COPPER LEVELS IN DIETS HIGH IN VEGETABLE INGREDIENTS FOR GILTHEAD SEABREAM (*Sparus aurata*) FINGERLINGS

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Introduction

Gilthead seabream (GSB) is the third most produced fish in EU. In commercial feeds for this species, marine ingredients (fish meal, FM and fish oil, FO) are increasingly substituted with ingredients of terrestrial origin. Copper (Cu) is an essential trace element and also a potential toxicant of concern at high intake levels. In general, ingredients of terrestrial origin have higher Cu levels than marine ingredients.

With the changing ingredient profile of commercial GSB feeds, it is necessary to define optimal dietary supply of Cu to ensure better performance, and to avoid excess supplementation.

Objective

The aim of this study was to evaluate optimal dietary inclusion level of Cu in low FM-FO diets for GSB fingerlings.

Materials and methods

Experimental conditions

- 450 GSB fingerlings weight 12.6 ± 1.5 g (mean ± S.D.) in 15 500L tanks.
- 5 practical diets high in vegetable ingredients (FM: 10%, FO: 6%) (table 1).
- Fed until apparent satiation three times per day for 6 weeks.

At the end of the trial

- Fish were individually weighed and measured
- Productive parameters were analysed
- Tissue proximal and mineral composition
- Molecular markers

Table 1: Feed formulation of the 5 experimental diets fed to GSB fingerlings for 6 weeks.

Raw ingredient (%)	Cu 6	Cu 7	Cu 9	Cu 13	Cu 35
Linseed oil	0.82	0.82	0.82	0.82	0.82
Wheat	11.69	11.68	11.68	11.68	11.66
Corn gluten	15.00	15.00	15.00	15.00	15.00
Wheat gluten	21.66	21.66	21.66	21.66	21.67
Soya concentrate	23.00	23.00	23.00	23.00	23.00
Faba beans	5.00	5.00	5.00	5.00	5.00
Fish meal	10.00	10.00	10.00	10.00	10.00
Rapeseed oil	3.00	3.00	3.00	3.00	3.00
Fishoil South American	6.00	6.00	6.00	6.00	6.00
Palm oil	1.64	1.64	1.64	1.64	1.64
Micronutrient premix*	2.19	2.19	2.19	2.19	2.19
Copper sulphate (mg/kg)	8.48	10.50	12.50	16.50	42.00
Analysed Copper (mg/kg)	6.00	7.00	9.00	13.00	35.00

*micronutrient premix includes: Methionine (0.001%), lysine (1.235%), phosphate (0.67%), vitamin premix (0.18%), mineral premix excluding Cu (0.11%)

Results and discussion

No effect of dietary Cu on productive parameters (table 2). Weight gain has been used as a criterion to evaluate Cu requirements in other species (Antony Jesu Prabhu *et al*., 2014). However, in the case of yellow croaker (Cao *et al*., 2014) and malabar grouper (Lin *et al*., 2008) the diets were purified, ver low Cu levels in the basal diet.

Table 2: Growth and productive parameters of GSB fingerlings fed the experimental diets for 6 weeks.

	Cu 6	Cu 7	Cu 9	Cu 13	Cu 35
Final weight (g)	36.2 ± 4.0	36.0 ± 4.1	35.5 ± 4.9	31.5 ± 3.7	35.0 ± 3.8
FCR	2.0 ± 0.2	1.9 ± 0.2	1.9 ± 0.1	2.1 ± 0.2	2.0 ± 0.1
SGR	1.9 ± 0.2	1.9 ± 0.0	2.0 ± 0.1	1.9 ± 0.2	1.9 ± 0.3

Cu retention in the tissues has also been used as a criterion to evaluate Cu requirements (Antony Jesu Prabhu *et al*., 2014), however, there was no effect of dietary Cu on tissue Cu retention in the present trial.

Dietary Cu did not have a significant effect on hepatic *CuZnsod* expression, however there was an increase in *catalase* expression up to 9 mg Cu kg^{-1} supplementation, while further inclusion reduced the expression (figure 1). Both CuZnsod and catalase are involved in the reactions that dispose of free radicals. These results suggest that the metabolic route covered by *CuZnsod* is not affected, thus production of H_2O_2 from O_2· is correct. The dispose of H_2O_2, however, is altered, as evidenced by *catalase* expression. The reduction in *catalase* expression, accompanied by an unaltered *CuZnsod* might indicate an hepatic damage which inactivates the expression of *catalase*, furthermore evidenced by the results obtained by histopathology (figures 2-6).

Figure 1: Hepatic *catalase* expression of GSB fingerlings fed the experimental diets for 6 weeks. Superscript letters indicate significant differences for *p*<0.05

The higher dietary Cu supplementation increased the prevalence of liver steatosis, broken cell margin, peripheral nuclei, and sinusoid dilatation, symptoms of liver damage (figures 2-6). Cu excess is excreted by the hepatocytes via the bile by ATP7 (Prohaska, 2009). The high levels of Cu supplementation may be forcing the hepatocytes to excrete this excess Cu and might be producing a dilatation in the sinusoids. This high dose of Cu might be also responsible for the altered morphology of the hepatocytes.

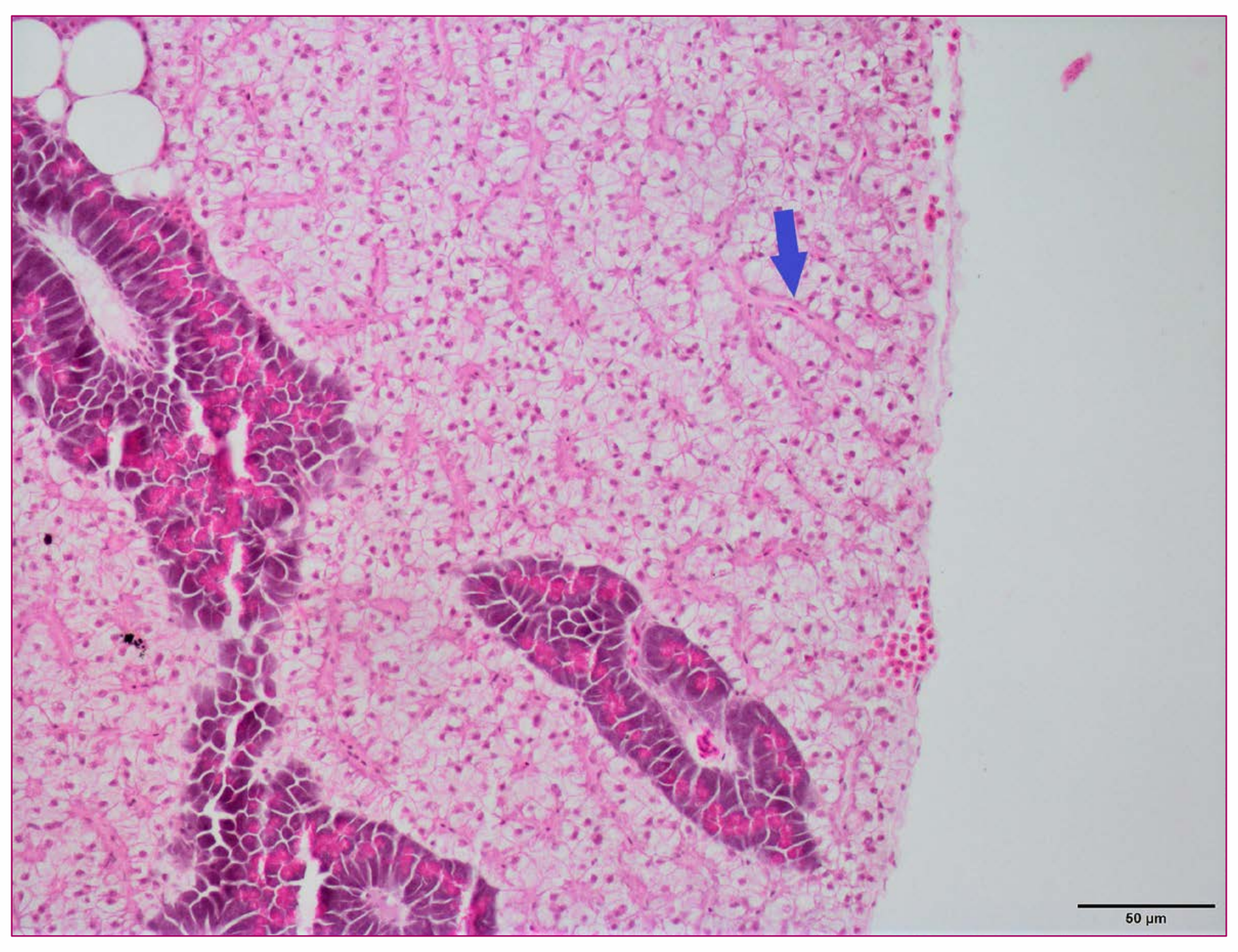
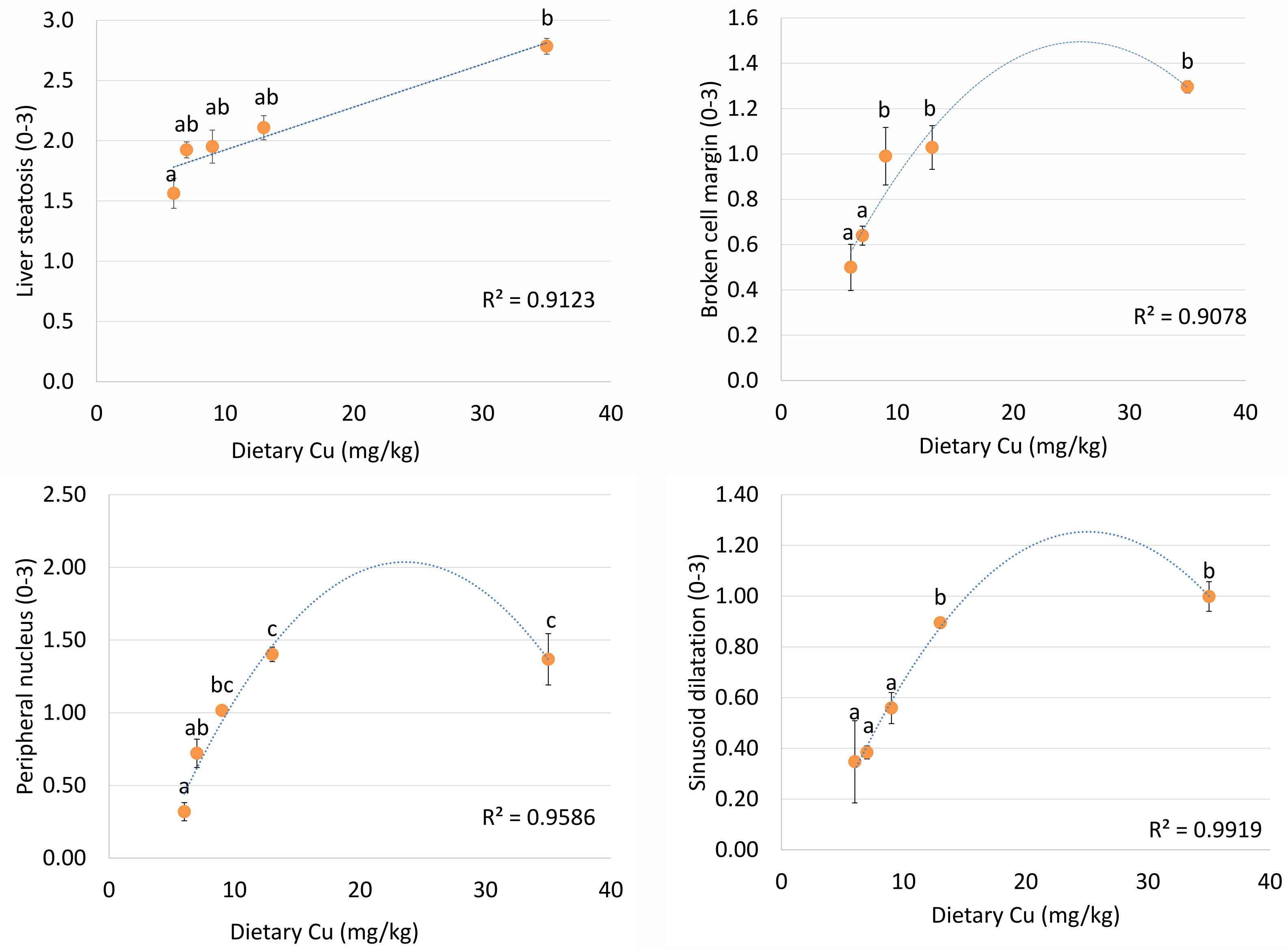


Figure 2: Dilatated sinusoids in liver of GSB fingerlings fed the experimental diets for 6 weeks (arrow, *200x*, H&E).

Nile tilapia presented similar alterations in liver morphology after fed toxic levels of Cu for 6 weeks, including lipidosis and loss of nuclei definition, but, contrary to our results, found a reduced sinusoidal space (Shaw & Handy, 2006). On the other hand, rainbow trout exposed to toxic Cu levels did present increased sinusoidal space (Handy *et al*., 1999).



Figures 3, 4, 5 & 6: Steatosis, broken cell margin, peripheral nucleus and sinusoid dilatation at the liver of GSB fingerlings fed the experimental diets for 6 weeks. Superscript letters indicate significant differences for *p*<0.05. Linear regressions are significant to *p*<0.05.

Conclusions

- Dietary did not have an effect on growth or productive parameters in diets with high substitution of FMFO in gilthead sea bream fingerlings.
- Cu retention in the different tissues was not affected by dietary Cu levels.
- *CuZnsod* expression remained unchanged with the different supplementary levels.
- Increasing Cu supplementation raised liver steatosis, broken cell margin, peripheral nuclei and sinusoid dilatation, markers of hepatic damage and cholestasis, indicating a potential toxic effect of Cu at levels above 9 mg Cu kg^{-1}.
- The results obtained indicate that Cu basal levels (6 mg Cu kg^{-1}) would be sufficient to cover the requirements for gilthead sea bream when fed diets with high substitution of FMFO. Higher levels can produce toxic effects on the liver.

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