ANSWER to Reviewer 2

Not all issues which have been pinpointed in the initial manuscript, have finally been addressed by the authors.

We apologize that our previous response did not fully address your query. In our next reply, we will provide a more detailed explanation and respond to your concerns more precisely.

The issues which have not been addressed, are:

3.2. Lines 171-175: regarding the stress trial, why have authors chosen “five minutes” as the interval time for the air exposure, and not two, three, four, or six minutes ? Is there a referred specific protocol for this? As fish have been returned back to the water after the air-exposure, have fish survived after this “5-minute air exposure”?

All stressed fish have been sampled after the air exposure, and they have been returned to the water for over-anesthetizing and sampling, as it is written in section 2.5.

The following extension has been added to section 2.5: “There is no referred, specific protocol for conducting air exposure stress experiments with tilapias. Therefore, the exposure time was selected according to data in the literature. In a similar experiment [7], one minute was applied, and five minutes was regarded as adequate, because tilapias can survive on air for much longer times [42, 43].” Also, two new references have been added to the manuscript:

42. Guan, L., Zhao, M., Liu, T., Fan, X., Chen, D. Cooling combined with hyperoxic CO2 anesthesia is effective in improving the air exposure duration of tilapia. Scientific Reports, 2017, 7, 14016.

43. Fan, X., Zhao, M., Guan, W., Liu, T., Chen, D. Carbon dioxide anesthesia: A potential application to improve the air exposure duration of tilapia. Iranian Journal of Fisheries Sciences, 2022, 21(1), 82-92.

3.3. Lines 100-112: The Initial and Final Stocking Density of fish in the cages, are somehow low (< 1 kg/m^{-3}). This does not correspond to real commercial fish farming conditions, where stocking densities range between 10-20 kg/m^{-3}. However, this is not a crucial drawback for the experimental design and credibility of the experimental outcome, BUT the authors have to justify this methodology. Why have they chosen such a low density? Is there a specific reason?

Please find the answer for the question with stocking density applied in the trial:

The stocking density was set lower than that typically used in commercial conditions to promote intensive weight gain. The aim was to obtain fish individuals large enough for head kidney collection, rather than to study growth performance. Furthermore, the limited availability of the tested *N. gaditana* meal imposed constraints on feed production and decreased the number of fish involved in the nutritional trial. In conclusion, following the commercialization of *N. gaditana,* a large-scale trial with high fish density is recommended in order to assess the production parameters..

3.4. Lines 144-145 (Table 2): There is a drawback (in the experimental design), as far as the protein and amino acid content of the experimental feeds, is concerned: the COM diet contains 40% fishmeal and 36% soybean meal. In the rest of experimental diets, soybean meal is diminished by half (18% of the diet) and fishmeal is increased by 10-13%. Does this increase in fishmeal, compensate for the loss of amino acids supplied by soybean meal? (which has been halved compared to the COM diet). This is clearly acknowledged in the Discussion Section (lines 296-301) and is good that the authors realize the negative side-effect of this specific experimental drawback.

The essential amino acid profile of fishmeal is more suitable for fish compared to soybean meal, as it contains all essential amino acids in high concentrations. Soybean meal itself is deficient in methionine and cystine; therefore, in aquafeeds would be necessary supplementation with synthetic amino acids to satisfy the requirement of fish.

In our diets the proportion of the fishmeal is high, ranging between 40-53%, and according to the formulation, all of the diets satisfied the requirement of limiting amino acids for Nile tilapia (lysine 1.70%, methionine 0.71%, source: International Aquaculture Feed Formulation Database, https://www.iaffd.com/). The calculated level of lysine varied between 3.078% and 3.150%, respectively and between 1.087-1.234% for methionine. Consequently, hypothesizing that the COM diet with lower fish meal content compared to the experimental diets may have been deficient in methionine and cystine, was not true. However, no analytical measurements were performed, and supporting data are not available. Nevertheless, the diets were supplemented with premix produced by Vitafort Ltd, which contains amino acids as well (7.31% lysine).

From our point of view, the altered fishmeal-to-soybean meal ratio in the COM diet to the other experimental diets, is reflected in differences in nutrient digestibility. These differences may be attributed to antinutritive factors present in soybean meal, which can temporarily cause enteritis in fish.

Due to this drawback in the selection of the control diet, the comparison of growth and nutrient utilization parameters was limited to the experimental diets and discussed in relation to their formulation differences compared to the control. (Line 321-331 in the revised version)

3.5. Lines 430-431: "However, the immunomodulatory effect of the additives observed after the feeding period was negated by the air exposure stress in all treatments". = this statement overturns almost all the positive results of the experiment

The Discussion section has been re-written as follows in Lines 421-426

“The expression of pro-inflammatory cytokine genes (t*nf-α, il-1β, il-8, ifn-γ*) detected in head kidney after the air exposure did not differ significantly among the experimental groups in our study, which indicates that the acute stress decreased the immunomodulatory effect of the experimental feeds detected at the end of feeding. However, a positive trend in the non-specific immune response of tilapias fed with feed additives compared to the control group could be observed following air-exposure.”

and 442-446:

“Finally, we concluded that all three studied feed additives in their respective doses improved the innate immune response of Nile tilapias after seven weeks of feeding. Moreover, the elevated antioxidant capacity of the *N. gaditana*-fed group was maintained even after the stress.”

Also, a sentence in the Conclusion section (lines 453-456) has been re-written as follows:

“Following air exposure, the increased antioxidant effect was maintained in the fish fed with *N. gaditana* supplementation, while the immunomodulatory effect of the additives observed after the feeding period was decreased after air exposure.”