

GILTHEAD SEA BREAM MICROBIOTA SHIFTS ASSOCIATED WITH THERMAL STRESS AND DIETARY INTERVENTION DURING A RECORD HEAT SUMMER

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Introduction

Gut microbiota can contribute to regulate growth, nutrient utilisation, disease resistance and physiological stress responses to cope with dietary changes and heat stress, among other environmental stressors. Hence, the composition and function of gut microbiota varies in farmed gilthead sea bream not only with diet, sex, age and season, but also with host genetics that shapes a more plastic microbiota that co-selects with fast growth (Naya-Català et al., 2022; Piazzon et al., 2020). Experimental evidence also indicates that natural and synthetic fat emulsifiers are able to improve growth in gilthead sea bream through changes, at least in part, in gut microbiota composition (Ruiz et al., 2023a; 2023b). The potential of emulsifiers to mitigate the effects of heat stress is proven in broilers (Yin et al., 2021). However, it is not yet known whether similar microbiome shifts are observable with changes in dietary fat levels and the extreme temperature rises associated to global warming. To bridge this gap, we investigated the combined effect of fat level and emulsifier supplementation (Volamel Aqua, Nukamel) in gut microbiota and conventional blood stressor markers during the extremely hot summer of 2022 at the Spanish Mediterranean coast.

Methods

Four isoproteic plant-based diets with 6% FM and two different dietary lipid levels (14%, 16%) with/without Volamel Aqua (0.1%) were formulated and produced by Research Diet Services (RDS, the Netherlands), resulting in four experimental diets: High fat diet (HFD), high fat + emulsifier (HFD-EMS), low fat diet (LFD) and low fat + emulsifier (LFD-EMS). Juveniles of gilthead sea bream (*Sparus aurata*; 12 g initial body weight) were allocated in triplicate 500 L tanks under natural photoperiod and temperature conditions at IATS latitude (40°5'N;0°10'E) from May to August and hand-fed daily until visual satiety. During the first half of the trial (46 days), emulsifier supplementation supported a 10% improvement of feed conversion ratio (FCR) with the increase of water temperature from 20 °C to 25 °C. Such improvement was masked during the second half of the trial with the achievement of the historical record of water temperature at our latitude (30.49 °C, August 9th, 2022). At this time, 12 fish per diet were anaesthetized with MS-222 and sampled for blood (circulating glucose and cortisol) and for intestinal mucus (adherent intestinal microbiota) analyses. Previous samples from fish with the same genetic background not exposed to extreme heat temperatures and fed a commercial standard formulation were used as reference values (REF). Microbial DNA was extracted (High Pure PCR Template Preparation Kit, Roche) and V3-V4 region of the 16S rRNA was amplified and sequenced with Illumina platform. Sequences were quality filtered and taxonomically assigned following a custom pipeline using SILVA database.

Results and discussion

As shown in previous studies, *Proteobacteria* followed by *Firmicutes*, *Actinobacteria* and *Bacteroidota* were the most abundant phyla in the gut microbiota of REF fish (Figure 1).

Conversely, with the increase of temperature, we found that *Spirochaetota* increased dramatically in HFD and secondly in LFD fish. This trend was partially reversed with the addition of the emulsifier, which shaped a microbiota profile in LFD-EMS fish closer to that of fish not exposed to extreme temperatures. At a closer look, partial least-squares discriminant analysis (PLS-DA) highlighted up to 11 genera with a high discriminant value after filtering by VIP>1 and 0.5% abundance. *Brevinema* (representing almost the total contribution to the *Spirochaetota* phylum) was the most abundant genus with a significant discriminant score, and its decrease with the emulsifier addition was concurrent with an increase in relative abundance of *Photobacterium*, *Vibrio*, *Cetobacterium*, and *Bacillus*. Other genera exclusively lowered by the emulsifier or synergistically with the decrease of dietary fat content were *Thauera*, and *Streptomyces* and *Staphylococcus*, respectively. Additionally, both dietary fat level and emulsifier supplementation altered glucose and cortisol levels, being achieved the lowest values of these blood stress markers in LFD-EMS fish.

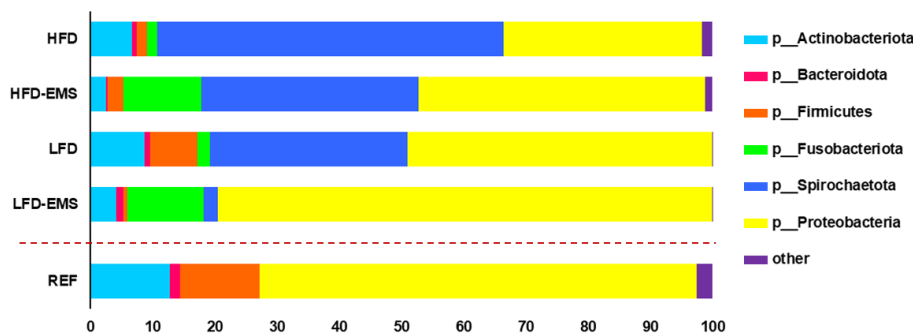


Figure 1: Stacked bar chart representing the relative abundance of bacterial phyla for each of the experimental diets (HFD, HFD-EMS, LFD, LFD-EMS) compared with the reference group (REF) of fish with the same genetic background not exposed to extreme heat temperatures.

Concluding remarks

The increase in *Brevinema* genus appeared associated to extreme summer temperature in our experimental model, confirming and extending the possible use of *Spirochaetota* phylum as a marker of heat stress in both fish (Steiner et al., 2022) and pigs (Le Sciellour et al., 2019). Intriguingly, the mitigation of microbiota dysbiosis was favoured by a low dietary fat level, which would depict that dietary intervention can contribute to alleviate in a large extent the negative impact of global warming in farmed fish, although this was not accompanied herein by the improvement of growth performance during episodes of extreme high temperatures. However, the above microbiota shifts were associated to a decrease in plasma glucose and cortisol levels, especially in LFD-EMS fish, which would be indicative of a low energy cost of growth in fish fed low fat diets with the emulsifier supplementation during extreme warming conditions.

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References

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