ABSTRACT

The objective of this study was to evaluate the effect of fish meal replacement with a mixture of plant, and animal protein supplemented with a commercial nucleotide NucleoforceFish™, on growth, feed utilization, whole-body composition, biometric indices; liver mitochondrial enzyme activities, selected gene expression and influence on the gut microbiome of gilthead seabream, *Sparus aurata*. Six hundred and thirty gilthead seabream with an average initial body weight of 0.358 ± 0.002 g/fish, were used. Six experimental diets were formulated to be isonitrogenous, 45% crude protein (CP) and isocaloric 20 MJ/kg gross energy (GE). The control diet (D1) contained 25% of fish meal (FM₂₅) and had no NucleoforceFish™ added (FM_{25/0}), diets 2-3 contained FM at a level of 25% and supplemented with 250 (FM_{25/250}) and 500 (FM_{25/500}) mg/kg NucleoforceFish™, respectively. Diets 4-6 were a non-fishmeal formulation and had no NucleoforceFish™, (diet 4, FM_{0/0}), 0%FM with 250mg/kg NucleoforceFish™ (diet 5, FM_{0/250}), and 0%FM with 500mg/kg NucleoforceFish™ (diet 6, FM_{0/500}). The results revealed that dietary nucleotides supplemented at 250mg/kg or 500mg/kg enhanced final body weight, weight gain, and specific growth rate of seabream either with dietary level of 25% FM diet (FM₂₅) or non-fishmeal diet (FM₀). All mitochondrial liver complex enzyme activities were recorded as having a significant positive response to the nucleotide dietary supplement. Gilthead seabream fingerlings fed FM_{0/250} diet for 150 days had significantly elevated liver mitochondrial enzyme (Complex III, Complex IV, Complex I and II) activities. In addition, gilthead seabream fed FM_{0/500} had significantly increased the gene expression of TCR-B and IL1-*B*, while fish fed FM_{25/250} increased significantly the gene expression of IGF1, while fish fed FM_{25/500} increased significantly the gene expression of Hepcidin (Hep) and the iron homeostasis hormone. There was a marked influence on specific bacteria comprising the gut microbiome namely *Photobacterium*; *damselae*; *Vibrio anguillarum*; *Lactobacillus plantarum*; *Lactobacillus Enterococcus faecium subtilis*. It can be concluded that FM_{25/250} and FM_{25/500} diets are expressing the best result, which explains that FM must be in the feed of gilthead seabream, and addition of NucleoforceFish™ at 250 mg/kg feed may further increase the performance.

1. Introduction

The production of marine fish has increased to meet the growing

demand of global seafood (FAO, 2018). Marine fish species need high levels of protein in their diets which is usually provided by fish meal obtained from wild fisheries or by-products of the animal processing

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industries. Fish meal is an optimal protein source for aquafeeds because of its nutritional value in terms of essential amino acids, and high palatability to fish. Elimination or reduction of the contribution of fish meal in marine fish diets was the gateway for nutritionists to provide economic and environmental benefits by reducing the cost of feed for fish farmers and reducing the pressure on species harvested for fish meal production and also serve as important resources in the marine food web (Tacon et al., 2011; Kok et al., 2020). Currently, a variety of vegetable and animal protein sources are used for substitution in aquafeeds with different inclusion rates (Rossi Allem Jr et al., 2021; Deng et al., 2011; Santigosa et al., 2011; Wang et al., 2012; Estruch et al., 2018; Hassaan et al., 2021; Hassaan et al., 2020; Goda et al., 2020a, 2020b; Davies et al., 2020; Hassaan et al., 2020; Goda et al., 2019). The common obstacle that nutritionists challenged regarding using alternative ingredients was changes in the immune status of marine fish by the introduction of a host of anti-nutritional factors especially in many plant by-products (Sitjà-Bobadilla et al., 2005). Not only the immune status can be altered by the fish meal replacement but also the intestinal absorption can be affected by these replacements as discussed by Santigosa et al. (2011). Consequently, aid solutions were recommended to solve this situation by using several feed additives among which are nucleotides (Ringø et al., 2012; Reda et al., 2018; Cardinaletti, 2019; Ashouri et al., 2020; Hossain et al., 2016; Hassaan et al., 2018).

Various researchers have studied nucleotides (NT) as functional nutrients for many species, especially mono-gastric animals such as those for aquaculture and poultry (Jung and Batal, 2012; Hossain et al., 2019). As such, nucleotides have been viewed as semi-essential nutrients; under specific conditions of rapid growth, stress and disease where *de novo* synthesis is not sufficient to meet physiological and metabolic demand. In this respect, Ki and Sung (2019) investigated the attributes of supplemental dietary nucleotides on intestinal health and growth performance of newly weaned pigs to examine primarily the effects on gut health with positive findings. Recently, Mohamed et al. (2020) investigated the impact of exogenous dietary nucleotides in ameliorating *Clostridium perfringens* infection by controlled challenge and elevating the intestinal barrier gene expression in broiler chicken with good results for a commercial nucleotide source (Nucleoforce poultry™). These workers also observed significant improvement in microscopic lesion scores, intestinal histomorphology, intestinal barriers (occludin and mucin mRNA expression) and growth parameters in broilers fed nucleotide supplementation. With respect to fish, Ferreira et al. (2020) found a positive influence of genes expression related to lipid metabolism and oxidative stress modulating of rainbow trout (*O. Mykiss*) fed diets contained *Saccharina latissima*. Furthermore, Safari et al. (2015) found that crayfish fed diets contained dietary nucleotide enhanced the growth performance, digestibility and immune responses. Also, Ashouri et al. (2020) found that Asian seabass fed diets supplemented with dietary sodium alginate and *Pediococcus acidilactici* improved liver antioxidant enzymes, intestinal lysozyme gene expression, histomorphology, microbiota, and digestive enzyme activity.

Animals can obtain their required amount of nucleotides exogenously or endogenously (Reda et al., 2018). Because of the active synthesis of NT mainly in the liver, most animals appear to be almost independent of exogenous NT (Jyonouchi, 1994). For aquatic animals, a positive correlation between the addition of nucleotides and growth performance was also witnessed in many aquatic species (Oliva-Teles et al., 2006; Xu et al., 2015; Jin et al., 2018; El-Nokrashy et al., 2020). Most recently, Bowyer et al. (2019) reported the benefits of supplementation of a commercial nucleotide product on the intestinal morphology of sea bass, *Dicentrarchus labrax*. The information on the effect of nucleotides on feed efficiency, metabolism, and mitochondrial enzyme complexes for sea bream is scarce. Dietary nucleotides positively affect fat metabolism, immunity, tissue development, and repair. The immune system and intestinal tract are unable to meet the needs of cellular nucleotides exclusively by synthesizing *de novo* and prefer to use 'the rescue' pathway to restore nucleotides and the nucleotide base of

blood and system (Gil, 2002). The molecular mechanisms through which dietary nucleotides modify the immune system are practically unknown. The small intestine plays a key role in the regulatory effects of nucleotides on the immune response. Dietary nucleotides have been shown to promote gene expression in the small intestine and may affect the biosynthesis of protein as well as the interaction of external nucleosides, and their receptors may also contribute to the modification of the expression of several genes. Additionally, mitochondria play a central role in energy metabolism and are the principal organelles of intracellular reactive oxygen species (ROS) generation. As such, they contain a complement of factors that can also promote cell death, thus displaying a shared platform for metabolism and apoptosis (Vakifahmetoglu-Norberg et al., 2017).

To maintain optimal function of mitochondria, an adequate supply of many micronutrients should be sustained (Wai and Langer, 2016). The complex relationship of the gastrointestinal tract and gut mucosal interface in terms of optimized nutrition and immune-competence in fish is of the vital importance. Burrells et al. (2001a) reported that dietary supplementation with nucleotides induced an increased resistance against pathogens in rainbow trout. These workers specifically showed that a combined inclusion level of 0.03%, mitigated challenge infections with bacterial, viral and rickettsial diseases as well as ectoparasitic infestation. Cheng et al. (2011) demonstrated the effectiveness of the influence of dietary nucleotides on immune responses and intestinal morphology of red drum, *Sciaenops ocellatus* showing higher enterocyte height and microvilli height. For salmonids, Hunt et al. (2016) investigated the effects of dietary nucleotide yeast supplementation on immune responses and related antioxidant enzyme activities of rainbow trout juveniles (*Oncorhynchus mykiss*) with promising findings. Ringø et al. (2012) presented a comprehensive review on the utilization of immunostimulants and nucleotides in aquaculture covering many scenarios.

Gut integrity and improved function is also a well recognized attribute of dietary nucleotide addition to the diets of fish. Burrells et al. (2001b) showed that fish fed nucleotides displayed a healthier intestinal system indicated by 18-21% increase of the lateral branching and height of the intestinal mucosal folds and also positive effects on vaccination, sea water transfer, growth rates and physiology of Atlantic salmon (*Salmo salar L.*). It has been reported that improved morphology of the intestine is a contributing factor to the improved growth of fish due to more efficient nutrient digestion and absorption. Xu et al. (2015) in their studies to elucidate the effects of dietary yeast nucleotides on growth, nonspecific immunity, intestine growth and intestinal microbiota of juvenile hybrid tilapia *Oreochromis niloticus x Oreochromis aureus* reported several production related attributes.

The role of nucleotides in influencing the key mechanisms of cellular communication has not been delineated and requires attention. For these reasons, a group of genes of interest was targeted for their expression in response to nucleotide supplementation in the diet for seabream. TCR, IL1-B, and IGF1 and immunoglobulin M IgM, and Interleukin1 beta T-Cell receptor proteins, are known to be important. Additionally, Hepcidin, an important peptide with antimicrobial properties and iron regulation capacity, was also selected. With the advent of new molecular sequencing techniques, it is easier to understand how the immune system of fish, may be orchestrated by the complex microbial communities that live in symbiosis with their hosts (Gomez et al., 2013). An even more paucity exists for the effect of dietary nucleotides on the gut microbial community, so the following important selection of gut-associated bacteria that may support immune function, i.e., *Photobacterium damsela*, *Photobacterium damse*, *Vibrio anguillarum*, *Lactobacillus plantarum*, *Lactobacillus subtilis*, *Lactobacillus subtilis*, and *Enterococcus faecium* were determined (Li et al., 2015). The mechanism involved in the growth-promotion effect of nucleotides in fish is still unclear. Given the importance of Gilthead seabream to the aquaculture industry of the Mediterranean and Adriatic regions we need more information with regard to this species.

The objective of this study was to evaluate the effect of the reduction

and elimination of dietary fishmeal using alternate ingredients and supplementation of a commercial nucleotide source (NucleoforceFish™) on growth, feed utilization, biometric indices; liver mitochondrial enzyme activities and selected gene expression levels relating to specific immune function and specific genes associated to the gut microbial profile. The synergistic interplay of these factors warranted investigation. Additionally, an economic analysis of gilthead seabream, *Sparus aurata* fingerling production, was also determined with dietary nucleotide incorporation to assess cost benefits.

2. Materials and methods

2.1. Fish and experimental facilities

Five hundred and forty seabream fingerlings with an average initial body weight of 0.358 ± 0.002 g/fish, were obtained from El-Shref Hatchery, Alexandria, Egypt. Prior to the start of the experiment, fish were acclimated to the experimental conditions for two weeks. Fish fed commercial seabream diet (44% crude protein and 18% fat) at a level of 10 % of body weight. The daily ration was divided into two equal amounts and offered two times a day. The fingerlings were stocked into six cement ponds (each with 18 m^3) at the National Institute of Oceanography and Fisheries (NIOF). Each cement pond was installed with three equal net-enclosures (each of 0.5 m^3), and each net-enclosure was stocked with 30 fish. Three replicate net enclosures were randomly assigned to each treatment. Ponds were supplied with saline water (32ppt) from underground saltwater well. Water quality parameters measured and maintained within the optimum range for seabream. The turnover rate of water was 20%/pond/day and fish were held under natural light (12,12 h light, dark schedule). The experiment was carried out in 2017 and continued for 150 days.

2.2. Experimental diets and feeding regime

The ingredients used in this experiment were purchased from a commercial feed manufacturer except Poultry By-product Meal (PBM) that was obtained from a rendering plant-based in Egypt. Nucleoforce-Fish™ was obtained from Bioiberica® Spain, and is a balanced concentrate of free nucleotides (>24%) and active precursors obtained from dried yeasts (*Saccharomyces cerevisiae*). It has the following nutrient specification: Protein 20.34%; Protein Nitrogen 3.25% Non-protein Nitrogen (mainly from nucleotides) 2.09%; crude ash 3.38% and crude fiber 0.10%.

Six experimental diets were formulated to be isonitrogenous, 45% crude protein (CP) and isocaloric 20 MJ/kg gross energy (GE). The control diet (D1) contained 25% of fish meal (FM₂₅) and had no NucleoforceFish™ added (FM_{25/0}), diets 2-3 contained FM at a level of 25% and supplemented with 250 (FM_{25/250}) and 500 (FM_{25/500}) mg/kg NucleoforceFish™, respectively. Diets 4-6 were a non-fishmeal composition and had no NucleoforceFish™, (diet 4, FM_{0/0}), 0%FM with 250mg/kg NucleoforceFish™ (diet 5, FM_{0/250}), and 0%FM with 500mg/kg NucleoforceFish™ (diet 6, FM_{0/500}). The diets were processed by blending the dry ingredients into a homogenous mixture for 10 minutes. Oil source and hot water were added, and then diets were made in an electric kitchen meat grinder without using additional heat (cold-pressed). All diets were air-dried for 4 hrs after that oven-dried at 45°C for 12 hrs. Dried diets were sieved using different feed sievers to provide the right pellet size for fish. The chemical composition of the experimental diets is presented in Table 1. The fish were fed twice a day, 6 days a week at rates starting from 10% of their body weight at the beginning of the experiment, and finished with 5% at the end.

2.3. Sample collection and analytical procedures

2.3.1. Chemical analysis and Growth performance

The chemical compositions of the experimental diets were measured

Table 1

Formulation and proximate composition of the experimental diets for gilthead seabream (g/kg).

	Experimental diets*					
	FM _{0/0}	FM _{0/250}	FM _{0/500}	FM _{25/0}	FM _{25/250}	FM _{25/500}
Fish meal	0	0	0	185	185	185
Poultry By-product	294	294	294	220	220	220
Soybean meal	234	234	234	175	175	175
Corn Gluten	134	134	134	100	100	100
Wheat Bran	76	75.75	75.50	89	88.75	88.50
Squid meal	134	134	134	100	100	100
Fish oil	118	118	118	121	121	121
Vitamins and mineral mixture ^a	10	10	10	10	10	10
Nucleotides ^b	0	0.25	0.5	0	0.25	0.5
Proximate composition (%)						
DM, %	93.9	93.6	92.9	93.3	93.5	93.8
Protein, %	45.50	45.90	46.60	46.10	45.75	46.00
lipids, %	18.00	17.80	17.60	18.10	18.20	17.70
Ash, %	15.35	15.70	15.10	15.40	15.65	15.85
Fiber, %	2.93	2.53	2.68	2.55	2.92	3.30
NFE ^c , %	18.22	18.08	18.02	17.85	17.48	17.16
Gross energy (MJ/kg) ^d	20.97	20.96	21.04	21.09	20.98	20.79
P/E ratio (mg CP:kJ)	90.74	91.58	92.63	91.43	91.19	92.55
Price (US\$/kg feed)	0.844	0.858	0.872	0.961	0.975	0.989

-FM_{0/0} (0% fish meal, 0mg nucleotide), FM_{0/250} (0% fish meal+250mg nucleotide), FM_{0/500} (0% fish meal+500mg nucleotide), FM_{25/0} (0mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250mg nucleotide), FM_{25/500} (25% fish meal+500mg nucleotide).

-DM= Dry matter.

^a Vitamin and mineral mixture each 1-kg of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B₁₂, 4.0 g Vit B₂, 6 g Vit B₆, 4.0 g Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium, 1.2 mg Niacin, 12 mg D-calcium Pantothenate, 26 mg Pyridoxine, HCl, 6 mg Riboflavin, 7.2 mg Thiamin, HCl, 1.2 mg Sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg Ferrous sulfate (FeSO₄·7H₂O, 20% Fe), 65 mg Manganese sulfate (MnSO₄, 36% Mn), 89 mg Zinc sulfate (ZnSO₄·7H₂O, 40% Zn), 150 mg Copper sulfate (CuSO₄·5H₂O, 25% Cu), 28 mg Potassium iodide (KI, 24% K, 76% I), 1000 mg Celite AW521 (acid-washed diatomaceous earth silica).

^b Nucleoforce Fish™ is concentrated balanced free nucleotides and active precursors, obtained from yeast produced by the bioiberica company, Spain. <https://www.bioiberica.com/animal-health/animal-nutrition/fish/nucleoforce-fish-1/#sthash.oj8NWjSA.dpbs>.

^c Nitrogen free extract (NFE) = 100 - (Protein% + lipids% + Ash% + Fiber %).

^d Calculated using gross caloric values of 23.62, 39.52, and 17.15 kJ/g for protein, fat, and carbohydrate, respectively, according to Brett (1973).

according to the procedures of AOAC (2000). Dry matter was determined after drying the samples in an oven (105 °C) for 24 h. Ash by incineration at 550°C for 12 h. Crude protein was determined by micro-Kjeldhal method, $N\% \times 6.25$ (using Kjeltach autoanalyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by soxhlet extraction with diethyl ether (40 – 60 °C). Fish were weighed every month, and the feeding rates were re-adjusted depending on the fish weight and the fish's health status. Growth performance was measured, according to Lu et al. (2016). Final Body Weight (FBW), Weight Gain (WG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Productive Value (PPV) and Energy Utilization, (EU, %), Economical Conversion Rate (ECR) were calculated using the following equations:

FBW = Final fish biomass (g)/fish number; WG = Final Body Weight (g) - Initial Body Weight (g); Specific Growth Rate (%/day): $SGR = 100 \times (\ln W_t - \ln W_o) / n$, Where: W_o: Initial Mean Weight of fish (grams); W_t: Final Mean Weight of fish (grams); n: Experimental period (days); ln: natural logarithm; FCR = Feed Intake (g)/Weight Gain (g); Protein Productive Value (PPV, %) = $100 \times (P_t - P_0) / \text{protein intake (g)}$; Where: P₀: Protein content in fish carcass at the start, P_t: Protein content in fish carcass at the end; Energy Utilization, EU (%): $EU = 100 \times (\text{Energy Gain}$

(Kcal)/ Energy Intake (Kcal)), $ECR = \text{Cost of diet } (\$/\text{kg}) \times \text{FCR}$ according to the following equation described by [Bonaldo et al. \(2010\)](#).

2.3.2. Isolation of liver mitochondria

The liver mitochondria isolation was performed in a medium A containing 10 mM KH_2PO_4 , 250 mM sucrose, and 5 mM ethylenediaminetetraacetate (EDTA) adjusted to pH 7.4. Two fish from each net enclosure were randomly chosen, anesthetized with 50 ppm Tricaine Methanesulfonate (MS-222), and sacrificed with a sharp blow to the head. Liver samples were dissected immediately and placed in ice-cold extraction medium A. Approximately 3 g of liver tissue was homogenized in 10 volumes of medium A with a handheld Teflon/glass homogenizer (Potter-Elvehjem). The homogenate was centrifuged at $600\times g$ for 10 min at 4°C . The superficial lipid layer was removed, and the remaining supernatant was centrifuged at $9000\times g$ for 10 min. The pellet obtained from the second spin was washed three times in medium A and re-suspended in a small volume of medium A plus 1 mg mL^{-1} Bovine Serum Albumin (BSA) according to [Suarez and Hochachka \(1981\)](#). Isolation and preparation of mitochondrial fractions from muscle and intestine were performed in medium B containing 120 mM KCl, 20 mM HEPES (4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid), 2 mM MgCl_2 , 1 mM ethylene glycol tetra-acetic acid (EGTA) and 5 mg mL^{-1} BSA adjusted to pH 7.4. Muscle and intestine samples (3–5 g) were dissected, chopped finely with a pair of scissors, and homogenized separately using a Potter-Elvehjem grinder in 10 volumes of medium B. The homogenate was centrifuged at $600\times g$ for 10 min, and the supernatant was filtered through four layers of cheesecloth to remove fat and fibrous tissue. The supernatant was centrifuged at $17000\times g$ for 10 min, and the pellet containing the mitochondria was re-suspended in 10 volumes of medium B and centrifuged at $7000\times g$ for 10 min. The pellets were re-suspended in 10 volumes of medium C (300 mM sucrose, 2 mM HEPES, 0.1 mM EDTA adjusted to pH 7.4) and centrifuged at $3500\times g$ for 10 min. The mitochondrial fraction was finally suspended in a small volume of medium C ([Birch-Machin and Turnbull, 2001a, 2001b](#); [Kirby et al., 2007](#)).

The mitochondrial suspensions were divided into aliquots, immediately and frozen in liquid nitrogen and stored at -80°C for the spectrophotometric measurement of the activities of individual complexes I–IV.

2.3.3. Measurements of liver mitochondrial enzyme activities

The activities of respiratory chain enzymes were determined at 28°C using a SmartSpec spectrophotometer after the mitochondrial membrane was ruptured by two freezes (in liquid nitrogen) and melting (in ice-cold water) cycles. All duplicate analyses in a Spectrosil Quartz Cuvette were executed to a final volume of 1 ml for each reading.

All enzyme activities were expressed as a function of mg mitochondrial protein for an ml unit with one unit of enzyme activity corresponding to the appearance of 1 μmol of the product or consumption of 1 μmol of the substrate per minute. Complex I (NADH: Ubiquinone Oxidoreductase, EC 1.6.5.3) activity was analyzed following the decrease in absorbance due to oxidation of NADH at 340 nm with 425 nm as the reference wavelength (extinction coefficient for $\text{NADH}=6.81\text{mM}^{-1}\text{cm}^{-1}$, to account for the contribution of ubiquinone1 to the absorbance at 340 nm) according to [Birch-Machin and Turnbull \(2001a, 2001b\)](#) and [Kirby et al. \(2007\)](#). Briefly, NADH (0.13mM), ubiquinone1 (65 μM), and antimycin A (2 $\mu\text{g mL}^{-1}$) were added to the assay medium containing 25mM potassium phosphate buffer, 5 mM MgCl_2 , pH 7.2, 2 mM KCN, and 2.5 mg mL^{-1} BSA and the absorbance change recorded for 2 min. Mitochondria (20– 50 μg protein) were added, and the NADH: ubiquinone oxidoreductase activity was measured for 4 min before the addition of rotenone (2 $\mu\text{g mL}^{-1}$), after which the activity was measured for an additional 3 min. Complex I activity was the rotenone-sensitive NADH: ubiquinone oxidoreductase activity. Complex II (Succinate: ubiquinone1 oxidoreductase, EC 1.3.5.1) activity was measured by following the decrease in absorbance due to oxidation of 2,6-

dichlorophenolindophenol (DCPIP) at 600 nm according to the procedure of [Birch-Machin and Turnbull \(2001a, 2001b\)](#) and [Kirby et al. \(2007\)](#).

Briefly, mitochondria (10–50 μg protein) were pre-incubated in assay medium containing 25 mM potassium phosphate buffer, 5 mM MgCl_2 , pH 7.2, plus 20 mM sodium succinate, at 28°C for 10 min. Antimycin A (2 $\mu\text{g mL}^{-1}$), rotenone (2 $\mu\text{g mL}^{-1}$), KCN (2 mM), and DCPIP (50 μM) were added, and a baseline rate was recorded for 3 min. The reaction rate was started with 65 μM ubiquinone1, and the enzyme-catalyzed reduction of DCPIP was measured for 4 min. The specific activity of this complex was determined using the DCPIP extinction coefficient of $\epsilon=19.1\text{mM}^{-1}\text{cm}^{-1}$.

Mitochondrial complex III (ubiquinol-cytochrome c reductase, EC 1.10.2.2) activity was measured by monitoring cytochrome c reduction using decylubiquinol at 550 nm with 580 nm as the reference wavelength ([Jeejeebhoy, 2002](#)). Briefly, mitochondria were pre-incubated at 28°C in an assay medium containing potassium phosphate (50 mM, pH 8), 0.1 mM EDTA, 2 g L^{-1} defatted BSA, 3 mM sodium azide and 60 μM ferricytochrome c for 3 min. The reaction was initiated by adding 0.1 mM L^{-1} decylubiquinol, and the enzyme activity was measured for 4 min. The non-enzymatic reduction of cytochrome c was measured after the addition of 10 mg L^{-1} of antimycin A, and an extinction coefficient factor of $\epsilon=19\text{mM}^{-1}\text{cm}^{-1}$ was used to calculate absolute changes.

Complex IV (cytochrome c oxidase, EC 1.9.3.1) activity was measured by following the oxidation of reduced cytochrome c as a decrease in absorbance at 550 nm with 580 nm as the reference wavelength ([Birch-Machin and Turnbull, 2001a, 2001b](#)). Cytochrome c subunit II (15 μM) and dodecyl maltoside (0.45 mM) were added to the assay medium containing potassium phosphate (20 mM, pH 7), and the non-enzymatic rate was recorded. Mitochondria (5–15 μg of protein) were added, and the complex IV activity was measured as the apparent first-order rate constant after fully oxidizing cytochrome c subunit II by the addition of few grains of potassium hexacyanoferrate. The specific activity was calculated using an extinction coefficient factor of $\epsilon=19.1\text{mM}^{-1}\text{cm}^{-1}$.

2.3.4. Measurements of antioxidant parameters

Malondialdehyde (MDA) content was examined as an indicator of lipid peroxidation, which is based on the 2-thiobarbituric acid (2,6-dihydropyrimidine-2-thiol, TBA) reactivity, with the results expressed as nmol/g tissue ([Jain et al., 1989](#)). The glutathione (GSH) content was determined spectrophotometrically by monitoring the chromophoric product resulting from the reaction of the 5,50-dithiobis-(2- nitrobenzoic acid) with GSH in the presence of NADPH and glutathione reductase at 412 nm ([Wu et al., 2011](#)). The GSH content was expressed as nmol/g tissue. Catalase (CAT, EC 1.11.1.6) activity was determined by measuring the decrease in H_2O_2 concentration at 240nm, according to [Aebi \(1984\)](#). The reaction mixture contained 50mM-potassium phosphate buffer (pH 7.0) and 10mM- H_2O_2 freshly added.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by the ferricytochromic method using xanthine/xanthine oxidase as the source of superoxide radicals. The reaction was monitored at 550nm, according to [McCord and Fridovich \(1969\)](#). The reaction mixture consisted of 50mM-potassium phosphate buffer (pH 7.8), 0.1mM-EDTA, 0.1mM-xanthine, 0.012mM-cytochrome, and 0.025 IU/ml xanthine oxidase. Activity is reported in units of SOD/mg of protein. Here, one unit of activity is defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome c reduction rate.

2.3.5. Gut microbiota qRT-PCR

At all sampling points, the entire intestine was excised and immediately placed in RNAlater™ (Sigma-Aldrich) at 4°C for 24 hours before they were frozen at -20°C . The DNA was extracted from the collected intestines for use as a PCR template. The intestines of each fish were shaken with 300 μL of lysis buffer provided in the pathogen DNA Extraction Kit (Intron Biotech, South Korea). The DNA was extracted

according to the manufacturer's instructions. The concentration of the DNA was then quantitated on a NanoDrop ND-1000 Spectrophotometer (Thermo Fischer, USA).

The amount of specific bacterial DNA (coding for 16S rRNA) (*Photobacterium damsela*, *Vibrio anguillarum*, *Lactobacillus plantarum*, *Lactobacillus subtilis* and *Enterococcus faecium*) in the intestinal samples was measured by qPCR for each fish with amplification of the 16S rRNA gene hypervariable region. Isolated DNA samples were diluted to 80 ng/ μ l for normalization and used as a template in the qPCR reaction. The PCR reactions were setup in 25 μ l reaction volumes containing 1 μ l of the forward and reverse primers as displayed in Table 2 (10 μ M), 0.5 μ l of MgCl₂ (25 mM), 12.5 μ l of 2X RealMode SYBR® Green Mix (Intron Biotech, South Korea), 80 ng of DNA template and 9 μ l of nuclease-free water. The PCR reactions were carried out in a CFX96 real-time PCR detection system (Bio-Rad, USA). Reaction times and cycling conditions were 94°C for 2 min, 40 cycles of 94°C for 30 s and 60°C for 50 s. A melting curve analysis further terminated the run.

2.3.6. Gene expression analysis

Liver samples were obtained, stored at -80 °C until analysis. For the mRNA extraction, the RNeasy Mini Kit (Qiagen) was used. Liver samples of 15mg inserted in a microcentrifuge tube, homogenized and centrifuged at 5000 \times g for 15s. The supernatant was collected and mixed with 70% ethanol. After the extraction of RNA, cDNA was synthesized using the HiSenScript RH cDNA Synthesis Kit (Intron Biotech, South Korea) following the manufacturer's protocol. Real-time PCR was done using SYBR select Master Mix kit (Thermo Fisher Scientific, Lithuania) using the following primers (TCR, IL1-B, Hepcidin, and IGF1) as shown in Table 2. β -Actin (ACTB) was used as housekeeping gene for mRNA gene expression normalisation (Table 2). Amplification was performed using the CFX96 Real-Time PCR System (Biorad, USA) with the protocol as follows: Initial 3 min denaturation at 94 °C, 40 cycles of 94 °C for 20 s, and 60 °C for 30 s. each assay was done in triplicate. SYBR Green was used as a fluorogenic dye that exhibits little fluorescence when in solution, but emits a strong fluorescent signal upon binding to double-stranded DNA. Spectrophotometric measurements at 260 nm was used to assess the concentration of the House Keeping Gene and target amplicon DNA for mRNA expression.

The relative gene expressions relative to β -Actin (ACTB) were calculated by the delta delta ct values. The expression stability was analyzed using geNorm and NormFinder software.

2.4. Statistical analysis

All data were analyzed using the SAS software, version 6.03 (Statistical Analysis System, 1996). Two-way ANOVA was used for

Table 2

The primers using for determining TCR, IL-1 β , Hepcidin and IGF1 are listed and were used relative to β -Actin (ACTB) and for bacteria.

Gene name	Gene abbreviation	Gene bank number	Primer sequences (5'-3')
Immunoglobulin M IgM	<i>IgM</i>	26691348 17547755	CAGCCTCGAGAAGTGGAAAC GAGGTTGACCAGGTTGGTGT
Hepcidin1	<i>Hep</i>	26691348 17547755	GCCATCGTGTCACCTTTAT CCTGCTGCCATACCCATCTT
Interleukin1 beta	<i>IL-1β</i>	28706519 26691348	GGGCTGAACAACAGCAGCTCTC TTAACACTCTCCACCCTCCA
T-Cell Receptor	<i>Tcr</i>	26691348 28453393	AAGTGCAATGCCAGCTTCTT TTGGCGGTCTGACTTCTT
Photobacterium damsela		28341681 27084008	CCTATGGGACATGAATGG GCTCTAGGCTAAATGAATC
Photobacterium damsela		27737014	ACATCATCCATTGTGTAC
Vibrio anguillarum		24649315	CCTTATCACTATCCAAATTTG
Vibrio anguillarum		26840426	GAAAGAGCCCCAACCAAGTGATT
Lactobacillus plantarum		23386831	CITCCAGATAATTCAACTATCGCTTA
Lactobacillus plantarum		23386831	GGAATCTTCCACAATGGACG
Lactobacillus subtilis		30286089	CGCTTTACGCCCAATAAATCCGG
Lactobacillus subtilis		25973610	ATCAACCATGTTGATGATAGC
Enterococcus faecium		30286089	AAGGGATACCGGACAATTCA

analyzing the individual effects of fishmeal and nucleotide inclusion levels and the interaction between them. Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed (Duncan, 1955). All percentage data were arc-sin transformed prior to analysis (Zar, 1984). However, data are presented untransformed to facilitate comparisons.

3. Results

3.1. Growth performance and feed efficiency

The growth performance of seabream is presented in (Table 3). The data obtained showed that the growth performance was ($P \leq 0.05$) affected either by the addition of nucleotides or the dietary fishmeal replacements and/or the interaction between two variables. The highest growth performance was recorded for fish fed FM_{25/250} and FM_{25/500} diets, while the lowest growth values were recorded for FM₀ and FM_{0/250} groups. Dietary nucleotides supplemented at 250mg/kg or 500mg/kg enhanced the final body weight of seabream either FM₂₅ or FM₀ compared to non-FM-N group (FM_{0/0}). The same trend was observed for WG and SGR. In terms of feed efficiency, Table 3 illustrates a significant difference in feed utilization indices between treatments ($P \leq 0.05$) with positive effects when dietary nucleotides was added. Fingerling seabream fed nucleotides-enriched diets recorded significantly ($P \leq 0.05$) better FCR values compared to non-nucleotides enriched diets, either FM₀ or FM₂₅. These results revealed that supplementation of the FM₂₅ diet with 500 mg/kg NucleoforceFish™ (FM_{25/500}) obtained the best significant ($P \leq 0.05$) FCR values. An improvement in dietary protein and energy utilization efficiency in terms of PPV and EU was observed. PPV increased by 32.9, and 17.8% for fish fed FM_{25/500}, and FM_{25/250} diets, respectively, compared to (FM_{25/0}), while it was 18.6 and 13.7% for FM_{0/500}, and FM_{0/250}, respectively, compared with non-FM-N diet (FM_{0/0}). Energy utilization was improved significantly ($P \leq 0.05$) by 44.7, and 28.8% for fish fed the FM_{25/500}, and FM_{25/250} diets, respectively, compared with the non-nucleotides enriched 25% FM diet (FM_{25/0}), while 21.0 and 14.3% values were recorded for fish fed the FM_{0/500}, and FM_{0/250} diets, respectively, compared with FM-N free diet (FM_{0/0}).

3.2. Liver enzyme activity

Table 4 shows that complex I liver enzyme activities gave a significant response ($P \leq 0.05$) to the dietary nucleotides enriched diets either FM-free or 25% FM contents and the interaction between them ($P \leq 0.05$). Complex I values ranged from 13.6 to 8.9 mL units/mg mitochondrial protein. The highest significant complex I activity values were recorded in FM_{25/500} and FM_{25/0}, respectively. Complex II and III

Table 3
Growth performance and feed utilization efficiency of juvenile gilthead seabream, *sparus aurata* fed different experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₅₀₀	NT ₂₅₀	NT ₀	NT ₅₀₀	NT ₂₅₀	NT ₀
IBW (g/fish)	0.36±0.01	0.36±0.01	0.35±0.01	0.36±0.01	0.36±0.01	0.35±0.01
FBW (g/fish)	13.14±0.19 ^{bc}	12.38±0.23 ^{cd}	11.76 ±0.27 ^d	14.25±0.24 ^a	13.63±0.16 ^{ab}	12.60 ±0.36 ^c
Weight gain (g/fish)	12.78±0.19 ^{bc}	12.02±0.24 ^{cd}	11.41±0.23 ^d	13.89±0.21 ^a	13.27±0.18 ^{ab}	12.25±0.31 ^c
SGR (%/fish/day)	1.999±0.013 ^{bc}	1.965±0.005 ^{cd}	1.947±0.007 ^d	2.043±0.005 ^a	2.022±0.010 ^{ab}	1.986±0.014 ^c
FCR	1.99±0.02 ^c	2.17±0.04 ^b	2.34±0.03 ^a	1.81±0.02 ^d	1.95±0.05 ^c	2.23±0.02 ^b
PPV (%)	19.17±1.34 ^{bc}	18.38±0.56 ^{bc}	16.16±0.40 ^c	23.32±1.64 ^a	20.67±1.46 ^{ab}	17.54±0.26 ^{bc}
EU (%)	15.75±1.32 ^{ab}	14.88±0.58 ^{bc}	13.02±0.36 ^{bc}	17.97±1.26 ^a	16.00±1.10 ^{ab}	12.42±0.20 ^c

	FM levels		NT Levels		
	25%	0%	250mg	0mg	500mg
IBW (g/fish)	0.36±0.1	0.35±0.1	0.36±0.1	0.35±0.1	0.36±0.1
FBW (g/fish)	13.49±0.25 ^a	12.43±0.22 ^b	13.01±0.22 ^b	12.18±0.30 ^c	13.70±0.14 ^a
Weight gain (g/fish)	13.13±0.22 ^a	12.08±0.21 ^b	12.65±0.22 ^{ab}	11.83±0.29 ^c	13.34±0.12 ^a
SGR (%/fish/day)	2.02±0.01 ^a	1.97±0.01 ^b	2.00±0.01 ^{ab}	1.97±0.01 ^c	2.02±0.01 ^a
FCR	2.00±0.02 ^b	2.17±0.03 ^a	2.06±0.04 ^b	2.29±0.02 ^a	1.90±0.02 ^c
PPV (%)	20.51±1.11 ^a	17.90±0.95 ^b	19.53±0.88 ^b	16.85±0.38 ^c	21.25±1.42 ^a
EU (%)	15.46±0.95 ^a	14.55±0.58 ^b	15.44±0.85 ^b	12.72±0.25 ^c	16.86±1.20 ^a

*Values are means ± SEM, n = 3.

** -FM_{0/0} (0% fish meal, 0mg nucleotide), FM_{0/250} (0% fish meal+250mg nucleotide), FM_{0/500} (0% fish meal+500mg nucleotide), FM_{25/0} (0mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250mg nucleotide), FM_{25/500} (25% fish meal+500mg nucleotide).

- Final body weight (FBW), Weight gain (WG), feed conversion ratio (FCR), protein productive value (PPV) and Energy utilization, (EU %).

-FM=fishmeal; NT = nucleotides.

Table 4

The specific activity (Activity Units/g Protein) of respiratory chain enzymes (Complex I, II, III, and IV) in liver mitochondria of juvenile gilthead seabream, *sparus aurata* fed different experimental diets.

	NT ₀	NT ₅₀₀	NT ₂₅₀	FM ₂₅	FM ₀	Items
				NT ₀	NT ₂₅₀	NT ₅₀₀
Complex I	8.98±0.18 ^c	10.32±0.14 ^c	9.59±0.32 ^d	12.40±0.11 ^b	19.16±0.16 ^a	13.62±0.16 ^b
Complex II	42.49±0.29 ^e	50.84±0.13 ^c	51.23±0.13 ^c	43.15±0.21 ^d	62.25±0.23 ^a	58.62±0.19 ^b
Complex III	151.48±1.71 ^e	178.95±1.84 ^c	165.76±0.95 ^d	152.95±0.42 ^c	212.64±1.45 ^a	198.35±0.47 ^b
Complex IV	250.92±1.57 ^e	268.43±1.09 ^c	261.31±1.27 ^d	268.50±1.25 ^c	296.31±1.55 ^a	290.44±0.50 ^{ab}

	0%	25%	FM levels	NT Levels	
			0mg	250mg	500mg
Complex I	9.63±0.21 ^b	11.73±0.13 ^a	10.69±0.14 ^b	9.38±0.24 ^c	11.97±0.13 ^a
Complex II	48.19±0.18 ^b	54.67±0.20 ^a	42.82±0.21 ^c	56.74±0.17 ^a	54.73±0.14 ^b
Complex III	165.40±1.31 ^b	187.98±0.64 ^a	152.22±1.11 ^c	189.20±1.01 ^a	188.65±1.23 ^a
Complex IV	260.22±1.29 ^b	285.08±1.01 ^a	259.71±1.27 ^c	275.81±1.43 ^b	282.44±0.87 ^a

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal, 0mg nucleotide), FM_{0/250} (0% fish meal+250mg nucleotide), FM_{0/500} (0% fish meal+500mg nucleotide), FM_{25/0} (0mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250mg nucleotide), FM_{25/500} (25% fish meal+500mg nucleotide).

-FM=fishmeal; N = nucleotides.

-Complex I =NADH: Ubiquinone Oxidoreductase, EC 1.6.5.3, Complex II =Succinate: ubiquinone1 oxidoreductase, EC 1.3.5.1, Complex III = ubiquinol: ferricytochrome c reductase, EC 1.10.2.2, Complex IV= cytochrome c oxidase, EC 1.9.3.1.

activity ranged from 62.25 to 42.5 and 212.64 to 151.48 mL units/mg mitochondrial protein, respectively. Fish fed the diets FM_{25/250}, and FM_{25/500} recorded the highest significant values for either complex II or III activity compared to other treatments. Complex IV ranged from 304.7 to 250.92 mL units/mg mitochondrial protein. Complex IV showed higher significant values for the FM_{25/0} diet group.

3.3. Gene expression

The gene expression results of IGF1, TCR-B, IL1-*B*, and Hecpidin of fish fed diets containing different levels of nucleotides and fish meal presented in Table 5. The results showed that the highest gene expression ($P \leq 0.05$) of IGF1 and Hecpidin was recorded for fish fed FM_{25/250}, while the highest gene expression ($P \leq 0.05$) of TCR-B and IL1-*B* were

recorded for fish fed FM_{0/0} and FM_{0/500}, respectively.

3.4. Gut microbiome

Table 6 shows the gut microbiome abundance of seabream fed different experimental diets. The obtained data showed that the selected gut microbial communities were significantly affected either with NucleoforceFish™ enriched or FM-free tested diets. The highest abundance of *Photobacterium damsela* was observed in FM₀ diets supplemented with 250 and 500 mg NucleoforceFish™. Furthermore, the lowest abundance was associated with the absence of nucleotides FM_{25/0} diet. Furthermore, *Vibrio anguillarum* was also dominating in fish fed with FM₀ supplemented with 500mg NucleoforceFish™; however, the lowest richness was linked to the FM_{0/0} diet. On the other hand, the

Table 5IGF1, TCR-B, IL-1 β , and Hcpicidin gene expressions in liver of juvenile gilthead seabream, *Sparus aurata* fed different experimental diets.

Items	FM0			FM25		
	NT0	NT250	NT500	NT0	NT250	NT500
IBW (g/fish)	0.35 \pm 0.01	0.36 \pm 0.01	0.36 \pm 0.01	0.35 \pm 0.01	0.36 \pm 0.01	0.36 \pm 0.01
FBW (g/fish)	11.76 \pm 0.27d	12.38 \pm 0.23cd	13.14 \pm 0.19bc	12.60 \pm 0.36c	13.63 \pm 0.16ab	14.25 \pm 0.24a
Weight gain (g/fish)	11.41 \pm 0.23d	12.02 \pm 0.24cd	12.78 \pm 0.19bc	12.25 \pm 0.31c	13.27 \pm 0.18ab	13.89 \pm 0.21a
SGR (%/fish/day)	1.947 \pm 0.007d	1.965 \pm 0.005cd	1.999 \pm 0.013bc	1.986 \pm 0.014c	2.022 \pm 0.010ab	2.043 \pm 0.005a
FCR	2.34 \pm 0.03a	2.17 \pm 0.04b	1.99 \pm 0.02c	2.23 \pm 0.02b	1.95 \pm 0.05c	1.81 \pm 0.02d
PPV (%)	16.16 \pm 0.40c	18.38 \pm 0.56bc	19.17 \pm 1.34bc	17.54 \pm 0.26bc	20.67 \pm 1.46ab	23.32 \pm 1.64a
EU (%)	13.02 \pm 0.36bc	14.88 \pm 0.58bc	15.75 \pm 1.32ab	12.42 \pm 0.20c	16.00 \pm 1.10ab	17.97 \pm 1.26a

	FM levels			NT Levels		
	0%	25%	0mg	250mg	500mg	
IBW (g/fish)	0.35 \pm 0.1	0.36 \pm 0.1	0.35 \pm 0.1	0.36 \pm 0.1	0.36 \pm 0.1	
FBW (g/fish)	12.43 \pm 0.22b	13.49 \pm 0.25a	12.18 \pm 0.30c	13.01 \pm 0.22b	13.70 \pm 0.14a	
Weight gain (g/fish)	11.41 \pm 0.21b	13.13 \pm 0.22a	11.83 \pm 0.29c	12.65 \pm 0.22ab	13.34 \pm 0.12a	
SGR (%/fish/day)	1.97 \pm 0.01b	2.02 \pm 0.01a	1.97 \pm 0.01c	2.00 \pm 0.01ab	2.02 \pm 0.01a	
FCR	2.17 \pm 0.03a	2.00 \pm 0.02b	2.29 \pm 0.02a	2.06 \pm 0.04b	1.90 \pm 0.02c	
PPV (%)	17.90 \pm 0.95b	20.51 \pm 1.11a	16.85 \pm 0.38c	19.53 \pm 0.88b	21.25 \pm 1.42a	
EU (%)	14.55 \pm 0.58b	15.46 \pm 0.95a	12.72 \pm 0.25c	15.44 \pm 0.85b	16.86 \pm 1.20a	

*Values are means \pm SEM, n = 3. Data is normalized to (1) for the control fishmeal nucleotide free diets.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal, 0mg nucleotide), FM_{0/250} (0% fish meal+250mg nucleotide), FM_{0/500} (0% fish meal+500mg nucleotide), FM_{25/0} (0mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250mg nucleotide), FM_{25/500} (25% fish meal+500mg nucleotide).

-FM=fish meal; N = nucleotides.

Table 6Gut microbiota profile of juvenile gilthead seabream, *sparus aurata* fed different experimental diets.

Items	FM0			FM25		
	NT0	NT250	NT500	NT0	NT250	NT500
IBW (g/fish)	0.35 \pm 0.01	0.36 \pm 0.01	0.36 \pm 0.01	0.35 \pm 0.01	0.36 \pm 0.01	0.36 \pm 0.01
FBW (g/fish)	11.76 \pm 0.27d	12.38 \pm 0.23cd	13.14 \pm 0.19bc	12.60 \pm 0.36c	13.63 \pm 0.16ab	14.25 \pm 0.24a
Weight gain (g/fish)	11.41 \pm 0.23d	12.02 \pm 0.24cd	12.78 \pm 0.19bc	12.25 \pm 0.31c	13.27 \pm 0.18ab	13.89 \pm 0.21a
SGR (%/fish/day)	1.947 \pm 0.007d	1.965 \pm 0.005cd	1.999 \pm 0.013bc	1.986 \pm 0.014c	2.022 \pm 0.010ab	2.043 \pm 0.005a
FCR	2.34 \pm 0.03a	2.17 \pm 0.04b	1.99 \pm 0.02c	2.23 \pm 0.02b	1.95 \pm 0.05c	1.81 \pm 0.02d
PPV (%)	16.16 \pm 0.40c	18.38 \pm 0.56bc	19.17 \pm 1.34bc	17.54 \pm 0.26bc	20.67 \pm 1.46ab	23.32 \pm 1.64a
EU (%)	13.02 \pm 0.36bc	14.88 \pm 0.58bc	15.75 \pm 1.32ab	12.42 \pm 0.20c	16.00 \pm 1.10ab	17.97 \pm 1.26a

	FM levels			NT Levels		
	0%	25%	0mg	250mg	500mg	
IBW (g/fish)	0.35 \pm 0.1	0.36 \pm 0.1	0.35 \pm 0.1	0.36 \pm 0.1	0.36 \pm 0.1	
FBW (g/fish)	12.43 \pm 0.22b	13.49 \pm 0.25a	12.18 \pm 0.30c	13.01 \pm 0.22b	13.70 \pm 0.14a	
Weight gain (g/fish)	11.41 \pm 0.21b	13.13 \pm 0.22a	11.83 \pm 0.29c	12.65 \pm 0.22ab	13.34 \pm 0.12a	
SGR (%/fish/day)	1.97 \pm 0.01b	2.02 \pm 0.01a	1.97 \pm 0.01c	2.00 \pm 0.01ab	2.02 \pm 0.01a	
FCR	2.17 \pm 0.03a	2.00 \pm 0.02b	2.29 \pm 0.02a	2.06 \pm 0.04b	1.90 \pm 0.02c	
PPV (%)	17.90 \pm 0.95b	20.51 \pm 1.11a	16.85 \pm 0.38c	19.53 \pm 0.88b	21.25 \pm 1.42a	
EU (%)	14.55 \pm 0.58b	15.46 \pm 0.95a	12.72 \pm 0.25c	15.44 \pm 0.85b	16.86 \pm 1.20a	

*Values are means \pm SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal, 0mg nucleotide), FM_{0/250} (0% fish meal+250mg nucleotide), FM_{0/500} (0% fish meal+500mg nucleotide), FM_{25/0} (0mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250mg nucleotide), FM_{25/500} (25% fish meal+500mg nucleotide).

-FM=fishmeal; N = nucleotides.

highest abundance of the *Lactobacillus plantarum* and *Lactobacillus subtilis* was revealed in fish fed on FM_{25/0}, but the lowest abundance was associated with FM_{0/0}. Finally, the highest richness *Enterococcus faecium* was recognized in fish fed on FM_{0/500}; nevertheless, the lowest was connected to the FM_{25/250} diet.

3.5. Activity of oxidative enzymes

Table 7 shows the activities of superoxide dismutase (SOD), catalase (CAT) (U/mg protein), glutathione (GSH) and malondialdehyde (MDA) (nmol/g tissue). Superoxide dismutase and CAT results significantly improved in both FM_{25/500} and FM_{25/250} with 554.5, 363.7 U/mg protein, and 268.1, 218.4 U/mg protein, respectively. Meanwhile, they were decreased in a significant way in FM_{0/0} diets with 138.5 and 75.5

U/mg protein. Reduced GSH and showed a significant decrease in both FM_{0/0} and FM_{0/250} diets with 1491.7 and 2179.3 nmol/g tissues, associated with a significant increase in MDA values recorded in the same two diets with 19.88 and 17.80, correspondingly.

3.6. Economic evaluation

Table 8 presents the data on the economic conversion rate (ECR). The cost of one kg of FM_{25/0} diet increased by 3.32, 6.64, 13.86, 17.18 and 20.50% in the FM_{25/500}, FM_{25/250}, FM_{0/0}, FM_{0/250}, and FM_{0/500} diets, respectively. The lowest values of ECR were recorded for FM_{0/500}, and FM_{25/500} diets, respectively, compared with either FM_{0/0} or FM_{25/0} diets, which recorded the highest ECR values.

Table 7

Specific activities of superoxide dismutase (SOD), catalase (CAT) (U/mg protein), reduced glutathione (GSH) and malondialdehyde (MDA) (nmol/g tissue) in the liver of gilthead seabream, *Sparus aurata* fed different experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₅₀₀	NT ₂₅₀	NT ₀	NT ₅₀₀	NT ₂₅₀	NT ₀
SOD (U/mg protein)	223.70±14.6 ^c	154.60±18.60 ^d	138.50±8.40 ^d	554.50±15.10 ^a	363.70±11.90 ^b	220.30±12.00 ^c
CAT (U/mg protein)	128.40±8.50 ^c	99.40±11.60 ^{cd}	75.50±9.30 ^d	268.10±10.30 ^a	218.40±9.60 ^b	127.60±8.20 ^c
GSH (nmol/g tissue)	2822.20±47.0 ^a	2179.30±54.3 ^b	1491.70±42.70 ^c	2852.50±37.40 ^a	2816.70±51.30 ^a	2797.03±42.90 ^a
MDA (nmol/g tissue)	9.88±0.30 ^d	17.80±0.50 ^b	19.88±0.30 ^a	9.97±0.40 ^d	10.88±0.50 ^{cd}	11.420±0.40 ^c
		FM levels		NT Levels		
		25%	0%	500mg	250mg	0mg
SOD (U/mg protein)		379.50±13.00 ^a	172.27±13.87 ^b	389.10±14.85 ^a	259.15±15.25 ^b	179.4±10.20 ^c
CAT (U/mg protein)		204.70±9.37 ^a	101.10±9.80 ^b	198.25±9.40 ^a	158.90±10.60 ^b	101.55±8.75 ^c
GSH (nmol/g tissue)		2822.17±43.87 ^a	2164.40±48.00 ^b	2837.35±42.20 ^a	2498.00±52.80 ^b	2144.50±42.80 ^c
MDA (nmol/g tissue)		10.76±0.43 ^b	15.85±0.37 ^a	9.925±0.35 ^c	14.34±0.5 ^b	15.65±0.35 ^a

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal, 0mg nucleotide), FM_{0/250} (0% fish meal+250mg nucleotide), FM_{0/500} (0% fish meal+500mg nucleotide), FM_{25/0} (0mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250mg nucleotide), FM_{25/500} (25% fish meal+500mg nucleotide).

-FM=fishmeal; N = nucleotides.

Table 8

Economic analysis of juvenile gilthead seabream, *Sparus aurata* production output cost/metrics fed different the experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₅₀₀	NT ₂₅₀	NT ₀	NT ₅₀₀	NT ₂₅₀	NT ₀
Cost of Diet (kg/\$)*	0.872	0.858	0.844	0.989	0.975	0.961
Total consumed feeds(gm/fish)	25.39±0.56 ^{bc}	26.06±0.56 ^b	26.68±0.89 ^b	25.13±0.39 ^c	25.88±0.40 ^{bc}	27.26±0.94 ^a
ECR	1.74±0.19 ^d	1.86±0.23 ^b	1.98±0.26 ^b	1.79±0.24 ^c	1.90±0.16 ^b	2.14±0.36 ^a
		FM levels		NT Levels		
		25%	0%	500mg	250mg	0mg
Cost of Diet (kg/\$)*		0.98	0.86	0.93	0.92	0.9
Total consumed feeds (gm/fish)		26.09±0.58	26.04±0.67	25.26±0.48 ^c	25.97±0.48 ^b	26.97±0.92 ^a
ECR		1.94±0.25 ^a	1.86±0.23 ^b	1.77±0.22 ^c	1.88±0.20 ^b	2.06±0.31 ^a

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal, 0mg nucleotide), FM_{0/250} (0% fish meal+250mg nucleotide), FM_{0/500} (0% fish meal+500mg nucleotide), FM_{25/0} (0mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250mg nucleotide), FM_{25/500} (25% fish meal+500mg nucleotide).

-FM=fishmeal; NT = nucleotides.

One US\$ = 18 Egyptian pound (L.E.).

FCR= Feed conversion ratio.

ECR=Economical conversion rate= Cost of diet (\$/kg)* FCR.

4. Discussion

Nucleotides play a crucial role in cellular metabolism, including the storage, transfer, and expression of genetic information, standing as activated intermediates of energy transport in cells (e.g., ATP, NAD+), (Krüger and Mariët, 2018). Nucleotides are synthesized *de novo* in most fish tissues, but with low quantity under certain conditions (Do Huu, 2016). Therefore, they are considered as being mostly non-essential nutrients but sometimes becoming semi-essential (Li and Gatlin III, 2006).

Inclusions of nucleotides have a significant benefit to the body under certain conditions, particularly under physiological stress (Hess and Greenberg, 2012). Fish meal has a considerable content of nucleotides, but unfortunately, fish meal is becoming a limited, expensive natural resource. Most aquafeed formulas without fish meal contain relatively low amounts of nucleotides (Do Huu et al., 2012). To combat this issue, considerable research is now being performed toward the replacement of fish meal with other alternative products that contain high levels of nucleotides.

The results observed in the current investigation showed that nucleotide-enriched diets with 250mg/kg or 500mg/kg enhanced growth performance and feed utilization and metabolic indices of sea bream fed both fish meal and non-fish meal control diets respectively. These results are consistent with Hossain et al. (2016) who demonstrated

positive effects of nucleotides on growth, blood chemistry, oxidative stress and growth factor gene expression of juvenile red sea bream (*Pagrus major*). Furthermore, Hassaan et al. (2018) found that Nile tilapia fed diets contained four levels of yeast extract rich in nucleotides and β-glucan increased the weight gain linearly while the feed conversion ratio declined linearly with increasing levels of yeast extract. These latter authors also stated that in addition, fish fed diets supplemented with enriched nucleotide product improved Hepatic function and promote liver and gut restoration. In addition, Tahmasebi-Kohyani et al. (2012) reported inclusion of 0.1% or 0.2% nucleotide to the diet promoted improved weight gain and feed efficiency in fingerling rainbow trout. Xu et al. (2015) found that fingerling hybrid tilapia fed a 0.60% nucleotide supplemented diet exhibited the highest growth and feed utilization indices compared to a nucleotide free control diet. Also, Yin et al. (2015) concluded that *Ancherythroculter nigrocauda* (a Chinese cyprinid species) fed experimental diets containing yeast nucleotide with a concentration of 0.45% showed the highest values of final weight, SGR, PER and the best value of FCR compared with other diets. Moreover, Hossain et al. (2019) reported the advantage of inclusion nucleotide on growth performance and immune system in different fish species. Following the same trend, Selim et al. (2020) found that the highest growth performance and feed utilization efficiency were recorded in fish fed 2.5 g NT/kg diet. However, the present results for seabream are inconsistent with Ridwanudin et al. (2019) who found that rainbow

trout fed diets containing low-fish meal content supplemented with nucleotide clearly showed that there was no positive effect of dietary nucleotides on growth of rainbow trout. Furthermore, nucleotide supplementation in juvenile turbot, *Scophthalmus maximus* failed to affect the growth either in a positive or negative pattern, this finding could be due to species effect or the insufficient dose of nucleotide employed (Fuchs et al., 2015).

The present results could be attributed to a different scenario as follows: i) the inclusion of Nucleoforce Fish™ that has good palatability, flavor, and aromatic characteristics, which may cause better attractability, feed intake and development of the intestine of the nucleotide-supplemented juvenile seabream. As a consequence, better growth response induction and feed utilization of the tested diets is obtained, ii) Inclusion of nucleotide alters intestinal bacteria that enhances nutrient assimilation and consequently improves growth performance and feed utilization (Bowyer et al., 2019; Gupta et al., 2019; Hoseinifar et al., 2020),, iii) Nucleotide fortification improves the haemato-biochemical parameters as serum alanine amino-transferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP); v) stimulatory effects of digestive enzymes as lipase, amylase, and growth hormone which directly has positive effects on growth performance and feed efficiency (Ridwanudin et al., 2019).

The present study revealed a positive effect of nucleotides on liver mitochondrial enzyme complex activities, which are consequently associated with the positive effect on feed utilization parameters FCR ($n=3$, $r^2=0.99$), PPV ($n=3$, $r^2=0.93$) and energy utilization ($n=3$, $r^2=0.94$). Fish fed the diets FM_{25/500} was superior in the expression of Complex I by 13.62% and associated with the best values of FCR (1.95) which supports the hypothesis that enhancement of mitochondrial function is correlated with enhancing growth and feed utilization. The activity levels of Complex II enzymes for fish fed the diets of FM_{25/250} were highly significantly different compared with other diets (Table 4). Similarly, the best FCR ratios, PPV and EU values, were recorded with either fish fed the diets FM_{25/250} or FM_{25/500}, which both were superior to either FM₂₅ or FM₀ diets supporting the current study findings as they are linked to their best expression to Complex II. The present results are in parallel with (Bottje and Carstens, 2009; Eya et al., 2013). Moreover, Eya et al. (2013) indicated that correlating enhancement of performance and feed efficiency with mitochondrial enzyme complexes in many animals is vital to decrease the cost expenditure for energy production at the cellular level.

In terms of Hepatic gene expression, expression of IGF1 in diet FM_{25/250} was statistically elevated than in the livers of seabream from the other treatments. This finding was in accordance with the FBW with the same diet as it showed an enhanced growth pattern. The present results are consistent with Hossain et al. (2016) who found positive effects of nucleotide on growth and growth factor gene expression of juvenile red sea bream. Furthermore, Larva Lanes et al. (2012) found an enhancement of gene expression of IGF1 of rainbow trout fed diets enriched with nucleotides, which will positively affect the growth rate. On the contrary, Do Huu (2016) found that excessive use of nucleotide (10%) negatively affects the growth of rainbow trout under their experimental conditions.

T cell receptors B (TCR-B) protein in nature complexes are found in the plasma membranes of immune cells to recognize antigens and are responsible for interaction with major histocompatibility complex (MHC) considered as a very important indicator for cell-mediated immunity (Estevez et al., 2018). Nucleotide supplementation elevates stress tolerance in aquaculture as it enhances and modulates cell-mediated immunity as well as innate immunity, consequently increases the fish capability to face any infection (Ringø et al., 2012). Our findings were in accordance with the previously mentioned hypothesis in which FM_{25/250} and FM_{25/500} diets showed the least up-regulation of TCR-B with statistical difference other than the rest of the diets following then FM_{0/250} and FM_{0/500}. Furthermore, Torrecillas et al. (2015) and Zhou et al. (2018) suggested that the usage of soybean meal-

based diets showed down-regulation of TCR-B; this was not in the same line with our findings where FM₀ diet was statistically the highest up-regulation of TCR-B in particular with FM_{0/500} treatment with the apparently strongest nucleotide modulating influence. This could be due to differences in diet formulations between the two studies as ours was supplemented with poultry by-product meal while the other study was totally formulated with soybean meal and other plant protein sources. As a result of activation of pathogen associated molecular patterns (PAMPs) via host pattern recognition receptors (PRRs) Interleukin 1-beta (IL-1 β) is produced, (Zou and Secombes, 2016).

According to Angosto et al. (2012) the mechanism of IL-1 β is different in teleost fish from mammals, yet it still plays a vital role in regulating the inflammatory progression, furthermore it has a role in fish muscle metabolism and growth, as it controls muscle mass and insulin growth factor binding protein (IGFBP), (Pooley et al., 2013; Heidari et al., 2015). In the present study, IL-1 β was significantly up-regulated in FM_{0/500}, FM_{0/250} and FM_{25/250} seabream liver compared to the remaining dietary treatments fed to seabream. These findings are in accordance with Reda et al., 2018 with the same product tested in tilapia. In contrast, Reyes-Becerril et al. (2008) reported IL-1 β expression in liver was down-regulated in sea bream using live yeast, *Debaryomyces hansenii*.

The up-regulation of IL-1 β via nucleotide supplementation needs further clarification either it is up-regulated due to increase in the inflammatory action or it is due to its positive effect on IGF expression. Interestingly, in our study the significant up-regulation of IL-1 β expression in FM_{0/500} could be due to the compensatory effect of enriched diets with NucleoforceFish™ in the absence of dietary fish meal. In this connection, Sitja-Bobadilla et al. (2005) reported that increasing the inclusion of dietary plant protein sources in FM-free diets may negative affects the immune system of fish.

Hepcidin (Hep) an antimicrobial peptide, is one of the main mediators in the innate immune system expressed in liver (Cuesta et al., 2008). Gene expression of Hep is up-regulated as a result of lipopolysaccharide (LPS) presence, iron overload and bacteria (Yang et al., 2007). Pereiro et al. (2012) reported that Hep is up-regulated due to bacterial infection not because of iron surplus.

Although Hep showed antimicrobial activity against most bacteria, according to Cuesta et al. (2008) the viability of *Photobacterium damsela* and *Vibrio anguillarum* was not affected yet growth inhibition of nearly all strains of pathogenic bacteria was achieved in the latter study.

Our findings showed significant differences in Hep relative gene expression values between all experimental treatments, where FM_{25/500} showed the highest relative up-regulation and FM_{25/0} showed the lowest degree of up-regulation.

The present results are consistent with Guo et al. (2019) who found zebra fish, *Danio rerio* fed diets supplemented with nucleotides modulate the regulation of Hep which may consequently enhance the immune system. On the contrary, Reyes-Becerril et al. (2008) found a down-regulation of the Hep gene in liver when the diet was supplied by live yeast, *Debaryomyces hansenii* to gilthead seabream. The effect of absence of dietary fish meal on Hep was reported by Wang et al. (2020) for juvenile hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂). Their results exhibited down-regulation of Hep gradually with the decrease of fish meal inclusion where the diet with 34% fish meal showed the lowest significant decline in Hep. Their results were in partial accordance with our outcomes as diet FM_{25/0} was observed to present the most relative down-regulation of Hep. The significant up-regulation for Hep for fish receiving the 500mg/Kg supplementation of NucleoforceFish™ in the presence and absence of fishmeal was notable and a trend for significant up-regulation with increased nucleotide addition was detected. This finding suggests that nucleotides could support the absence of FM by substitution with terrestrial animal and plant based ingredients but only to a certain limit.

Therefore, the interaction between the FM substitution and nucleotide supplementation needs further research regarding the immune

status of sea bream. Insulin-like growth factor 1 (IGF1) peptides released from the liver in nature regulates the growth of vertebrates via stimulating cell division through the somatotrophic axis (Tsai et al., 2014; Midhun et al., 2016). Using nutrients that stimulate IGF1 is an old approach to enhance the growth in aquaculture as well as deficiencies affecting the IGF1 expression decreases the performance and consequently negatively affects the growth (Azizi et al., 2016).

To our knowledge, there is very scarce research regarding the effect of nucleotide supplementation accompanied by FM limitation on the antioxidant response in sea bream diets. The current trial evaluated the effect of nucleotide supplementation and FM replacement on SOD and CAT as indicators for the increase of free radicals superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Hassan, 1980). GSH, another non-enzymatic antioxidant marker indicator for antioxidant stress and MDA, one of the metabolites of lipid peroxidation were measured to give a full picture of oxidative stress (Requena et al., 1996). The present results showed a significant increase in SOD and CAT in both FM₀, FM_{25/250} and FM_{25/500} in comparison to the other groups. This result is in accordance with most of the literature where the increase in metabolic activity exerts an amount of free radicals compensated by the production of SOD and CAT indicating that the supply of nucleotides enhances the antioxidant activity (Zhao et al., 2017; Reda et al., 2018). On the contrary, Wei et al. (2015) did not observe any differences in the expression of SOD and CAT as a result of supplementation of dietary nucleotides in the diets of sea cucumber, *Apostichopsis japonicas*. Similarly, there was no significant difference in SOD and CAT activities reported when a dietary commercial nucleotide yeast based protein (Nu-Pro®) (NP) additive was tested on rainbow trout (*Oncorhynchus mykiss*) by Özlüer-Hunt et al., 2016 to evaluate 20 (NP 20), 40 (NP 40) and 60 % (NP 60) fish meal substitution with this nucleotide enriched product. Furthermore, Zhou et al. (2011) stated that the replacement of FM by poultry by-product meal (PBM) in diets for juvenile cobia, *Rachycentron canadum* does not affect the antioxidant defenses. In addition, Hossain et al. (2016) reported that CAT levels were decreased by supplementation of nucleotides in juvenile red sea bream, *Pagrus major*.

The highest MDA ($P \leq 0.05$) values observed in fish fed diets without nucleotide supplementation as FM_{0/0} and FM_{0/250} consistent with the increase in our results of lipid peroxidation where there was no FM in the diets and the minimum amount of nucleotide addition. The same results was observed by (Xu et al., 2015; Reda et al., 2018; Zhao et al., 2017), where diets contained a high amount of nucleotides decreased the MDA level significantly in juvenile hybrid tilapia, seabass, and yellow catfish, respectively.

The vertebrate intestinal environment is colonized by a complex microbiome community, which plays a crucial role in host physiology and health (Pickard et al., 2017). The total or partial replacement of fish meal in seabream diets is commonly accompanied by gut microbial community changes (Estruch et al., 2015). However, the impact of nucleotide supplementation is not fully elucidated. The Proteobacteria, Firmicutes and Actinobacteria dominate fish gut microbial communities, therefore the present study focused on a group of these phyla, including *Photobacterium damsela*, *Vibrio anguillarum*, *Lactobacillus plantarum*, *Lactobacillus subtilis* and *Enterococcus faecium* (Egerton et al., 2018). The pathobiome is recently identified as a group of host-associated organisms accompanied by a fundamental decrease of the animal health status, owing to the interaction between these group members and hosts (Bass et al., 2019). Thus, the present study presented two crucial species of seabream pathobiome; *Photobacterium damsela* and *Vibrio anguillarum*. *Photobacterium damsela* (formerly *Vibrio damsela*) is one of the main bacterial diseases affecting seabream, and it has been reported as an endemic infection in Egypt (Essam et al., 2016). *Photobacterium damsela* was highly represented in FM₀ diets supplemented with either 250 or 500 nucleotides than in comparison to other diets.

The total replacement of fish meal was reported to be linked to an increase in the abundance of *Photobacterium* (Estruch et al., 2015).

Differences in *Photobacterium* abundance may be attributed to the differences in fibre between diets, and it might also suggest a change of gut immune mechanisms of seabream (Estruch et al., 2015). Moreover, the nucleotide represents an essential source of the building blocks of the bacterial nucleic acid (Reda et al., 2018). On the contrary, a moderate abundance of *Photobacterium damsela* was observed in fish fed on FM_{25/500} and FM_{25/250} and the lowest abundance was associated with the absence of nucleotides. Besides, *Vibrio anguillarum* was predominating in fish fed with FM₀ supplemented with 250 and 500 nucleotides. These results agreed with Estruch et al. (2015), who reported that the presence of vibrio was only associated with the total replacement of fishmeal.

On the other hand, fish fed with FM_{25/250} have shown a lower abundance of both species suggesting that the supplementation of nucleotide is crucial for lowering some species of seabream pathobiome. Lactic acid bacteria (LAB) play a pivotal role in finfish health and physiology, owing to their role in gastrointestinal tract development and enhancement of digestive functions, improving host immune response and disease resistance (Ringø et al., 2018). Furthermore, lactic acid bacteria have been widely used as probiotics in aquaculture; thus, the current study targeted three species of lactic acid bacteria. The highest abundance of the *Lactobacillus plantarum* and *Lactobacillus subtilis* was detected in fish fed with FM_{25/0}. Although the replacement of fish meal has been reported to negatively impact *Lactobacillus* abundance in seabream gut (Estruch et al., 2015). A high abundance of *Lactobacillus* was revealed in fish fed on FM_{0/250}. The lowest abundance of *Lactobacillus plantarum* and *Lactobacillus subtilis* was observed in fish fed on FM₀ and FM_{25/500}, respectively. On the other hand, the highest abundance of *Enterococcus faecium* was identified in fish fed on FM_{0/500}. However, previous studies suggested that *Enterococcus faecium* was not detected in fish fed on FM substitutions (Torrecillas et al., 2015). Thus, this result reveals the role of nucleotides in increasing the abundance of *Enterococcus faecium* within the seabream gut microbiome. The moderate richness of *Enterococcus faecium* was detected in fish fed on FM_{25/500}, but the lowest was connected to the FM_{25/250} diet.

The increasing price of feed is considered one of the most important factors limiting profitability in fish culture. The cost of all experimental supplemented nucleotide diets with and without fish meal, remained low compared to the control treatment with no added nucleotide (FM_{0/0}). This is due to the higher price and limited quantity of FM as a main ingredient in the control diets. The elevated price of fish feed mainly responds to the cost of FM (Abdel Rahman et al., 2010), and therefore finding a relatively lower cost alternative ingredient has been an ongoing research goal (Goda et al., 2020c). Often in scientific research, technical results will appear to favour a particular treatment, but what confirms and disproves the validity of technical results is economical and financial analysis. Although the addition of NucleoforceFish™ has increased the price of the diets compared to non-enriched N-diets, the value of ECR as a result of the inclusion of dietary nucleotide has decreased, which means that the efficiency of the experimental enriched diets with NucleoforceFish™ in terms of relative economic values.

In the present study, the data confirmed that the addition of NucleoforceFish™ to the feeds of gilthead seabream would have an apparent positive effect decreasing the ECR values, which are associated with increasing the economic return.

5. Conclusion

The data in this study indicated that the best overall dietary treatment performance was FM_{25/500} that included fish meal at 25% with 500mg/kg of nucleotide addition. This was generally without adverse effects on fish quality parameters and the efficiency of growth and feed utilization. The supplementation of zero fish meal diets for seabream with NucleoforceFish™ as a commercial nucleotide supplement was also advantageous in compensation for a decrease in the abundance and richness of the gut microbiome without fish meal in formulated diets for Gilthead seabream. There was clear evidence of metabolic and health

related benefits to the incorporation of dietary nucleotides for positive trends in metabolic enzymes and specific gene expression.

Use of nucleotides in aquaculture may support the economic performance, and aid the cost of production (investment volume). We must evaluate the cost benefits of such feed additives in a wider context throughout the production cycle and for other marine species of commercial importance.

Authorship statement

The authors including in the authorship of the manuscript share and participate into the following category:

Declaration of Competing Interest

None.

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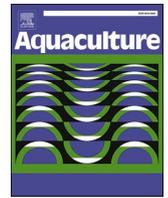
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Erratum to ‘Impact of nucleotide enriched diets on the production of gilthead seabream, *Sparus aurata* fingerlings by modulation of liver mitochondrial enzyme activity, antioxidant status, immune gene expression, and gut microbial ecology’ [Aquaculture, Volume 535, 30 March 2021, 736398]

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The publisher regret the journal place the data of Table 3 in the place of table Number 5 & 6. Please place the right data of Tables 5 & 6 in their proper place instead of the data of Table 3. (See [Tables 1–8.](#))

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Table 1
Formulation and proximate composition of the experimental diets for gilthead seabream (g/kg).

	Experimental Diets*					
	FM _{0/0}	FM _{0/250}	FM _{0/500}	FM _{25/0}	FM _{25/250}	FM _{25/500}
	0	250	500	0	250	500
Fish meal	0	0	0	185	185	185
Poultry By-product	294	294	294	220	220	220
Soybean meal	234	234	234	175	175	175
Corn Gluten	134	134	134	100	100	100
Wheat Bran	76	75.75	75.50	89	88.75	88.50
Squid meal	134	134	134	100	100	100
Fish oil	118	118	118	121	121	121
Vitamins and mineral mixture ¹	10	10	10	10	10	10
Nucleotides ²	0	0.25	0.5	0	0.25	0.5
Proximate Composition (%)						
DM, %	93.9	93.6	92.9	93.3	93.5	93.8
Protein, %	45.50	45.90	46.60	46.10	45.75	46.00
lipids, %	18.00	17.80	17.60	18.10	18.20	17.70
Ash, %	15.35	15.70	15.10	15.40	15.65	15.85
Fiber, %	2.93	2.53	2.68	2.55	2.92	3.30
NFE ³ , %	18.22	18.08	18.02	17.85	17.48	17.16
Gross energy (MJ/kg) ⁴	20.97	20.96	21.04	21.09	20.98	20.79
P/E ratio (mg CP:kJ)	90.74	91.58	92.63	91.43	91.19	92.55
Price (US\$/kg feed)	0.844	0.858	0.872	0.961	0.975	0.989

-FM_{0/0} (0% fish meal; 0 mg nucleotide), FM_{0/250} (0% fish meal+250 mg nucleotide), FM_{0/500} (0% fish meal+500 mg nucleotide), FM_{25/0} (0 mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250 mg nucleotide), FM_{25/500} (25% fish meal+500 mg nucleotide).

-DM = Dry matter.

¹ Vitamin and mineral mixture each 1-kg of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B₁₂, 4.0 g Vit B₂, 6 g Vit B₆, 4.0 g Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 g Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium, 1.2 mg Niacin, 12 mg D-calcium Pantothenate, 26 mg Pyridoxine. HCl, 6 mg Riboflavin, 7.2 mg Thiamin. HCl, 1.2 mg Sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg Ferrous sulfate (FeSO₄.7H₂O, 20% Fe), 65 mg Manganese sulfate (MnSO₄, 36% Mn), 89 mg Zinc sulfate (ZnSO₄.7H₂O, 40% Zn), 150 mg Copper sulfate (CuSO₄.5H₂O, 25% Cu), 28 mg Potassium iodide (KI, 24% K, 76% I), 1000 mg Celite AW521 (acid-washed diatomaceous earth silica).

² Nucleoforce Fish™ is concentrated balanced free nucleotides and active precursors, obtained from yeast produced by the bioiberica company, Spain. <https://www.bioiberica.com/animal-health/animal-nutrition/fish/nucleoforce-fish-1/#sthash.oj8NWjSA.dpbs>.

³ Nitrogen free extract (NFE) =100- (Protein% + lipids% + Ash% + Fiber %).

⁴ Calculated using gross caloric values of 23.62, 39.52, and 17.15 kJ/g for protein, fat, and carbohydrate, respectively, according to Brett (1973).

Table 2
The primers using for determining TCR, IL-1β, Hepcidin and IGF1 are listed and were used relative to β-Actin (ACTB).

Gene name	Gene abbreviation	Gene Bank number	Primer sequences (5' - 3')
Immunoglobulin M IgM	<i>IgM</i>	26691348 17547755	CAGCCTCGAGAAGTGGAAC GAGGTTGACCAGGTGGTGT
Hepcidin1	<i>Hep</i>	26691348 17547755	GCCATCGTGTCACCTTTAT CCTGCTGCCATACCCATCTT
Interleukin1 beta	<i>IL-1β</i>	28706519 26691348	GGGCTGAACAACAGCACTCTC TTAACACTCTCCACCCTCCA
T-Cell Receptor	<i>Tcr</i>	26691348 28453393	AAGTGAATGCCAGCTTCTT TTGGCGGTCTGACTTCTT
"Photobacterium damsela"		28341681 27084008	CCTATGGGACATGAATGG GCTCTAGGCTAAATGAATC
Photobacterium damsela		27737014	ACATCATCCATTGTTAC
Vibrio anguillarum		24649315	CCTTACTACTATCCAAATTG
Vibrio anguillarum		26840426	GAAAGAGCCCCAAACCAAGTGATT
Lactobacillus plantarum		23386831	CITCCAGATAATTCACTATCGCTTA
Lactobacillus plantarum		23386831	GGAATCTTCCACAATGGACG
Lactobacillus subtilis		30286089	CGCTTTACGCCCAATAAAATCCGG
Lactobacillus subtilis		25973610	ATCAACCATGTTGATGTAGC
Enterococcus faecium		30286089	AAGGGATACCGGACAATTCA

Table 3
Growth performance and feed utilization efficiency of juvenile gilthead seabream, *Sparus aurata* fed different experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₀	NT ₂₅₀	NT ₅₀₀	NT ₀	NT ₂₅₀	NT ₅₀₀
IBW (g/fish)	0.35 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
FBW (g/fish)	11.76 ± 0.27 ^d	12.38 ± 0.23 ^{cd}	13.14 ± 0.19 ^{bc}	12.60 ± 0.36 ^c	13.63 ± 0.16 ^{ab}	14.25 ± 0.24 ^a
Weight gain (g/fish)	11.41 ± 0.23 ^d	12.02 ± 0.24 ^{cd}	12.78 ± 0.19 ^{bc}	12.25 ± 0.31 ^c	13.27 ± 0.18 ^{ab}	13.89 ± 0.21 ^a
SGR (%/fish/day)	1.947 ± 0.007 ^d	1.965 ± 0.005 ^{cd}	1.999 ± 0.013 ^{bc}	1.986 ± 0.014 ^c	2.022 ± 0.010 ^{ab}	2.043 ± 0.005 ^a
FCR	2.34 ± 0.03 ^a	2.17 ± 0.04 ^b	1.99 ± 0.02 ^c	2.23 ± 0.02 ^b	1.95 ± 0.05 ^c	1.81 ± 0.02 ^d
PPV (%)	16.16 ± 0.40 ^c	18.38 ± 0.56 ^{bc}	19.17 ± 1.34 ^{bc}	17.54 ± 0.26 ^{bc}	20.67 ± 1.46 ^{ab}	23.32 ± 1.64 ^a
EU (%)	13.02 ± 0.36 ^{bc}	14.88 ± 0.58 ^{bc}	15.75 ± 1.32 ^{ab}	12.42 ± 0.20 ^c	16.00 ± 1.10 ^{ab}	17.97 ± 1.26 ^a
	FM levels			NT Levels		
	0%			0 mg		
	25%			250 mg		
	500 mg			500 mg		
IBW (g/fish)	0.35 ± 0.1	0.36 ± 0.1	0.35 ± 0.1	0.35 ± 0.1	0.36 ± 0.1	0.36 ± 0.1
FBW (g/fish)	12.43 ± 0.22 ^b	13.49 ± 0.25 ^a	12.18 ± 0.30 ^c	13.01 ± 0.22 ^b	13.70 ± 0.14 ^a	13.70 ± 0.14 ^a
Weight gain (g/fish)	12.08 ± 0.21 ^b	13.13 ± 0.22 ^a	11.83 ± 0.29 ^c	12.65 ± 0.22 ^{ab}	13.34 ± 0.12 ^a	13.34 ± 0.12 ^a
SGR (%/fish/day)	1.97 ± 0.01 ^b	2.02 ± 0.01 ^a	1.97 ± 0.01 ^c	2.00 ± 0.01 ^{ab}	2.02 ± 0.01 ^a	2.02 ± 0.01 ^a
FCR	2.17 ± 0.03 ^a	2.00 ± 0.02 ^b	2.29 ± 0.02 ^a	2.06 ± 0.04 ^b	1.90 ± 0.02 ^c	1.90 ± 0.02 ^c
PPV (%)	17.90 ± 0.95 ^b	20.51 ± 1.11 ^a	16.85 ± 0.38 ^c	19.53 ± 0.88 ^b	21.25 ± 1.42 ^a	21.25 ± 1.42 ^a
EU (%)	14.55 ± 0.58 ^b	15.46 ± 0.95 ^a	12.72 ± 0.25 ^c	15.44 ± 0.85 ^b	16.86 ± 1.20 ^a	16.86 ± 1.20 ^a

*Values are means ± SEM, n = 3.

** -FM_{0/0} (0% fish meal; 0 mg nucleotide), FM_{0/250} (0% fish meal+250 mg nucleotide), FM_{0/500} (0% fish meal+500 mg nucleotide), FM_{25/0} (0 mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250 mg nucleotide), FM_{25/500} (25% fish meal+500 mg nucleotide).

- Final body weight (FBW), Weight gain (WG), feed conversion ratio (FCR), protein productive value (PPV) and Energy utilization, (EU %).

-FM = fishmeal; NT = nucleotides.

Table 4

The activity of respiratory chain enzymes (Complex I, II, III, and IV) in liver mitochondria of juvenile gilthead seabream, *Sparus aurata* fed different experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₀	NT ₂₅₀	NT ₅₀₀	NT ₀	NT ₂₅₀	NT ₅₀₀
Complex I	8.98 ± 0.18 ^e	9.59 ± 0.32 ^d	10.32 ± 0.14 ^c	12.40 ± 0.11 ^b	19.16 ± 0.16 ^a	13.62 ± 0.16 ^b
Complex II	42.49 ± 0.29 ^e	51.23 ± 0.13 ^c	50.84 ± 0.13 ^c	43.15 ± 0.21 ^d	62.25 ± 0.23 ^a	58.62 ± 0.19 ^b
Complex III	151.48 ± 1.71 ^c	165.76 ± 0.95 ^d	178.95 ± 1.84 ^c	152.95 ± 0.42 ^e	212.64 ± 1.45 ^a	198.35 ± 0.47 ^b
Complex IV	250.92 ± 1.57 ^c	261.31 ± 1.27 ^d	268.43 ± 1.09 ^c	268.50 ± 1.25 ^c	296.31 ± 1.55 ^a	290.44 ± 0.50 ^{ab}
	FM levels			NT Levels		
	0%			0 mg		
	25%			250 mg		
	500 mg			500 mg		
Complex I	9.63 ± 0.21 ^b	11.73 ± 0.13 ^a	10.69 ± 0.14 ^b	9.38 ± 0.24 ^c	11.97 ± 0.13 ^a	11.97 ± 0.13 ^a
Complex II	48.19 ± 0.18 ^b	54.67 ± 0.20 ^a	42.82 ± 0.21 ^c	56.74 ± 0.17 ^a	54.73 ± 0.14 ^b	54.73 ± 0.14 ^b
Complex III	165.40 ± 1.31 ^b	187.98 ± 0.64 ^a	152.22 ± 1.11 ^c	189.20 ± 1.01 ^a	188.65 ± 1.23 ^a	188.65 ± 1.23 ^a
Complex IV	260.22 ± 1.29 ^b	285.08 ± 1.01 ^a	259.71 ± 1.27 ^c	275.81 ± 1.43 ^b	282.44 ± 0.87 ^a	282.44 ± 0.87 ^a

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal; 0 mg nucleotide), FM_{0/250} (0% fish meal+250 mg nucleotide), FM_{0/500} (0% fish meal+500 mg nucleotide), FM_{25/0} (0 mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250 mg nucleotide), FM_{25/500} (25% fish meal+500 mg nucleotide).

-FM = fishmeal; N = nucleotides.

-Complex I = NADH: Ubiquinone Oxidoreductase, EC 1.6.5.3, Complex II = Succinate: ubiquinone1 oxidoreductase, EC 1.3.5.1, Complex III = ubiquinol: ferricytochrome c reductase, EC 1.10.2.2, Complex IV = cytochrome c oxidase, EC 1.9.3.1.

Table 5

IGF1, TCR-B, IL-1β, and Hcpicidin of juvenile Gilthead seabream, *Sparus aurata* fed different experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₀	NT ₂₅₀	NT ₅₀₀	NT ₀	NT ₂₅₀	NT ₅₀₀
IGF1	1.25 ± 0.21 ^b	0.93 ± 0.04 ^c	0.93 ± 0.04 ^c	0.84 ± 0.03 ^c	2.09 ± 0.04 ^a	1.10 ± 0.05 ^{bc}
TCR-B	1.28 ± 0.27 ^c	1.19 ± 0.01 ^{cd}	3.13 ± 0.12 ^{ab}	1.71 ± 0.02 ^c	0.91 ± 0.02 ^d	1.12 ± 0.33 ^d
IL-1β	0.89 ± 0.05 ^d	1.85 ± 0.08 ^b	2.38 ± 0.32 ^a	1.21 ± 0.02 ^{cd}	1.92 ± 0.07 ^b	1.59 ± 0.03 ^{bc}
Hcpicidin	1.11 ± 0.02 ^e	1.41 ± 0.01 ^d	1.60 ± 0.01 ^c	0.51 ± 0.01 ^f	1.84 ± 0.06 ^b	2.08 ± 0.04 ^a
	FM levels			NT Levels		
	0%			0 mg		
	25%			250 mg		
	500 mg			500 mg		
IGF1	1.04 ± 0.33 ^b	1.34 ± 0.56 ^a	1.04 ± 0.42 ^b	1.51 ± 0.61 ^a	1.02 ± 0.14 ^b	1.02 ± 0.14 ^b
TCR-B	2.40 ± 0.43 ^a	1.72 ± 0.13 ^b	11.97 ± 0.75 ^a	1.05 ± 0.16 ^c	1.41 ± 0.62 ^b	1.41 ± 0.62 ^b
IL-1β	1.70 ± 0.77 ^a	1.57 ± 0.32 ^b	1.05 ± 0.19 ^c	1.89 ± 0.18 ^b	1.98 ± 0.67 ^a	1.98 ± 0.67 ^a
Hcpicidin	1.37 ± 0.21 ^b	1.47 ± 0.72 ^a	0.81 ± 0.32 ^c	1.62 ± 0.24 ^b	1.84 ± 0.26 ^a	1.84 ± 0.26 ^a

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal; 0 mg nucleotide), FM_{0/250} (0% fish meal+250 mg nucleotide), FM_{0/500} (0% fish meal+500 mg nucleotide), FM_{25/0} (0 mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250 mg nucleotide), FM_{25/500} (25% fish meal+500 mg nucleotide).

-FM = fish meal; N = nucleotides.

Table 6

Gut microbiota of juvenile gilthead seabream, *Sparus aurata* fed different experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₀	NT ₂₅₀	NT ₅₀₀	NT ₀	NT ₂₅₀	NT ₅₀₀
<i>Photobacterium damsela</i>	95.33 ± 2.56 ^a	110.50 ± 6.84 ^a	101.00 ± 6.12 ^a	72.67 ± 2.50 ^b	95.00 ± 6.11 ^a	100.33 ± 6.12 ^a
<i>Vibrio anguillarum</i>	55.50 ± 1.98 ^d	162.67 ± 1.21 ^b	213.17 ± 1.10 ^a	120.83 ± 6.17 ^c	111.50 ± 6.85 ^c	155.83 ± 5.40 ^b
<i>Lactobacillus plantarum</i>	77.50 ± 3.49 ^d	132.00 ± 7.59 ^b	115.50 ± 8.63 ^{bc}	230.83 ± 8.74 ^a	132.33 ± 5.59 ^b	107.67 ± 5.91 ^c
<i>Lactobacillus subtilis</i>	166.67 ± 9.27 ^b	180.67 ± 7.43 ^b	118.83 ± 4.66 ^c	341.83 ± 10.16 ^a	118.33 ± 5.18 ^c	109.17 ± 8.01 ^c
<i>Enterococcus faecium</i>	290.50 ± 9.52 ^c	210.67 ± 7.07 ^c	360.17 ± 7.60 ^a	256.67 ± 11.20 ^d	99.33 ± 4.45 ^f	325.00 ± 5.88 ^b
	FM levels			NT Levels		
	0%	25%		0 mg	250 mg	500 mg
<i>Photobacterium damsela</i>	102.28 ± 4.20 ^a	89.33 ± 7.18 ^b		84.00 ± 3.23 ^c	102.75 ± 7.18 ^a	100.67 ± 4.30 ^b
<i>Vibrio anguillarum</i>	143.78 ± 5.58 ^a	129.39 ± 7.84 ^b		88.17 ± 3.03 ^c	137.08 ± 9.76 ^b	184.50 ± 4.27 ^a
<i>Lactobacillus plantarum</i>	108.33 ± 3.08 ^b	156.94 ± 2.24 ^a		154.17 ± 9.26 ^a	132.17 ± 5.82 ^b	111.58 ± 6.11 ^c
<i>Lactobacillus subtilis</i>	155.39 ± 8.39 ^b	189.78 ± 7.00 ^a		254.25 ± 8.57 ^a	149.50 ± 5.57 ^b	114.00 ± 7.75 ^c
<i>Enterococcus faecium</i>	287.11 ± 7.60 ^a	227.00 ± 3.89 ^b		273.58 ± 5.76 ^b	155.00 ± 3.57 ^c	342.58 ± 4.36 ^a

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal; 0 mg nucleotide), FM_{0/250} (0% fish meal+250 mg nucleotide), FM_{0/500} (0% fish meal+500 mg nucleotide), FM_{25/0} (0 mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250 mg nucleotide), FM_{25/500} (25% fish meal+500 mg nucleotide).

-FM = fishmeal; N = nucleotides.

Table 7

Specific activities of superoxide dismutase (SOD), catalase (CAT) (U/mg protein), reduced glutathione (GSH) and malondialdehyde (MDA) (nmol/g tissue) in the liver of gilthead seabream, *Sparus aurata* fed different experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₀	NT ₂₅₀	NT ₅₀₀	NT ₀	NT ₂₅₀	NT ₅₀₀
SOD (U/mg protein)	138.50 ± 8.40 ^d	154.60 ± 18.60 ^d	223.70 ± 14.6 ^c	220.30 ± 12.00 ^c	363.70 ± 11.90 ^b	554.50 ± 15.10 ^a
CAT (U/mg protein)	75.50 ± 9.30 ^d	99.40 ± 11.60 ^{cd}	128.40 ± 8.50 ^c	127.60 ± 8.20 ^c	218.40 ± 9.60 ^b	268.10 ± 10.30 ^a
GSH (nmol/g tissue)	1491.70 ± 42.70 ^c	2179.30 ± 54.3 ^b	2822.20 ± 47.0 ^a	2797.03 ± 42.90 ^a	2816.70 ± 51.30 ^a	2852.50 ± 37.40 ^a
MDA (nmol/g tissue)	19.88 ± 0.30 ^a	17.80 ± 0.50 ^b	9.88 ± 0.30 ^d	11.420 ± 0.40 ^c	10.88 ± 0.50 ^{cd}	9.97 ± 0.40 ^d
	FM levels			NT Levels		
	0%	25%		0 mg	250 mg	500 mg
SOD (U/mg protein)	172.27 ± 13.87 ^b	379.50 ± 13.00 ^a		179.4 ± 10.20 ^c	259.15 ± 15.25 ^b	389.10 ± 14.85 ^a
CAT (U/mg protein)	101.10 ± 9.80 ^b	204.70 ± 9.37 ^a		101.55 ± 8.75 ^c	158.90 ± 10.60 ^b	198.25 ± 9.40 ^a
GSH (nmol/g tissue)	2164.40 ± 48.00 ^b	2822.17 ± 43.87 ^a		2144.50 ± 42.80 ^c	2498.00 ± 52.80 ^b	2837.35 ± 42.20 ^a
MDA (nmol/g tissue)	15.85 ± 0.37 ^a	10.76 ± 0.43 ^b		15.65 ± 0.35 ^a	14.34 ± 0.5 ^b	9.925 ± 0.35 ^c

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal; 0 mg nucleotide), FM_{0/250} (0% fish meal+250 mg nucleotide), FM_{0/500} (0% fish meal+500 mg nucleotide), FM_{25/0} (0 mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250 mg nucleotide), FM_{25/500} (25% fish meal+500 mg nucleotide).

-FM = fishmeal; N = nucleotides.

Table 8

Economic analysis of juvenile gilthead seabream, *Sparus aurata* production output cost/metrics fed different the experimental diets.

Items	FM ₀			FM ₂₅		
	NT ₀	NT ₂₅₀	NT ₅₀₀	NT ₀	NT ₂₅₀	NT ₅₀₀
Cost of Diet (kg/ \$)*	0.844	0.858	0.872	0.961	0.975	0.989
Total consumed feeds(gm/ fish)	26.68 ± 0.89 ^b	26.06 ± 0.56 ^b	25.39 ± 0.56 ^{bc}	27.26 ± 0.94 ^a	25.88 ± 0.40 ^{bc}	25.13 ± 0.39 ^c
ECR	1.98 ± 0.26 ^b	1.86 ± 0.23 ^b	1.74 ± 0.19 ^d	2.14 ± 0.36 ^a	1.90 ± 0.16 ^b	1.79 ± 0.24 ^c
	FM levels			NT Levels		
	0%	25%		0 mg	250 mg	500 mg
Cost of Diet (kg/ \$)*	0.86	0.98		0.90	0.92	0.93
Total consumed feeds (gm/ fish)	26.04 ± 0.67	26.09 ± 0.58		26.97 ± 0.92 ^a	25.97 ± 0.48 ^b	25.26 ± 0.48 ^c
ECR	1.86 ± 0.23 ^b	1.94 ± 0.25 ^a		2.06 ± 0.31 ^a	1.88 ± 0.20 ^b	1.77 ± 0.22 ^c

*Values are means ± SEM, n = 3.

**Means in a row with different superscript letter are significantly different.

-FM_{0/0} (0% fish meal; 0 mg nucleotide), FM_{0/250} (0% fish meal+250 mg nucleotide), FM_{0/500} (0% fish meal+500 mg nucleotide), FM_{25/0} (0 mg nucleotide, 25% fish meal), FM_{25/250} (25% fish meal+250 mg nucleotide), FM_{25/500} (25% fish meal+500 mg nucleotide).

-FM = fishmeal; NT = nucleotides.

One US\$ = 18 Egyptian pound (L.E.).

FCR = Feed conversion ratio.

ECR = Economical conversion rate = Cost of diet (\$/kg)* FCR.