UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

Effect of dietary essential oils supplementation on growth performance, protein digestibility and digestive enzymes in juvenile gilthead seabream fed a low fishmeal diet

Diego Alfredo de la Cruz Villeda

Dissertação apresentada para a obtenção do grau de Mestre em Aquacultura e Pescas com especialização em Aquacultura

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Sob a supervisão de: Dr. Jorge Proença Dias

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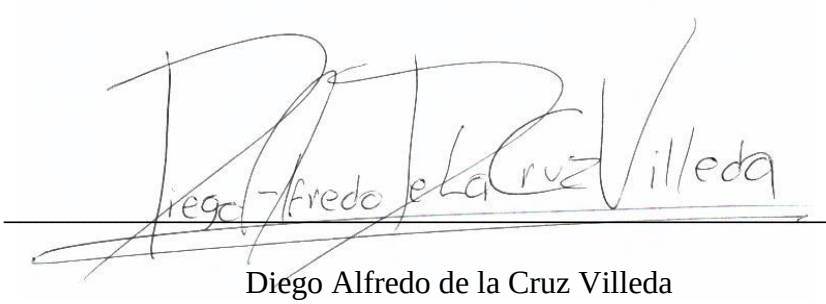
Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da lista de referências incluída.



Diego Alfredo de la Cruz Villeda

Dedicatoria

Este trabajo de tesis se la dedico a mi familia en especial a mi padre Alfredo de la Cruz Muñoz que me ha guiado en este camino, me ha orientado con toda su sabiduría ha sido mi profesor mi compañero me ha brindado su apoyo incondicional, a mi madre Maria del Rosario Villeda de de la Cruz que ha sido mi apoyo sentimental mi maestra y sobre todo recordarme seguir el camino correcto de la vida, a mi hermana Andrea Maria de la Cruz Villeda que ha sido clave en este trayecto que a pesar que estamos lejos siempre está en mente y corazón a mi cuñado Aldo Días me ha apoyado cuando lo he necesitado.

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Resumo

Este estudo foi realizado com o objectivo de avaliar a eficácia de uma suplementação alimentar em óleos essenciais no desempenho do crescimento, digestibilidade da proteína e atividade de enzimas digestivas em juvenis de dourada alimentados com uma dieta rica em proteínas vegetais. Quatro dietas práticas foram formuladas para serem isoprotéicas (proteína bruta, 45,4% de MS), isolipídicas (18,5% MS) e isoenergéticas (energia bruta de 21,4 kJ / g MS). A dieta controlo (CTRL) foi formulado com baixos níveis de proteínas de origem marinha (19%). Esta mesma formulação base, foi suplementada com uma mistura comercial de anis, óleos essenciais de orégão e citrinos a 1,2 g / kg (dieta Phyto C), uma mistura semelhante, mas disponível sob uma forma encapsulada a 0,2 g / kg (dieta Phyto E) e este mesmo produto encapsulado (0,2 g / kg), juntamente com uma levedura autolisada (1 g / kg) (dieta Phyto E + AY). Grupos de 20 douradas, com peso médio inicial de 27.9 ± 2.1 g foram alimentados com uma das quatro dietas experimentais durante 63 dias. No final do ensaio, a taxa de crescimento específico da dourada não foi afetada significativamente ($P>0.05$) pela suplementação alimentar. Por outro lado, a suplementação da dieta não teve efeito ($P>0.05$) sobre a digestibilidade aparente da proteína. No entanto, os peixes alimentados com as diferentes rações suplementadas, apresentaram uma redução significativa ($P<0.05$) do FCR (fator de conversão alimentar) em relação aos peixes alimentados com a dieta CTRL. Da mesma forma, em comparação com o tratamento controlo, os peixes alimentados com as dietas suplementadas apresentaram uma melhoria significativa ($P <0,05$) da retenção de proteínas e gordura. O ganho diário de azoto (acreção proteica) não foi afetada ($P> 0,05$) pelos tratamentos alimentares, mas os peixes alimentados com dietas suplementadas com os óleos essenciais mostraram uma redução significativa ($P <0,05$) das perdas metabólicas de azoto. Os peixes alimentados com dieta Phyto E + AY mostraram um aumento da actividade ($P <0,05$) da fosfatase alcalina e leucina alanina peptidase. Todos os suplementos fitogênicos reforçaram ($P <0,05$) a atividade da pepsina digestiva, enquanto a atividade da lipase foi pouco afetada pelos tratamentos alimentares. Em termos gerais, a suplementação dos alimentos com óleos essenciais contribuiu para uma redução do FCR e uma melhoria da retenção de proteínas e de gordura em juvenis de dourada alimentados com uma dieta baixa em farinha de peixe.

Palavras-chave: dourada, óleos essenciais, crescimento, enzimas digestivas.

Abstract

A study was undertaken to evaluate the efficacy of supplemental essential oils on the growth performance, protein digestibility and digestive enzyme activities in juvenile gilthead seabream fed a plant protein-rich diet. Four practical diets were formulated to be isonitrogenous (crude protein, 45.4% DM), isolipidic (18.5% DM) and isoenergetic (gross energy 21.4 kJ/g DM). The control diet (CTRL) was formulated with low levels of marine-derived proteins (19%). The same basal formulation was supplemented with a commercial blend of anis, citrus and oregano essential oils at 1.2 g/kg (diet Phyto C), a similar blend but available in an encapsulated form at 0.2 g/kg (diet Phyto E) and this same encapsulated product (0.2 g/kg) together with an autolysed yeast (1 g/kg) made up the third test diet (diet Phyto E+AY). Triplicate groups of 20 gilthead seabream, with a mean initial weight of 27.9 ± 2.1 g were fed one of the four experimental diets during 63 days. At the end of the trial, the specific growth rate of seabream was not significantly affected ($P>0.05$) by the dietary supplements. Moreover, dietary supplementation had no effect ($P>0.05$) on the apparent digestibility of protein. However, fish fed the various supplemented feeds showed significantly lower ($P<0.05$) FCR values than those fed the CTRL diet. Similarly, in comparison to the control treatment, the various supplemental products significantly improved ($P<0.05$) protein and fat retention. Daily nitrogen gain was not affected ($P>0.05$) by the dietary treatments, but fish fed diets supplemented with the essential oils showed a significant reduction ($P<0.05$) of metabolic N losses. Fish fed diet Phyto E+AY showed enhanced activity ($P<0.05$) of alkaline phosphatase and leucine alanine peptidase. All phytogenic supplements enhanced ($P<0.05$) digestive pepsin activity, while lipase activity was little affected by the dietary treatments. Supplemental essential oils contributed to a reduction of FCR and an improved retention of protein and fat in seabream juveniles fed a low fishmeal diet.

Keywords: Gilthead seabream, essential oils, growth, digestive enzymes.

1. Introduction

Gilthead seabream is the most important species cultured in the Mediterranean region and its production is still in rapid expansion. According to the latest statistics by the Federation of European of Aquaculture Producers, in 2010 the seabream production in the EU countries and Turkey reached a volume 158.000 tonnes and represented a total value of 477 M€. The majority of seabream consumers are still found in Mediterranean countries but sales have risen in more Northern markets such as the UK, Germany and Russia.

The seabream industry could be considered as a sector already entering its mature phase, but still needs more efficient production systems and new technologies. As in more traditional forms of animal production, nutrition plays a critical role in intensive aquaculture because it influences not only production costs (about 50% of operational costs) but also fish growth, health and waste production. To develop nutritious, cost-effective diets we must know the nutritional requirements of a given species and meet those requirements with will balanced diet formulations and appropriate feeding practices.

1.1 The use of low-fishmeal diets with gilthead seabream

A lower use of finite marine-harvested resources is a sustainability challenge facing the future growth of the aquaculture industry. Grain and oilseed by-products are promising sources of protein and energy for aquaculture feeds. Recent studies with gilthead seabream showed the feasibility of replacing high levels (up to 50-75%) of fishmeal by plant protein sources, provided that diets are duly supplemented with essential amino acids (EAA) and inorganic phosphorus sources (De Francesco et al., 2007). Substitution of up to 60% fish oil (FO) by vegetable oils does not seem to affect seabream growth (Izquierdo et al., 2005). Efforts have now been directed towards the assessment of the concomitant replacement of fishmeal and fish oil by vegetable ingredients in seabream feeds. It has been reported that the partial replacement (33 and 66%) of fish oil by a blend of vegetable oils (rapeseed, linseed and palm oils) associated to a plant-protein rich formulation had no detrimental effects on growth performance of juvenile seabream (Benedito-Palos et al., 2007; Benedito-Palos et al., 2008; Dias et al., 2010). However, high dietary inclusion levels of plant proteins may affect growth, feed efficiency, digestive enzyme activities and in some cases the overall immune status of fish (Sitjà-Bobadilla et al., 2005; Silva et al., 2010; Santigosa et al., 2011).

1.2 The potential for using phytogenics in fish nutrition

After decades of intensive growth promoter application, the resistance of important pathogens of farm animals to drugs has to be considered a serious threat to the profitability and consequently sustainability of animal production systems worldwide. Besides the growing problem of drug resistance amongst diseases, consumer concerns in regard to the use of chemicals in aquaculture and agriculture. The use of antibiotics as "growth-promoting feed additives" has been completely banned in the European Union since 2006 because they are suspected of contributing substantially to increasing resistance among human pathogens (Franz et al., 2009).

Residues in food items are also contributing to the increasing requirement for alternative measures to the exclusive use of growth promoters. The rapid growth of the popularity of organic farming can also be considered another major driving factor for the increased necessity of alternative control measures. As alternatives, a group of natural products known as phytogenics has been the focus of intense research in recent years (Windisch et al., 2008). Amorozo (2002) indicated that there is a growing academic interest on natural pharmacopoeia which was enlarged after its ascertainment developed over centuries, moreover nowadays having scientific proof to completely enable the usage in the industrialized society (Figure 1).

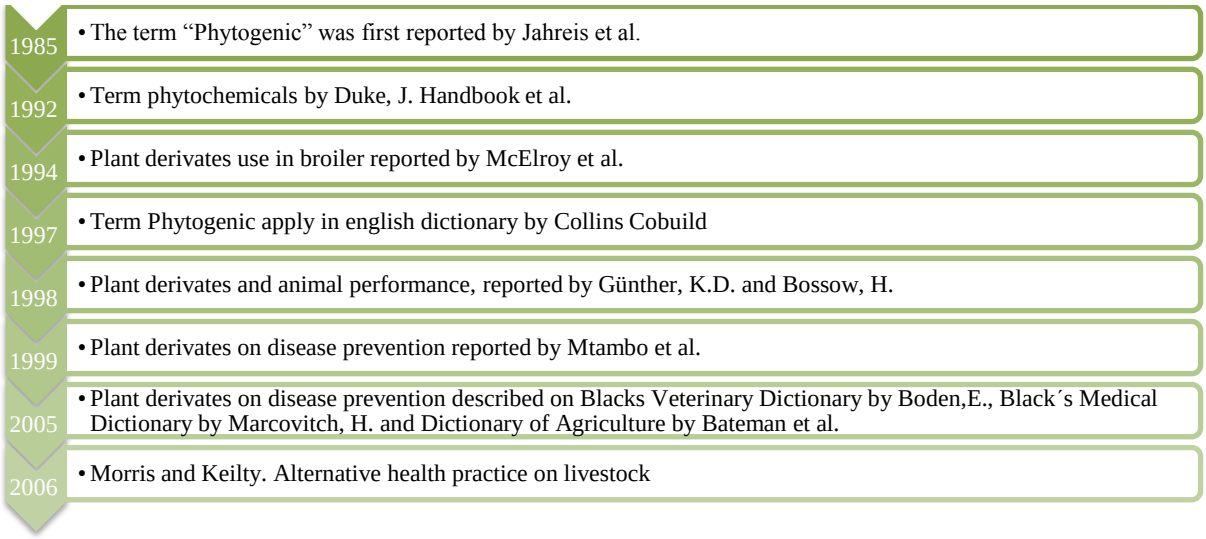

Figure 1. Timeline of plants derivates of animal feed. (Adapted from Amorozo, 2002)

Phytogenics are plant derivate products used as additive in animal feed to potentially improve animal performance. Also referred to as phytobiotics or botanicals, phytogenics comprise a wide range of substances that are further classified according to botanical origin, processing method and composition.

Phytogenic feed additives include herbs, spices, essential oils and oleoresins (Figure 2). Essential oils are volatile oils obtained from plants or from parts thereof by cold pressing and steam or water distillation. Most essential oils consist of mixtures of hydrocarbons (terpenes, sesquiterpenes), oxygenated compounds (alcohol, esters, aldehydes, ketones) and a small percentage of non-volatile residues (paraffin, wax, etc.). Within phytogenic feed additives,the content of active substances in products may vary widely, depending on the plant part used (e.g. seeds, leaf, root, or bark), harvesting season, and geographical origin. The technique for processing (e.g. cold expression, steam distillation, extraction with nonaqueous solvents, etc.) modifies the active substances and associated compounds within the final product (figure 3).

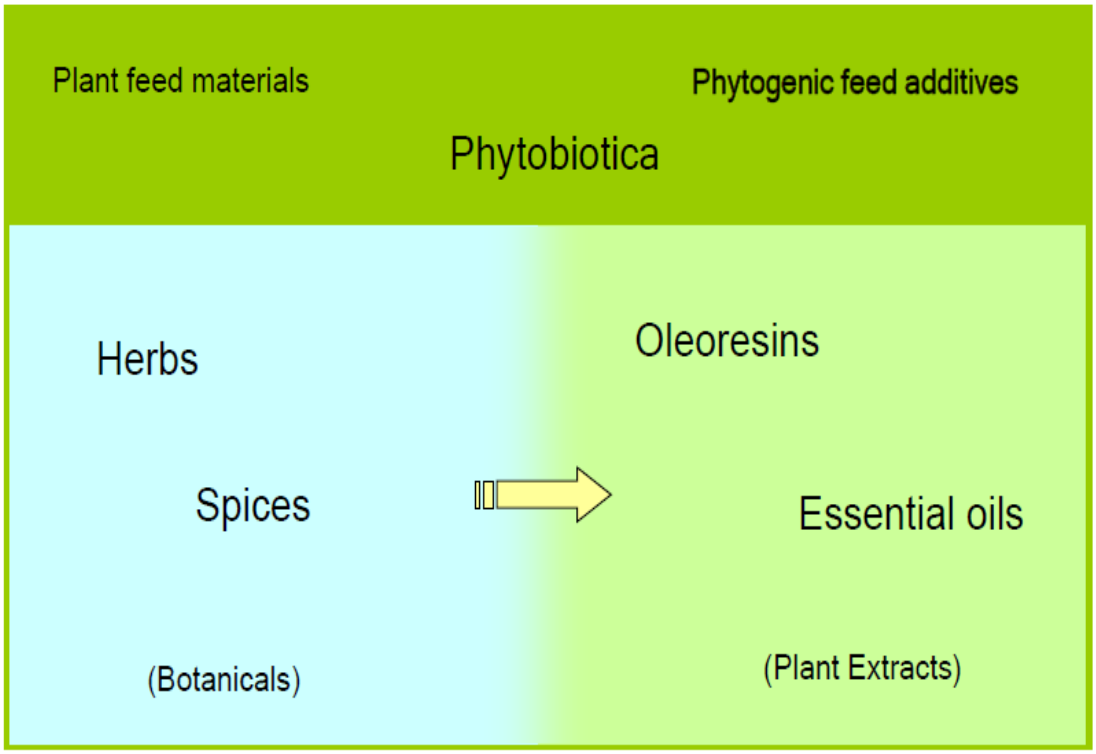

Figure 2. Phytogenic feed additives. (Adapted from Gaubinger, 2013)

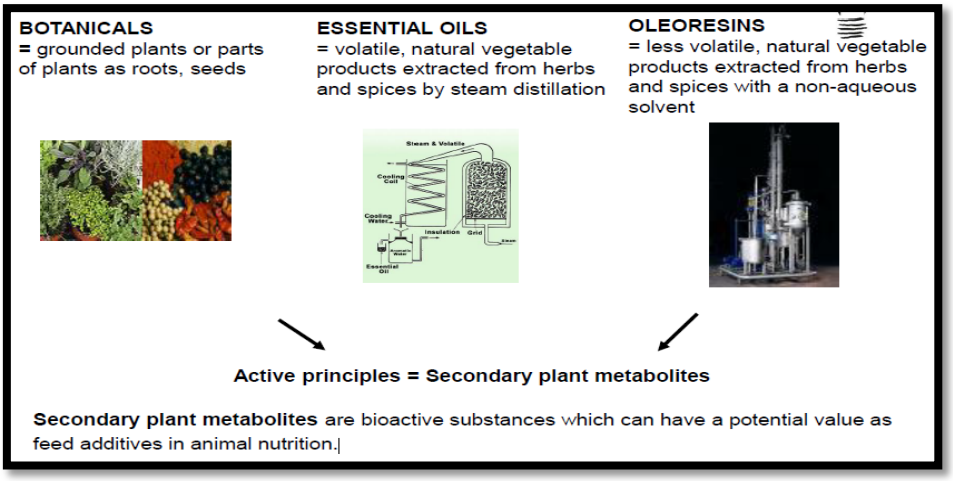

Figure 3. Secondary plant metabolites. (Adapted from Gaubinger, 2013).

Their use has a long history in human consumption as flavors, fragrances and medicines. Their beneficial effects have been shown in a vast number of scientific reports pertaining to their antimicrobial, antifungal, antioxidant and many other biological activities.(Kroismayr et al., 2008a; Windisch et al., 2008). Aside from having antimicrobial activity, essential oils potentially provide antioxidative effects, enhance palatability, improve gut functions, or promote growth (Franz et al., 2009; Brenes and Moura, 2010). Nowadays these compounds have been used more and more frequently as animal feed additives. Indeed, a growing number of scientific and field reports show that these compounds exert substantial performance-enhancing effects in animals. Hence, phytogenic substances are considered to be well accepted by consumers due to their natural character and proven efficacy. Figure 4 illustrates the three general features of phytogenics, which make them attractive in animal nutrition.

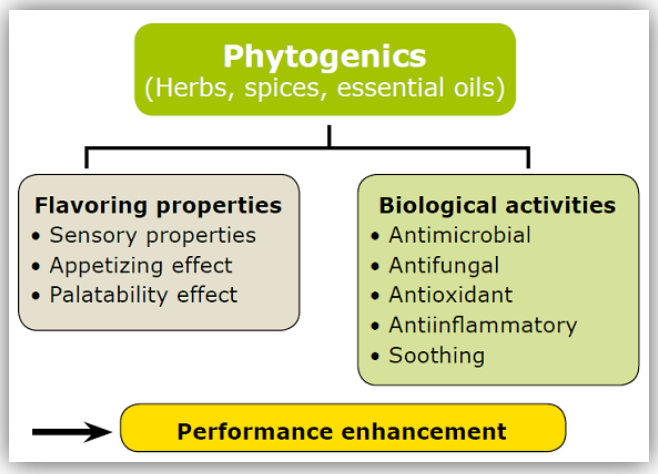

Figure 4. Features of phytogenics in animal nutrition. (Adapted from Kroismayr *et al.*, 2008a; Windisch *et al.*, 2008).

1.2.1. Antioxidative action of phytogenic feed additives

Among a variety of plants bearing antioxidative constituents, the volatile oils from the Labiatae family (mint plants) have been attracting the greatest interest, especially products from rosemary. Their antioxidative activity arises from phenolic terpenes, such as rosmarinic acid and labiatae species with significant antioxidative properties are thyme and oregano, which contain large amounts of the monoterpenes thymol and carvacrol (Cuppett and Hall, 1998). Plant species from the families of Zingiberaceae (e.g., ginger and curcuma) and Umbelliferae (e.g., anise and coriander), as well as plants rich in flavonoids (e.g., green tea) and anthocyans (e.g., many fruits), are also described as exerting antioxidative properties (Nakatani, 2000; Wei and Shibamoto, 2007). Furthermore, pepper (*Piper nigrum*), red pepper (*Capsicum annuum L.*), and chili (*Capsicum frutescene*) contain antioxidative components (Nakatani, 1994). In many of these plants, parts of the active substances are highly odorous or may taste hot or pungent, which may restrict their use for animal feeding purposes.

The antioxidant property of many phytogenic compounds may be assumed to contribute to protection of feed lipids from oxidative damage, such as the antioxidants usually added to diets. Although this aspect has not been explicitly investigated for piglet and poultry feeds, there is a wide practice of successfully using essential oils, especially those from the Labiatae plant family, as natural antioxidants in human food (Cuppett and Hall, 1998), as well as in the feed of companion animals. The principal potential of feed additives from the Labiatae plant family containing herbal phenolic compounds to improve the oxidative stability of animal derived products has been demonstrated for: poultry meat (Botsoglou et al., 2002, 2003a,b; Papageorgiou et al., 2003; Young et al., 2003; Basmacioglu et al., 2004; Govaris et al., 2004; Giannenas et al., 2005; Florou- Paneri et al., 2006), pork (Janz et al., 2007), rabbit meat (Botsoglou et al., 2004b), and eggs (Botsoglou et al., 2005).

Oxidative stability was also shown to be improved with other herbal products (Botsoglou et al., 2004a; Schiavone et al., 2007). Nevertheless, it remains unclear whether these phytogenic antioxidants are able to replace the antioxidants usually added to the feeds (e.g., α-tocopherols) to a quantitatively relevant extent under conditions of common feeding practice.

1.2.2. Antimicrobial actions

Herbs and spices are well known to exert antimicrobial actions in vitro against important pathogens, including fungi (Adam et al., 1998; Smith-Palmer et al., 1998; Hammer et al., 1999; Dorman and Deans, 2000; Burt, 2004; Si et al., 2006; Ozer et al., 2007). Again, the plant family of labiatae has received the greatest interest, with thyme, oregano, and sage as the most popular representatives (Burt, 2004). The antimicrobial mode of action is considered to arise mainly from the potential of the hydrophobic essential oils to intrude into the bacterial cell membrane, disintegrate membrane structures, and cause ion leakage.

High antibacterial activities are also reported from a variety of nonphenolic substances, for example, limonene and compounds from *Sanguinaria canadensis* (Newton et al., 2002; Burt, 2004). Microbiological analysis of minimum inhibitory concentrations (MIC) of plant extracts from spices and herbs, as well as of pure active substances, revealed levels that considerably exceeded the dietary doses when used as phytogenic feed additives (Burt, 2004). This may indicate that the antimicrobial action of phytogenics should not contribute significantly to the overall efficacy of this class of feed additives. On the other hand, some studies with broilers demonstrated in vivo antimicrobial efficacy of essential oils against *Escherichia coli* and *Clostridium perfringens* (Jamroz et al., 2003, 2005; Mitsch et al., 2004).

In swine, however, the few studies available thus far have failed to demonstrate the efficacy of phytogenic compounds on sheddingof specific pathogens (Jugl-Chizzola et al., 2005; Hag-müller et al., 2006). Another implication of the antimicrobial action of phytogenic feed additives may in be improving the microbial hygiene of carcasses. Indeed, there are isolated reports on the beneficial effects of essential oils from oregano on the microbial load of total viable bacteria, as well as of specific pathogens (e.g., Salmonella) on broiler carcasses (e.g., Aksit et al., 2006). However, available data are still too limited to allow reliable conclusions on the possible efficacy of certain phytogenic.

1.2.3. Growth-promoting efficacy

The primary mode of action of growth-promoting feed additives arises from stabilizing feed hygiene (e.g., through organic acids), and even more from beneficially affecting the ecosystem of gastrointestinal microbiota through controlling potential pathogens (e.g., Roth

and Kirchgessner 1998). This applies especially to critical phases of an animal's production cycle characterized by high susceptibility to digestive disorders, such as the weaning phase of piglets or early in the life of poultry. Because of a more stabilized intestinal health, animals are less exposed to microbial toxins and other undesired microbial metabolites, such as ammonia and biogenic amines (e.g., Eckel et al., 1992).

Literature on the biological efficacy of phytogenic feed additives presents a scattered picture. Data on swine reviewed by Rodehutscord and Kluth (2002) varied widely from depressions in production performance to improvements similar to those observed with common growth promoters, such as antibiotics, organic acids, and probiotics. The same applies to more recent investigations (e.g., Manzanilla et al,. 2004, 2006; Namkung et al., 2004; Straub et al., 2005; Hagmüller et al., 2006; Nofrarias et al., 2006; Schöne et al., 2006; Kroismayr et al., 2007; Lien et al., 2007). Recent studies with swine and poultry indicated stabilizing effects of phytogenic feed additives on the ecosystem of gastrointestinal microbiota. Kroismayr et al. (2007) compared a blend of essential oils from oregano, anise, and citrus peels with an antibiotic growth promotant and reported a decrease in microbial activity in the terminal ileum, cecum, and colon for both feed additives, as was obvious from reduced bacterial colony counts and reduced chyme contents of VFA as well as of biogenic amines. Comparable observations for herbal essential oils and oleoresins on the activity of intestinal microbiota were also found in other studies with pigs and broilers (Jamroz et al., 2003, 2005; Manzanilla et al., 2004; Mitsch et al., 2004; Namkung et al., 2004; Castillo et al., 2006).

1.2.4. Impact on dietary palatability and gut functions

Phytogenic feed additives are often claimed to improve the flavor and palatability of feed, thus enhancing production performance. However, the number of studies having tested the specific effect of phytogenic products on palatability by applying a choice-feeding design is quite limited. They show dose-related depressions of palatability in pigs fed essential oils from fennel and caraway, as well as from the herbs thyme and oregano (Jugl-Chizzola et al., 2006; Schöne et al., 2006). Other hand, there are numerous reports on improved feed intake through phytogenic feed additives in swine however, an increase in feed intake in swine is a common result of the use of growth-promoting feed additives, such as antibiotics, organic acids, and probiotics, and, in the first instance, it may be considered to reflect the higher

consumption capacity of animals grown larger compared with untreated controls (Freitag et al., 1998).

Therefore, the assumption that herbs, spices, and their extracts improve the palatability of feed does not seem to be justified in general. A wide range of spices, herbs, and their extracts are known from medicine to exert beneficial actions within the digestive tract, such as laxative and spasmolytic effects, as well as prevention from flatulence (Chrubasik et al., 2005). Furthermore, stimulation of digestive secretions, bile, and mucus, and enhanced enzyme activity are proposed to be a core mode of nutritional action (Platel 2004).

The essential oils used as feed additives for broilers were shown to enhance the activities of trypsin and amylase (Lee et al., 2003; Jang et al., 2004). Glucose absorption from the small intestine was accelerated in rats fed anise oil (Kreydiyyeh et al., 2003). Phytogenic feed additives were also reported to stimulate intestinal secretion of mucus in broilers, an effect that was assumed to impair adhesion of pathogens and thus to contribute to stabilizing the microbial eubiosis in the gut of the animals (Jamroz et al., 2006). These observations support the hypothesis that phytogenic feed additives may favorably affect gut functions, but the number of in vivo studies with swine and poultry is still quite limited.

Recent investigations have shown significant antimicrobial effects of several essential oils and essential oils chemical compounds against pathogenic organisms in farmed animals (Franz et al., 2009), including fish (Lee et al., 2009; Zheng et al., 2009). In marine fish species, information on the efficacy of supplemental essential oils is extremely scarce.

1.3. Objectives

The general objective of this thesis was to evaluate the efficacy of dietary supplemental essential oils as feed additive in low fishmeal diets for gilthead seabream juveniles.
Studies undertaken during the thesis comprised the assessment of:

- Overall growth performance of fish
- Apparent digestibility and digestive capacity
- Metabolic nutrient utilization

2. Material and Methods

2.1 Experimental diets

A low-fishmeal diet (CTRL) was formulated with practical ingredients to fulfill the nutritional requirements of gilthead seabream juveniles. Marine-derived protein sources (fishmeal and a fish soluble protein concentrate) accounted for 19% of the formula, while the majority of the dietary protein content was derived from plant ingredients (pea protein concentrate, soybean meal, wheat gluten and corn gluten) (Table 1).

Formulation of the CTRL diet included also the supplementation with selected EAA's and inorganic phosphorus to avoid any nutritional deficiencies. Based on this formulation, three additional experimental diets were further supplemented with: a commercial blend of anis, citrus and oregano essential oils (Biomin® P.E.P. 1000) at 1.2 g/kg (diet Phyto C); a similar blend but available in an encapsulated form (Biomin® P.E.P. MGE 150) at 0.2 g/kg (diet Phyto E) and this same encapsulated product (0.2 g/kg) together with an autolysed yeast at 1 g/kg (diet Phyto E+AY).

All supplemental products were provided by Biomin GmbH, Austria, in powdered form and incorporated in the formula during the mixing phase in a double-helix mixer. All diets were manufactured by extrusion (pellet size 2.0 mm) by means of a pilot-scale twin-screw extruder CLEXTRAL BC45 (Clextral, France) with a screw diameter of 55.5 mm and temperature ranging 105-110ºC. Four additional batches were produced with the incorporation of yttrium oxide at 250 mg/kg, as an inert marker, for its use on subsequent apparent digestibility measurements. Upon extrusion, all batches of extruded feeds were dried in a convection oven (OP 750-UF, LTE Scientifics, United Kingdom) for 2 hours at 60ºC.

Following drying, pellets were allowed to cool at room temperature, and subsequently the oil fraction (blend of fish and rapeseed oils) was added under vacuum coating conditions in a Pegasus vacuum mixer (PG-10VCLAB, DINNISEN, The Netherlands). Throughout the duration of the trial, experimental feeds were stored refrigerated at 4ºC. Samples of each diet were taken for proximate composition analysis (Table 1).

Table 1. Formulation and proximate composition of experimental diets.

Ingredients, %	CTRL	Phyto C	Phyto E	Phyto E+AY
Fishmeal 70 LT[1]	7.0	7.0	7.0	7.0
Fishmeal FAQ[2]	7.0	7.0	7.0	7.0
CPSP 90[3]	5.0	5.0	5.0	5.0
Pea protein concentrate[4]	14.0	14.0	14.0	14.0
Wheat gluten[5]	8.0	8.0	8.0	8.0
Corn gluten[6]	5.0	5.0	5.0	5.0
Soybean meal 48[7]	9.0	9.0	9.0	9.0
Wheat DDGS	5.3	5.3	5.3	5.3
Wheat meal	21.5	21.5	21.5	21.5
Fish oil	10.0	10.0	10.0	10.0
Rapeseed oil	3.2	3.2	3.2	3.2
Vitamin & Mineral Premix[8]	1.0	1.0	1.0	1.0
Di-calcium phosphate	2.0	2.0	2.0	2.0
L-Lysine	1.5	1.5	1.5	1.5
DL-Methionine	0.5	0.5	0.5	0.5
Supplemental products (g/kg)				
PEP 1000[9]		1.2		
PEP MGE[9]			0.2	0.2
Autolysed yeast[9]				1.0
Proximate composition				
Dry matter (DM) (%)	94.2 ± 0.1	94.3 ± 0.1	93.6 ± 0.1	93.4 ± 0.1
Crude protein (%DM)	45.4 ± 0.2	45.7 ± 0.2	45.2 ± 0.1	45.3 ± 0.2
Lipid (%DM)	18.2 ± 0.2	18.1 ± 0.2	18.2 ± 0.3	18.2 ± 0.1
Ash (%DM)	6.7 ± 0.0	6.7 ± 0.0	6.6 ± 0.0	6.3 ± 0.1
Phosphorus (%DM)	1.3 ± 0.1	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0
Gross energy (kJ/g DM)	21.4 ± 0.2	21.4 ± 0.2	21.5 ± 0.1	21.4 ± 0.2

[1]Peruvian fishmeal LT: 67% crude protein (CP), 9% crude fat (CF), EXALMAR, Peru.
[2]Fair Average Quality (FAQ) fishmeal: 60% CP, 11%CF, COFACO, Portugal.
[3]Fish solubles protein concentrate: 84% CP, 12% CF, Sopropêche, France.
[4]Peas protein concentrate: 78% CP, 8% CF, ROQUETTE, France.
[5]VITEN: 85.7% CP, 1.3% CF, ROQUETTE, France.
[6]GLUTALYS: 61% CP, 8% CF, ROQUETTE, France.
[7]Solvent extracted dehulled soybean meal: 47% CP, 2.6% CF, SORGAL, Portugal.
[8]PVO40.01 premix for marine fish, PREMIX Lda, Portugal. Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25mg; retinyl acetate, 20000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30mg; riboflavin, 30mg; pyridoxine, 20mg; B12, 0.1mg; nicotinic acid, 200mg; folic acid, 15mg; ascorbic acid, 1000mg; inositol, 500mg; biotin, 3mg; calcium panthotenate, 100mg; choline chloride, 1000mg, betaine, 500mg. Minerals (g or mg/kg diet): cobalt sulphate, 2.5mg; copper sulphate, 1.1mg; ferric citrate, 0.2g; potassium iodide, 5mg; manganese sulphate, 15mg; sodium selenite, 0.2mg; zinc sulphate, 40mg; magnesium hydroxide, 0.6g; potassium chloride 1.1g; sodium chloride, 0.5 g; calcium carbonate, 4g.
[9]Supplied by BIOMIN, Austria.

2.2. Fish and rearing conditions

The fish study in Ramalhete Experimental Research Station of CCMAR (Faro,Portugal) Triplicate groups of 20 gilthead seabream (*Sparus aurata*) juveniles, with a mean initial body weight (IBW) of 27.9 ± 2.1 g were fed one of the four experimental diets during 63 days. Fish were grown in plastic circular tanks (volume: 100 L) supplied with flow-through seawater, with temperature ranging 24 ± 2°C and dissolved oxygen levels above 7 mg/L. A 12/12 fluorescent light/dark cycle was adopted (Figure 5). Fish were hand-fed to apparent satiety, three times per day (two at weekends), with utmost care to avoid feed wastage. Feed intake was quantified on a weekly basis.

Figure 5. Plastic circular tanks in Ramalhete Marine Station with seabream

2.3. Sampling

Anesthetized fish were individually weighed at the start of the trial; group weighed at day 21 and again individually weighed at day 63. At the beginning, 10 fish from the same initial stock were sampled and stored at –20°C for subsequent whole-body composition analysis. After 63 days of experimental feeding, 6 fish from each group were sampled for the same purpose. Additionally, samples of the proximal intestine were collected (Figure 6) from 3 fish per tank (9 per treatment), internally rinsed with phosphate buffer saline and stored at -80ºC prior to assay of enzyme activities.

Figure 6. Internal organs and isolated gastro-intestinal tract of gilthead seabream

2.4. Apparent digestibility measurements

At the end of the growth trial and following all associated samplings, the remaining fish were used to determine the apparent digestibility coefficients (ADC) of dry matter and protein by the indirect method. For this purpose, fish from duplicate tanks, were fed with the previous experimental diets, but in which yttrium oxide had been incorporated at 250 mg/kg. After one week, 8 fish per tank were euthanized by lethal anesthesia and the contents of the posterior intestine (feces) were collected, pooled per tank and freeze-dried prior to analysis.

2.5. Analytical methods

2.5.1. Proximate composition of diets, feces and whole-fish

Diets were grinded prior to analysis of proximate composition. Frozen feces and whole-body samples were minced, mixed, a representative sample freeze-dried and homogenized with a laboratory mill prior to analysis. The chemical composition analysis of the diets, faeces and whole fish was made using the following procedures:

- Dry matter was calculated using the official method by the Association of Official Agricultural Chemists (AOAC) in which, samples are dried at 105º C for 24 h in an Binder oven.

- Ash content was determined in the same samples used for dry matter, by weight differential before and after incineration of approximately 24 h in a Nabertherm muffle at 500º C for 12 hours.

- Crude protein was quantified using a LECO FP-528 combustion nitrogen analyzer. To get the determination of total nitrogen in the form of NO_2 this machine burns a small amount of sample in pure oxygen at high temperatures, around 900 ºC. The overall NO_2 is measured by a thermal conductivity cell controlled by a microprocessor and then converted to equivalent protein (N x 6.25).

- Fat was determined using the Soxtherm official AOAC method. The principle of this method consists in the quantification of total lipids in a sample by the extraction using an organic solvent, in this case, petroleum ether. A Soxtec 2500 machine was used to reproduce this method, as well as a Binder oven.

- Gross energy was obtained with the help of an adiabatic bomb calorimeter IKA C2000, IKA-Werke GMBH & CO.KG, Staufen, Germany. The principle of this machine consists in determining gross energy of organic matter, using the combustion action of oxygen, in a decomposition pot at pre-set conditions. A precision Beckmann thermometer measures the temperature before and after the combustion, causing the water temperature to rise first, and then gradually returns to the ambient temperature, to a constant value. The variation in the temperature ΔT observed in the calorimeter is proportional to the heat liberated by the reaction and using this value it is possible to measure directly the amount of energy depending on the gross weight of the sample.

- Total phosphorus was determined by the AFNOR V 04-406 method, in which the sample is subjected to digestion, in a Kjeldatherm Block unit, and wet oxidation at the temperature of 230 ° C with sulphuric acid and hydrogen peroxide, and subsequently orthophosphate is determined by spectrophotometry absorption at 820 nm.

- Yttrium oxide concentrations in feeds and feces were determined by an external analytical laboratory (SGS Multilab, France) using an ICP-AES method.

2.5.2. Digestive enzyme activities

Processing the tissues for analysis required defrosting the samples on ice (4°C), diluting with four parts (wt/vol) 150 mM NaCl, then using an automated tissue grinder (Polytron) to create a fine homogenate. These homogenates were transferred to 1.5-ml Eppendorf tubes and centrifuged for 10 min at 12 000 *g* at 4°C. The supernatants were aliquoted in 0.5-ml Eppendorf tubes and stored at −80°C until assayed for total protein concentration and enzyme activities. Assays were conducted in triplicate for each replicate sample and were performed at room temperature by diluting with two volumes of the respective assay buffer.

Alkaline phosphatase

Alkaline phosphatase (ALP) activity was assayed according to methods of Bessey et al. (1946). A 100 mM ammonium carbonate–magnesium chloride buffer was made by adding 0.79 g ammonium bicarbonate to 0.020 g magnesium bicarbonate and diluting in 100 ml distilled water. The substrate was a 20 mM solution of *p*-nitrophenyl phosphate disodium prepared by diluting 74.22 mg *p*-nitrophenyl phosphate disodium in 10 ml of the buffer solution. A microplate containing wells with 55 µl buffer and 25 µl sample was incubated at room temperature (≈22°C) for 5 min, then 20 µl of room temperature substrate was added. Blanks contained 80 µl buffer and 20 µl substrate. Optical density was measured at 405 nm in 30-s intervals over 30 min to obtain a reaction rate, which was then used to calculate the enzymatic activity of the sample. Enzyme activities are reported as units of activity per milligram protein. One unit (U) represents 1 µmol *p*-nitrophenol liberated during 1 min of hydrolysis.

Pepsin

Pepsin activity was assayed according to methods in Anson et al. (1938). The substrate was prepared from a mixture of 0.2 g of 2% bovine haemoglobin stock solution in 10 ml distilled water, mixing vigorously, and filtering through a glass wool filter. Eight millilitres of this solution then had ≈2 ml HCl added to adjust the pH to 2.0. Intestine samples (50 µl) were incubated for 10 min at room temperature (≈22°C) with 250 µl of the final 1.6% haemoglobin substrate in a microplate. The reaction was stopped by adding 500 µl of 5% (wt/vol) TCA, and then centrifuged at 3600 *g* for 6 min. The blank contained 250 µl substrate and 500 µl 5% TCA. Optical density was measured at 280 nm on the supernatant in quartz cuvettes. Enzyme

activities are reported as units of activity per milligram total protein. One unit (U) represents 1 μmol tyrosine liberated during 1 min of hydrolysis.

Lipase

Lipase activity was assayed according to methods of Iijima et al. (1998). A detergent solution was prepared by adding 1.0 ml of 10% Triton X-100 to 9.0 ml 100 mM ammonium bicarbonate buffer (pH 7.8). The substrate was a 10 mM solution of *p*-nitrophenyl myristate in 100% ethanol. A microplate containing wells with 25 μl buffer, 25 μl sample, and 50 μl detergent solution was incubated at room temperature (≈22°C) for 15 min, then 4 μl of room temperature intestine extract samples was added. Blanks contained 50 μl buffer, 50 μl detergent solution, and 4 μl substrate. Optical density was measured at 405 nm in 30-s intervals over 30 min to get a reaction rate, which was then used to calculate the enzymatic activity of the sample. Enzyme activities are reported as units of activity per milligram protein. One unit (U) represents 1 μmol *p*-nitrophenol liberated during 1 min of hydrolysis.

Leucine-alanine peptidase

Intestinal peptidase, leucine-alanine peptidase (LAP), determination was based on the study by Nicholson and Kim (1975) using leucine-alanine 0.01 M as substrate. The reaction tube with homogenate was done with 0.5 ml of substrate, 1ml of LAOR (L-amino oxidase, horseradish peroxidase, o-dianisine dissolved in a Tris-HCl 50 mM buffer), 5 minutes in a bath at 37 ºC, 25 μl of homogenate, 20 minutes bath and the reaction was stopped with sulphuric acid. The blank sample tube was made in the same way but instead of adding substrate, it was added 0.5 ml of Tris-HCl buffer. The blank substrate tube was made the same way as the reaction tube but instead of homogenate, 25 μl of water was added. The blank zero was made with only Tris-HCl buffer, LAOR and sulphuric acid to stop the reaction. A standard curve using leucine (0.01 M) was done to determine this enzyme activity. All tubes were read at 530 nm in the spectrophotometer against the blank zero.

Soluble protein

Enzymes were expressed as specific activity (U/mg protein). Therefore, protein was determined by means of the Bradford method (Bradford, 1976) using bovine serum albumin as standard.

2.6. Criteria under evaluation

The biological evaluation of finished feeds involves feeding fish and analyzing some aspect of fish performance and/or diet digestibility.

Accurate prediction of the growth potential of a fish stock, under given husbandry conditions, is an inevitable prerequisite to estimate the energy or feed requirement (e.g., weekly ration). The formula most commonly used for fish growth rate expression is the instantaneous growth rate, known as the **Specific Growth Rate**, which is based on the natural logarithm of body weight, and can be used to compare growth on a daily basis:

$$\mathbf{SGR} = \frac{[\mathbf{In(FBW)} - \mathbf{In(IBW)}]}{\mathbf{Days}} \times \mathbf{100}$$

In this formula, **FBW** is the final mean body weight (g); **IBW**, the initial mean body weight (g); and **D**, the number of days. The SGR has been widely used by most biologists to describe the growth rate of fish, being dependent on the IBW, with comparisons of growth rates among groups made with similar IBW.

One of the most important ratios is the **Feeding Conversion Ratio**, being the quantity of feed fed divided by fish weight gain over a specific time period, with values typically ranging from 1.5 to 0.8 in intensive fish culture (0.8 is a better ratio). The formula for FCR is defined as:

$$\mathbf{FCR} = \frac{\mathbf{Dry\ Feed\ Supplied}}{\mathbf{Fish\ Wet\ Weight\ Gained}}$$

A useful method to compare the adequacy of dietary protein supply is the **Protein Efficiency Ratio**, a measure of the weight gain per unit protein fed. PER is calculated as follows:

$$\mathbf{PER} = \frac{\mathbf{Wet\ Weight\ Gain}}{(\mathbf{Crude\ Protein\ Intake} \times \mathbf{\%Feed\ Protein})}$$

Finally, a measure of feed intake, by weight, and by day, is calculated through **Voluntary Feed Intake**, standing as a percentage, is calculated by:

$$\mathbf{VFI} = \frac{\mathbf{Crude\ Feed\ Intake}}{(\mathbf{IBW} \times \mathbf{Days})} \times \mathbf{100}$$

This formula gives a notion of the percentage of feed that fish consume to increase their weight, on a daily basis. Temperature, diseases, light, and many others factors, that unbalance the respective comfort-zone, will have an increase or reduction on feed intake, varying VFI. If fish get satiated with feeds that have a good efficiency (and they are fully on their comfort-zone), their VFI would be lower, since they need to intake less feed to grow and maintain their balance.

The retention of specific nutrients or energy in the whole body of fish over a specific time period is a useful way of evaluating the availability and balance of amino acids and the availability of some essential elements and other nutrients as well. Based on data from feed intake and whole-body composition of fish, nutrient and energy retention (expressed as percentage of intake) as well as daily nutrient gain were calculated as follow:

$$\mathbf{Retention} = \mathbf{100} \times \frac{(\mathbf{FBW} \times \mathbf{F.carcass\ nutrient\ content} - \mathbf{IBW} \times \mathbf{I.carcass\ nutrient\ content})}{\mathbf{Nutrient\ Intake}}$$

The apparent digestibility coefficients (ADC) of dry matter and protein were calculated as follows:

$$\mathbf{ADC\ (\%)} = \mathbf{100} \times \left[\mathbf{1} - \frac{\mathbf{dietary\ Cr_2O_3\ level}}{\mathbf{faecal\ Cr_2O_3\ level}} \times \frac{\mathbf{faecal\ nutrientor\ energy\ level}}{\mathbf{dietary\ nutrientor\ energy\ level}} \right]$$

2.7. Statistical analysis

Data are presented as mean of three replicates ± standard deviation. Data were subjected to a one-way analysis of variance, and when appropriate, means were compared by the Newman-Keuls test. Parameters expressed as percentages were subjected to arcsin square root transformation. Statistical significance was tested at 0.05 probability level. All statistical tests were performed using the SPSS V18 software.

3. Results

Data on growth performance, feed conversion and protein efficiency of seabream juveniles fed the various experimental diets is reported in Table 2. Fish mortality throughout the trial was negligible and not associated to dietary treatments. Given the relatively high feed intake values found for all treatments, it seems clear that dietary supplements had no significant effect on feed palatability (P>0.05).

Table 2. Growth performance at day 63 (IBW: 27.9 ± 2.1 g).

	CTRL	Phyto C	Phyto E	Phyto E+AY
FBW (g)	84.31 ± 2.26	86.83 ± 1.17	87.72 ± 2.20	88.09 ± 1.55
Weight gain (%IBW/day)	3.15 ± 0.19	3.19 ± 0.15	3.26 ± 0.20	3.31 ± 0.11
SGR (%/day)	1.76 ± 0.04	1.80 ± 0.02	1.82 ± 0.04	1.82 ± 0.02
VFI ((%IBW/day)	4.04 ± 0.07	3.97 ± 0.31	3.93 ± 0.24	4.01 ± 0.05
FCR	1.28 ± 0.07 [b]	1.17 ± 0.07 [a]	1.12 ± 0.05 [a]	1.09 ± 0.01 [a]
PER	1.83 ± 0.10	1.87 ± 0.06	1.96 ± 0.04	1.95 ± 0.08
ADC Dry matter (%)	83.61 ± 0.41	83.57 ± 0.22	83.93 ± 0.20	83.68 ± 0.12
ADC Protein (%)	92.30 ± 0.18	92.40 ± 0.12	92.45 ± 0.04	92.17 ± 0.09

Values are means ± sd (n=3). Row means with different letters differ significantly (P<0.05).
IBW (g): Initial mean body weight.
FBW (g): Final mean body weight.
Specific growth rate, SGR (%/day): (Ln FBW – Ln IBW) x 100/days.
Feed conversion ratio, FCR: crude feed intake/weight gain.
Voluntary feed intake, VFI (%BW/day): (crude feed intake/IBW/days) x 100.
Protein efficiency ratio, PER: wet weight gain/crude protein intake.
Apparent digestibility coefficient, ADC (%): 100–[(% Y2O3 feed/Y2O3 faeces) x (% nutrient faeces/ % nutrient feed)].

After 63 days of experimental feeding, data shows that dietary supplementations had no significant effect (P>0.05) on daily weight gain, specific growth rate, voluntary feed intake and protein efficiency ratio. Moreover, the apparent digestibility of protein was not affected by the various dietary supplementations (P>0.05) (Table 2). However, irrespective of supplemental product, FCR values for seabream juveniles fed the various essential oil supplemented feeds were significantly lower (P<0.05) than those fed the CTRL diet (Table 2).

Data on the whole-body composition of seabream after 63 days of experimental feeding are presented in Table 3 and show that dietary treatments had no effect on the nutrient and energy contents of whole-fish.

Table 3. Whole-body composition of seabream fed the various experimental diets.

	CTRL	Phyto C	Phyto E	Phyto E+AY
Dry matter (DM) (%)	34.84 ± 0.65	35.50 ± 0.30	34.71 ± 0.69	35.49 ± 0.45
Protein (%DM)	49.11 ± 2.20	47.95 ± 0.30	48.99 ± 1.52	47.18 ± 0.76
Lipid (%DM)	36.57 ± 0.80	36.58 ± 0.49	36.57 ± 0.10	36.96 ± 1.05
Ash (%DM)	11.09 ± 1.03	10.49 ± 1.04	10.87 ± 0.58	10.65 ± 0.60
Phosphorus (%DM)	1.97 ± 0.17	1.90 ± 0.17	1.99 ± 0.09	1.94 ± 0.15
Gross energy (kJ/g DM)	23.74 ± 0.17	23.55 ± 0.30	23.77 ± 0.10	23.94 ± 0.25

Initial fish: DM 30.94%; protein 50.82%: Lipid 23.23%, ash13.87%, phosphorus 2.36; energy 21.55 kJ/g.
Values are means ± sd (n=3).

Based on weight gain, feed intake and whole-body composition of fish, values for protein and fat retention, nutrient gain and nitrogenous losses are reported in Figures 6 and 7. In comparison to the control treatment, the various supplemental products significantly enhanced ($P<0.05$) protein and fat retention (as % of nutrient intake). After 63 days of feeding, dietary supplements had no effect ($P>0.05$) on daily nitrogen gain. However, in comparison to control fish, those fed with the diets supplemented with the essential oils showed a significant reduction ($P<0.05$) of total nitrogenous losses, which was clearly associated to lower metabolic losses. No differences were found among the various supplemental products regarding nutrient retention and nitrogenous losses.

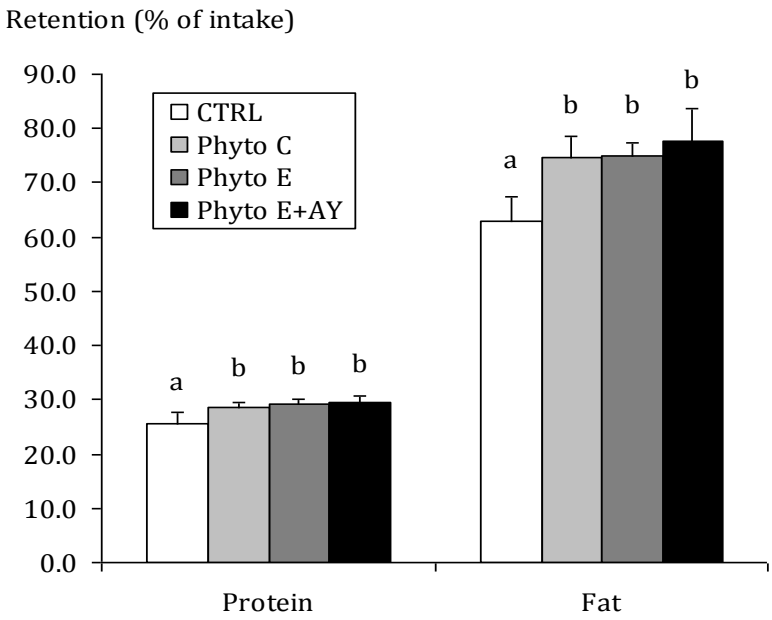

Figure 6. Protein and fat retention in seabream fed the various dietary treatments.
Bars are means ± sd (n=3). Bars with different letters differ significantly ($P<0.05$).
Retention (%): 100 x (FBW x final body nutrient content - IBW x initial body nutrient content) / nutrient intake.

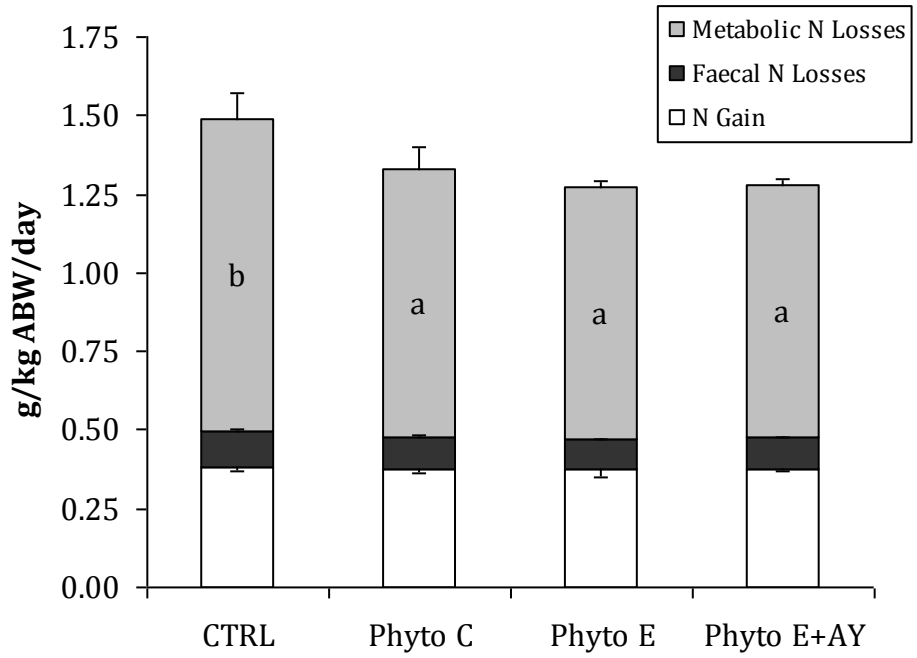

Figure 7. Nitrogen (N) budget in seabream fed the various dietary treatments.
Bars are means ± sd (n=3). Bars with different letters differ significantly (P<0.05).
N gain: (final whole-body N content - initial whole-body N content) / [(FBW+IBW)/2] / days.
Faecal N losses: [N intake – (protein intake x %ADC protein) /6.25] / [(FBW+IBW)/2] / days.
Metabolic N losses: N intake – (N gain + faecal N losses).

Activities of digestive enzymes were affected by the various dietary treatments. In relation to the fish fed the CTRL diet, those fed the Phyto E+AY diet showed a significant enhancement (P<0.05) of the activity of intestinal alkaline phosphatase (ALP), leucine-alanine peptidase (LAP) and pepsin (Table 4). All phytogenic supplements significantly enhanced (P<0.05) pepsin activity at the intestinal level. Lipase activity was significantly reduced in fish fed the Phyto C diet (P<0.05), but little affected by the other dietary treatments.

Table 4. Digestive enzyme activities in seabream fed the various experimental diets.

	CTRL	Phyto C	Phyto E	Phyto E+AY
ALP (mU/mg protein)	1.25 ± 0.19 a	1.00 ± 0.21 a	1.37 ± 0.21 ab	1.65 ± 0.32 b
LAP (U/mg protein)	1.21 ± 0.09 a	1.29 ± 0.08 a	1.27 ± 0.13 a	1.56 ± 0.11 b
Pepsin (μU/mg protein)	44.1 ± 5.0 a	72.3 ± 7.4 c	74.2 ± 4.7 c	56.0 ± 7.1 b
Lipase (mU/mg protein)	1.91 ± 0.27 b	1.56 ± 0.24 a	2.15 ± 0.22 b	2.14 ± 0.12 b

Values are means ± sd (n=9). Row means with different letters differ significantly (P<0.05).

4. Discussion

At the end of the trial, the overall growth performance can be considered as good and within the normal range for seabream of this size, with SGR values for the total duration of trial varying from 1.76 to 1.82 (%/day). In all treatments, fish had a 3-fold increase of their initial body weight. Apparent digestibility of protein was high in all dietary treatments (92%) and little affected by the incorporation of essential oil supplements. Earlier studies with gilthead seabream confirm the feasibility of using low fishmeal diets (Benedito-Palos et al., 2007; De Francesco et al., 2007; Dias et al., 2010), provided that diets are duly supplemented with EAA and inorganic phosphorus sources. A formulation containing a similar level of both fishmeal and soluble fish protein concentrate has also proven suitable to sustain an excellent growth performance in seabream juveniles (weight range 16 to 90 grams) (Benedito-Palos et al., 2007).

After 63 days of feeding, supplemental essential oils had no significant effect on weight gain or growth rate of juvenile seabream. However, it is worth pointing out that a general trend for an enhanced growth rate was observed with essential oils supplemented feeds. An extension of the experimental feeding period could potentially heighten such performance gains. Available literature data on the efficacy of dietary essential oils on the zootechnical performance of fish is extremely scarce. In channel catfish, feeds supplemented with carvacrol, a combination of carvacrol and thymol and oregano essential oil were found to enhance weight gain (Zheng et al., 2009). Additionally, the use of an essential oil blend (anis, citrus and oregano) identical to the one tested in our Phyto E and Phyto E+AY diets, was found to enhance weight gain of channel catfish and this effect was mainly associated to a higher feed intake (Peterson et al., 2011). Our data showed a relatively high level of feed intake for all treatments, which may have limited a potential effect of supplemental essential oils on feed palatability. The dietary supplementation of a low fishmeal diet with the essential oils under testing resulted in a significant reduction of FCR values. In absolute terms, such reductions ranged from 9% with the diet supplemented with Phyto C, 12% with the diet Phyto E and 15% with the diet supplemented with Phyto E+AY. A similar reduction on the FCR values have been reported in channel catfish fed essential oil supplemented feeds (Zheng et al., 2009).

Given this FCR reduction, without changes on the feed intake, our data resulted in a significant enhancement of protein and fat retention (as % of nutrient intake) in seabream fed the various essential oil supplemented feeds. However, overall results suggest that the enhanced utilization of dietary protein with essential oils was mainly associated to lower metabolic losses, rather than an improvement on the protein deposition. The metabolic processes underlying such changes remain unknown and are probably multifactorial.

The rationale behind the beneficial effects of phytogenic substances on the growth performance and feed utilization in terrestrial farm animals relies on mechanisms associated to improved gut function, antioxidant and antimicrobial effects. In poultry, improvement in gut function has mainly been attributed to the stimulatory effect of phytogenic substances on digestive secretions, such as digestive enzymes, bile, and mucus (Williams and Losa, 2001; Basmacioğlu Malayoğlu et al., 2010). In pigs, essential oils derived from oregano, anise and citrus peels, seem to similarly modulate relevant gastrointestinal parameters, such as microbial colony counts, fermentation products including undesirable or toxic substances, digestibility of nutrients, gut tissue morphology and immunological reactions (Kroismayr et al., 2008a, b).

Our results show that the blend of anis, citrus and oregano essential oils enhanced pepsin activity in the proximal intestine of seabream, but had little effect on the activities of alkaline phosphatase (ALP) and leucine alanine peptidase (LAP). However, the use of the microencapsulated essential oil blend together with the autolysed yeast significantly enhanced the activities of ALP, LAP and pepsin. Yeast components have been associated to enhanced capacity to bind enteropathogenic bacteria (Ganner et al., 2010), improved gut morphology (Reisinger et al., 2011) and beneficially modulate the immune system (Sealy et al., 2007).

Little is known about the antimicrobial effect of essential oils in fish. Recently, Yeh et al. (2009) reported that extracts from *Cinnamomum kanehirae* (stout camphor tree) have antibacterial effects against different pathogens of aquatic animals. Additionally, white shrimp treated with selected extracts exhibited an enhanced disease resistance to *Vibrio alginolyticus* (Yeh et al. 2009). This capacity of essential oils to enhance disease resistance in fish has also been demonstrated with oregano essential oil in channel catfish and gilthead seabream (Zheng et al., 2009; Yiagnisis et al., 2010) and cinnamon essential oil in Nile tilapia (Rattanachaikunsopon and Phumkhachorn, 2010). The basis for the antibacterial action of

essential oils is still poorly understood. It has been suggested that their lipophilic properties and chemical structure could play a role in disturbing the permeability of the bacterial cell membrane (Burt, 2004). Moreover, oral administration of antibacterial phytogenic compounds may alter gut microbiota in poultry (Tiihonen et al., 2010). However, despite demonstrating the *in vitro* antibacterial activity of thyme essential oil in intestinal isolates and fish pathogens, Navarrete et al. (2010) reported that a dietary supplementation with thyme essential oil did not alter the intestinal bacterial populations of rainbow trout.

In the overall this study suggests that in seabream juveniles fed a low fishmeal diet, the supplementation with a blend of anis, citrus and oregano essential oils, alone or in combination with autolysed yeast, contributed significantly to a reduction of FCR, an improvement of protein and fat retention, lower metabolic nitrogen losses and the modulation of selected digestive enzymes. However, further studies are needed to gain a better insight of the physiological and metabolic mechanisms causing such effects in fish.

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