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Can lysine and branched-chain amino acids improve lumpfish health and stress resilience?

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ABSTRACT

The expansion of lumpfish use as a cleaner fish has recently come to a halt, in part due to the concerns around their welfare. While refining handling procedures and operational standards is necessary, improving aquafeeds has the potential to alleviate the burden caused by chronic stress. This study examined the influence of different inclusions of dietary branched-chain amino acids (BCAAs) and lysine (Lys) on lumpfish health, immunity, stress response, growth and plasma amino acid profiles for 10 weeks. Stress and metabolic biomarkers (cortisol, lactate, glucose, triglycerides) showed modest but measurable alterations, such as lower triglycerides and higher glucose in fish fed the diet with limiting Lys and BCAAs. Metabolic differences were also visible from plasma amino acid profiles. Limiting Lys and BCAAs in diets seems to increase the glucogenic activity in stressed fish, increasing glucogenic amino acid levels in plasma. Diets with increased Lys and BCAAs levels increased antiprotease activity and lowered GABA plasma concentrations. These findings suggest that Lys- and BCAAs-enriched diets might provide relevant nutritional support, which allows the organism to adapt to a mild chronic stress. Immunonutrition can prove to be an effective strategy to ameliorate welfare in the face of chronic stress.

1. Introduction

Within the rapidly evolving field of biological sciences, the topic of animal welfare has been for decades, complex and divisive (Haynes, 2011). Defining animal welfare is challenging (Fraser et al., 1997; Jena, 2017). Historically viewed as a physiological expression, it has evolved to encompass the subjectivity and sentiment that animals experience (Broom and Johnson, 2019; Mellor et al., 2020; Segner et al., 2012). The recognition of this subjective domain became possible through scientific advancements in the fields of animal cognition (Bshary et al., 2002; Bshary and Brown, 2014). Where once animals were viewed as merely utilitarian beings, modern science challenges our society to recognize the depth of animals' feelings (Brown, 2015; Haynes, 2011). The growing evidence that farmed animals are self-aware may present a challenge to a civilization which has long relied on animals (Singer and Harari, 2023). However, it also offers an opportunity to reshape the way we interact with the living world.

One relationship that stands out as a pressing matter for revaluation, is the one with lumpfish (*Cyclopterus lumpus*). These fish inhabit the Northern Atlantic, have a ball-shaped body without scales, display a blue-green hue and are equipped with a suction disk used to attach to hard surfaces (Davenport, 1985). This species gained the attention of

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salmon farmers due to their promising ability in sea lice grazing (Imsland et al., 2021). It seemed like a potential solution to alleviate the burden imposed by sea lice on the Atlantic salmon industry. As such, lumpfish production rapidly expanded, in just a few years went from several hundred thousand individuals produced - topping 43 million in 2019 (Fiskeridirektoratet, 2023). However, the welfare issues started to emerge. The rapid expansion was not accompanied by knowledge on the species biological and physiological requirements. One report from the Norwegian Food Safety Authority (NFSA) revealed annual mortalities in the range of 45 %, with several farmers reporting 100 % mortalities of deployed lumpfish in salmon sea pens (Mattilsynet, 2020; Stien et al., 2020). Five years have passed since the first NFSA report on cleaner fish welfare, and the situation seems to be worse. Indeed, the latest report from the Norwegian Veterinary Institute puts cleaner fish mortality in the range of 80–90 % (Moldal et al., 2025). This situation is both unfortunate and unacceptable, a fact that has been made clear by NFSA. Nonetheless, lumpfish continue being deployed as delousing agents. While this reality persists, stakeholders should strive to ensure the best possible conditions for lumpfish, to at least, guarantee good health. For that responsibility to be upheld, a great deal of research in lumpfish biological requirements, physiology, health and nutrition is needed (Garcia de Leaniz et al., 2022; Treasurer et al., 2018).

Stress is ubiquitous in animal aquaculture. From rearing practices, inadequate water physicochemical properties, handling, to sub-optimal diets, stressors are common (Afonso, 2020; Martos-Sitcha et al., 2020). While some stressors can have a potential positive effect, others, depending on their intensity, can lead to maladaptive changes in the organism (Dhabhar, 2008; Guo and Dixon, 2021). These maladaptive changes impact overall health. In teleosts, stressors, once perceived, prompt the release of catecholamines and, later, corticosteroid hormones such as cortisol (Balasch and Tort, 2019; Wendelaar Bonga, 1997). These hormones alter energy mobilization, and cell trafficking, leading to the organism preparedness for an incoming assault (Pickering and Pottinger, 1989; Vijayan et al., 2010). The animal is ready to resist, cope and heal from a potential threat. Depending on the intensity, duration and nature of the assault, the organism can overcome it and become more resilient. However, when the stressor is such that overpowers the organisms' defences, either through its intensity or frequency – as with certain chronic stressors - the health deteriorates (Barton, 2002; Gorissen and Flik, 2016; Pickering and Pottinger, 1989). The stress response itself is a highly energy expensive process (Beyers and Rice, 2002; Costas et al., 2011). Farmed animals are heavily reliant on the nutritional adequacy of their diets to have the necessary nutrients to regain homeostasis. As such, optimal diets factoring in the realistic needs of farmed animals, are paramount for their health (Hardy, 2012; Kiron, 2012; Lall, 2010; Wagner and Congleton, 2004).

Research in fish nutrition has helped to improve productivity and overall health of aquaculture fish. From ensuring biological requirements, to immune modulation, research on dietary strategies has had a significant impact (Ciji and Akhtar, 2021). One notable example is the cataract issue that once impacted the salmonid industry, partially resolved by dietary amino acid (AA) adjustment such as histidine – integral for lens osmoregulation (Bjerkås et al., 2006; Midtlyng et al., 1999; Ogata, 2002; Remø et al., 2017; Waagbø et al., 2010). AAs have a very interesting role in many physiological processes, being the building blocks of proteins. But beyond their role as bricks in the building of proteins, some AAs display biological activities per se. Tryptophan, for instance, is a precursor to serotonin, and has been documented having both immune and stress modulatory properties (Herrera et al., 2020; Machado et al., 2015). As such, AAs have received tremendous attention from aquaculture nutritionists (Andersen et al., 2016; Aragão et al., 2008; Hoseini and Reverter, 2022; P. Li et al., 2009). There is, however, a group of essential AAs which, despite being fundamental in several physiological processes, have received little attention in the immunonutrition research – the branched chain amino acids (BCAAs; leucine [Leu], isoleucine [Ile] and valine [Val]) (Ahmad, Ahmed, Fatma, et al., 2021; Castillo and Gatlin, 2018). BCAAs are essential AAs critical for protein synthesis and energy metabolism, being involved in cellular repair and regulation of metabolic pathways. Another relevant AA which has been thoroughly studied in relation to growth, but less so for its role in stress and immunity, is lysine (Lys). It is typically the first limiting AA in aquafeeds, and is highly relevant in the maintenance of healthy tissues, while also being implicated in immunity and stress resilience (Hu et al., 2021). Our previous work revealed that chronic stress significantly impacts the utilization of BCAAs and Lys levels in lumpfish, providing direct evidence to target these AAs in the present study (da Santa Lopes et al., 2024). The aim of this work was to explore the potential of BCAAs/Lys enriched diets in improving the health and stress resilience of lumpfish subject to chronic stress. To our knowledge, this is the first study to explore the effects of reduced and elevated BCAAs/Lys diets on lumpfish health, stress and immunity.

2. Material and methods

2.1. Fish and tanks

Juvenile lumpfish with a mean weight of 42.4 ± 4.8 g were transferred on calendar week 23, from Lerøy Aurora AS (Vangsvik, Norway) to Nord University research facilities in Mørkvedbukta (Bodø, Norway). An initial health screening was performed, including cataract assessment. Fish were weighed and randomly sorted into 18 flow-through 1000 L tanks, where they remained undisturbed for two weeks (n = 15; N = 270). For the first 2 weeks of acclimation, fish were fed a commercial diet (Skretting Clean Assist, 1.8 mm pellet size, Skretting, Stavanger, Norway) at 2 % body weight with the use of calibrated Arvotec feed automats (TD2000, Arvo-tec, Joroinen, Finland). During the experiment, the photoperiod followed the natural photoperiod of Nordland region at the time (24 h light). The water parameters were monitored daily, including water temperature (9.2 ± 0.6 ºC), salinity (34.4 ± 0.2 ‰) and dissolved oxygen (10.4 ± 0.7 mg L^{-1}). Fish were also monitored daily for feeding, hovering, resting and swimming behaviour.

2.2. Study design and experiment layout

Six groups were formed in triplicate tanks, including two control groups. A negative control group "CTR-", which remained undisturbed and fed a commercial diet throughout the experiment. A positive control group "CTR+ " which was exposed to air four times per week and fed a commercial diet. There were 4 experimental groups, with varying levels of BCAAs inclusion in their diets, namely group "A", "B", "C" and "D". Each tank was considered as an experimental unit.

The study was divided in two phases, lasting 10 weeks in total, reflecting a realistic set up for assessing the cumulative effects of chronic stress on metabolism and immunity. The influence of repeated 1 min air exposure was studied on lumpfish groups supplemented with different dietary inclusions of BCAAs (Fig. 1). During the first phase, which comprised the first two weeks, the groups were left undisturbed, and each was fed with their respective diet. In the first phase, group "CTR-" and "CTR+ " are treated in the same manner, and designated as the same group "CTR". After these first two weeks, the first sampling took place, and with it, the start of the second phase of the trial – with stress exposure. Here, group "CTR" was split into the two groups, "CTR-" left undisturbed and "CTR+ " exposed to stress. The stress exposure consisted of 1 min air exposure 4 times per week. Fish were netted in less than 30 s and lifted in the air for exactly 1 min, as described in Lopes et al. (da Santa Lopes et al., 2023). A second sampling took place 4 weeks after the start of the stress exposure. The third and final sampling was performed 8 weeks after the start of the stress exposure. Three fish per tank were assessed in each timepoint. Samplings always took place 1 h after exposure to stress. Fish were not fed 24 h before sampling.

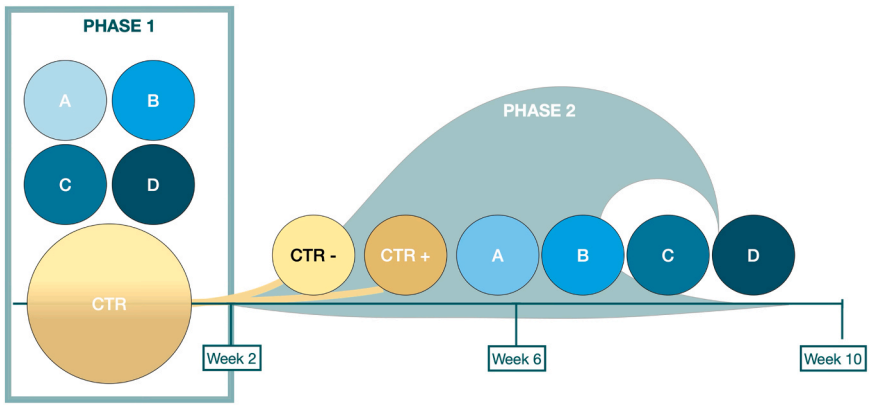

Fig. 1. Experimental design. Phase 1 (first two weeks of experiment) there was no stress exposure, with 5 groups: "CTR" fed commercial diet; groups "A"; "B"; "C" and "D" fed their respective experimental diets. At week 2, the first sampling occurred. After which "CTR" group was divided in two groups "CTR-" left undisturbed through the entire study and group "CTR+ ", subject to air exposure. Phase 2 started after week 2, with exposure to stress for all groups except "CTR-". Sampling occurred at weeks 6 and 10.

2.3. Experimental diets

Experimental diets were designed in collaboration and manufactured by Skretting (Stavanger, Norway), with variation on BCAAs and Lys inclusions as shown in the table below (Table 1). The control diet is a commercial feed produced by Skretting AS (Clean Assist, Skretting AS) and it was fed to CTR- and CTR+ , provides standard BCAA and Lys levels (e.g., Ile: 20.2 g/kg, Leu: 38.7 g/kg, Lys: 32.5 g/kg, Val: 22.3 g/kg). Diet A reduces these by approximately 15 % (e.g., Leu: 33.4 g/kg, Lys: 28.2 g/kg), while groups B, C, and D increase supplementation to ~30 %, 60 %, and 90 % above control (e.g., Leu in D: 73.1 g/kg). Changes in these amino acid levels were achieved by manipulating the inclusion levels of a specific protein ingredient.

2.4. Health and growth

Health and growth measurements were performed at each sampling point. An initial health screening was also performed on the fish at arrival in the facilities. Health score was performed in each sampling point, as described in the Lumpfish health scoring system (LHSS) (Reynolds et al., 2022). LHSS scores are separated in three different categories (below 3 is considered good welfare; between 3 and 5 is considered concerning welfare status, and a score above 5 is deemed a compromised welfare). Cataract scoring was performed using a portable slit lamp (Heine, Gilching, Germany), and the incidence calculated. Specific growth rate (SGR) was calculated: $SGR = \frac{(\ln final\ body\ weight - \ln initial\ body\ weight)}{days} \times 100$. Cataract size scores reflects the area of the eye lens covered by the cataract (1–4, where "1" is a cataract covering up to 10 % of the eye lens and "4" being a cataract covering over 90 % of the eye lens).

Table 1
Composition of the diets used in this study.

Nutrient	Unit	Control	A	B	C	D
			-15 %	30 %	60 %	90 %
Moisture	%	8.9	10.0	8.0	6.4	5.0
Crude protein	%	53.7	46.2	56.0	58.2	59.8
Crude fat	%	15.5	19.8	15.4	14.0	14.0
Ash	%	10.3	11.1	11.6	12.8	14.1
Crude fibre	%	0.8	1.8	0.8	0.8	0.7
Starch	%	7.0	8.0	5.2	5.2	4.3
Gross energy	MJ/kg	20.9	20.9	21.2	21.2	21.5
Crude protein/ gross energy	-	25.6	22.1	26.5	27.4	27.8
Total isoleucine	g/kg	20.2	17.4	26.4	32.4	38.4
Total leucine	g/kg	38.7	33.4	50.2	61.9	73.1
Total lysine	g/kg	32.5	28.2	42.0	51.9	61.4
Total valine	g/kg	22.3	19.2	29.5	35.5	42.2
Total of 17 amino acids	g/kg	485.2	411.4	510.7	534.3	552.7

2.5. Blood and plasma sampling

At each sampling timepoint, 3 lumpfish per tank (9 per group) were euthanized one hour after stress exposure, with 1600 mg L^{-1} of metacaine (tricaine methanesulphonate, Sigma Aldrich Co, St. Louis, Missouri, USA), following the recommendations provided by Skår et al. (Skår et al., 2017). Blood collection took less than 1 min per fish, drawn from the caudal vein into a heparinized vacutainer (BD, Plymouth, UK). Plasma was obtained after centrifuging the blood for 5 min at 2000 x g at 4 °C. We also established one plasma pool of 120 μL from 3 fish per tank, (using 40 μL plasma from each fish) to profile free amino acids. Plasma samples were immediately frozen in liquid nitrogen and stored at −80 °C.

2.6. Stress and metabolic biomarkers

Cortisol levels were measured in blood plasma, using a commercial kit (DetectX Cortisol Enzyme Immunoassay Kit, Arbor Assays Inc., Michigan, USA). After mixing and adding to the microtiter plates, the kit manufacturer's protocol was followed. Spinreact kits (Spinreact, Girona, Spain) were used to quantify metabolic biomarkers: glucose; lactate and triglycerides in plasma, using the protocol adapted by Costas et al. (Costas et al., 2011) for microplates.

2.7. Immune parameters

Following the protocol described by Quade and Roth (1997), we determined total peroxidase activity. This colorimetric assay result is expressed in units of peroxidase, which is the metric of the amount of peroxidase necessary to change absorbance by 1 OD. Anti-protease assay was performed, using the technique described by Ellis (1990b) and adapted by Machado et al. (Machado et al., 2015, 2020). Plasma lysozyme was assessed according to the assay outlined by (Ellis, 1990b), with adaptations by Costas et al. (Costas et al., 2011). To calculate the amount of lysozyme, a commercial kit was used (EnzCheck Phosphatase Assay Kit, Thermo Fisher Scientific Inc., Massachusetts, USA). The plasma's overall capacity in eliminating bacteria was determined following the method described by Graham et al. (Graham and Secombes, 1988), with adaptations from Machado et al. (Machado et al., 2015), validated for lumpfish (da Santa Lopes et al., 2024). The method

consists of incubating plasma with a known concentration of a specific bacteria (*Tenacibaculum maritimum* in this case). Bactericidal activity is expressed as the percentage of surviving bacteria relative to the positive control. Nitric oxide (NO) was indirectly evaluated using a colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany), which quantifies the total nitrites and nitrates from NO degradation.

2.8. Plasma free amino acids

We profiled the free amino acids in each plasma pool from each tank (3 pools per group per sampling point). For this, ultra-high-performance liquid chromatography (UPLC) was performed on a Waters Reversed-Phase Amino Acid Analysis System as described in Lopes et al. (da Santa Lopes et al., 2024). Norvaline served as an internal standard. Centrifugal ultrafiltration (10 kDa cut-off, 2500 x g, 20 min, 4 ºC) was applied to deproteinize the samples, which then underwent pre-column derivatization according to the AccQ Tag method (Waters, USA) using AccQ Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). The profile of amino acids was established by retention times from standard mixtures and pure standards (Sigma-Aldrich Co).

2.9. Statistical analysis

Homogeneity of variances and normality were tested using Levene's F test and Kolmogorov-Smirnov tests (Zar, 1984). Skewed data was logarithmically transformed. For the data that did not follow a normal distribution, non-parametric tests were conducted. Each group was assigned 3 replicate tanks (n = 3), with the exception of the control group CTR on week 2 (n = 6). Each tank was considered as an experimental unit in the statistical analysis. Due to the design of this experiment, in week "2" and week "6" a one-way ANOVA was performed between control groups – "CTR-" and "CTR+ " – to address the effect of stress. A one-way ANOVA was then performed to address the dietary influence between groups "CTR+ " and the experimental diet groups "A"; "B"; "C" and "D" since all these groups were subject to stress (p value < 0.05), followed by Tukey HSD post hoc test (Zar, 1984).). IBM SPSS v28.0 0 (IBM Corp., Armonk, New York, USA) was used to perform all statistical tests. A significance level (α) of 0.05 was used unless stated otherwise.

3. Results

3.1. Stress and metabolism biomarkers

The cortisol levels did not differ significantly between groups and were consistently found below 50 ng ml^{-1} (p value < 0.05, Fig. 2).

The triglyceride assay showed significant differences (p value < 0.05, Fig. 3) on week 6, between group fed diet A and groups fed diets C and D. No statistically significant differences were found between the two control groups nor between dietary treatments in the other timepoints (p value < 0.05).

No significant differences were found in lactate levels throughout the experiment (Table 2). Glucose in plasma was highest in group A on week 6 (1.70 ± 0.17 mM), being significantly higher than groups B (1.16 ± 0.06 mM) and C (1.14 ± 0.07 mM). On week 10, CTR- had statistically higher levels of glucose than CTR+ (Table 2).

3.2. Immune biomarkers

There were no statistically significant differences in lysozyme levels between dietary treatments nor between control groups at any given timepoint (Table 2). Analysis of peroxidase activity revealed significant differences only between dietary treatments in week 6, with group A (135.41 ± 11.24 units ml^{-1}) having higher activity than group B (66.43 ± 12.99 units ml^{-1}) (Fig. 4). The bactericidal activity did not reveal significant differences. On week 10, anti-protease levels were

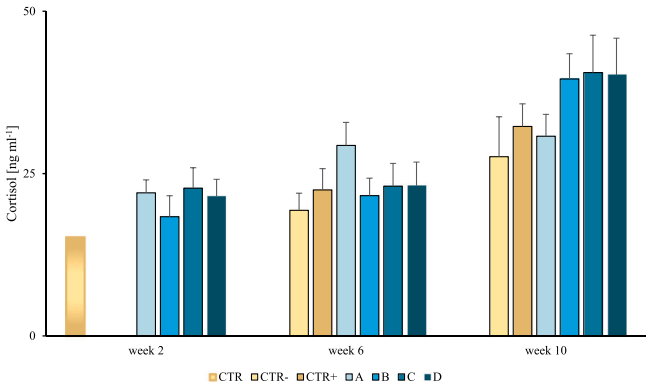

Fig. 2. Plasma cortisol (ng ml^{-1}) expressed as mean ± S.E. for all sampling points (week 2, week 6 and week 10). "CTR" refers to the tanks fed commercial diet prior to phase 2, where the CTR group was split into "CTR-" and "CTR+ ". A one-way ANOVA was employed for each timepoint. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

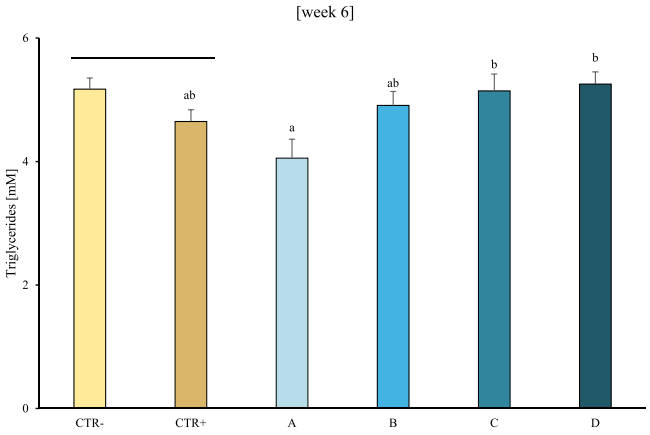

Fig. 3. Plasma triglyceride (mM) from week 6, expressed as average ± S.E. Letters indicate significant differences found between dietary treatments, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

significantly higher in group D, than CTR+ and A groups (Table 2). Nitric oxide assay presented significant differences between dietary treatments on week 6, with group A having a higher NO concentration (24.95 ± 2.87 μM) compared to group C (16.30 ± 1.46 μM). On week 6, CTR+ had significantly higher levels of NO (21.82 ± 1.53 μM) than the undisturbed control group CTR- (16.27 ± 1.40 μM).

3.3. Profile of plasma free amino acids

Data regarding plasma free AAs is presented in Table 3 and the results are described by timepoint (in weeks).

Week 2 (undisturbed phase)

1. Lys and BCAAs
   No differences found.
2. Tyrosine [Tyr] and aspartic acid [Asp]

   Tyr and Asp were higher in group A than in group C.

Week 6 (stress exposure phase)

Table 2
Metabolic and immune biomarkers across the different groups in each time point. Expressed as mean ± S.E. Letters indicate significant differences found between dietary treatments, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, with symbols indicating the significant differences.

Lactate (mM)	Group	Week 2			Week 6			Week 10		
	CTR	0.24	±	0.02						
	CTR-				0.21	±	0.03	0.24	±	0.04
	CTR+				0.25	±	0.03	0.31	±	0.07
	A	0.25	±	0.02	0.35	±	0.06	0.24	±	0.04
	B	0.18	±	0.03	0.24	±	0.03	0.26	±	0.05
	C	0.19	±	0.02	0.21	±	0.02	0.27	±	0.03
	D	0.22	±	0.02	0.30	±	0.05	0.23	±	0.03
Glucose (mM)	CTR	1.43	±	0.05						
	CTR-				1.33	±	0.09	**1.54^{b}**	**±**	**0.13**
	CTR+				**1.27$^{*\#}$**	**±**	**0.04**	**1.11^{a}**	**±**	**0.04**
	A	1.38	±	0.21	**1.70$^{\#}$**	**±**	**0.17**	1.65	±	0.27
	B	1.32	±	0.14	**1.16***	**±**	**0.06**	1.16	±	0.17
	C	1.95	±	0.37	**1.14***	**±**	**0.07**	1.14	±	0.13
	D	1.40	±	0.15	**1.35$^{*\#}$**	**±**	**0.07**	1.38	±	0.10
Anti-protease activity (%)	CTR	91 %	±	0.8						
	CTR-				97 %	±	0.7	96 %	±	0.4
	CTR+				96 %	±	0.4	**96 %***	**±**	**0.8**
	A	92 %	±	0.8	84 %	±	9.1	**94 %***	**±**	**2.0**
	B	92 %	±	1.2	96 %	±	0.8	**98 %$^{*\#}$**	**±**	**0.3**
	C	91 %	±	1.5	96 %	±	0.7	**97 %$^{*\#}$**	**±**	**0.8**
	D	91 %	±	1.2	97 %	±	0.4	**98 %$^{\#}$**	**±**	**0.2**
Lysozyme (μg ml^{-1})	CTR	52.20	±	6.57						
	CTR-				65.26	±	5.97	110.61	±	12.35
	CTR+				69.01	±	8.56	135.85	±	14.66
	A	48.15	±	3.92	60.76	±	8.78	97.25	±	12.75
	B	68.10	±	6.03	75.43	±	17.55	102.95	±	12.45
	C	59.61	±	8.25	78.19	±	12.73	102.72	±	8.86
	D	61.65	±	7.38	62.14	±	5.61	94.69	±	13.24
Bactericidal activity (%)	CTR	48.0 %	±	2.3						
	CTR-				47.0 %	±	4.5	31.0 %	±	3.2
	CTR+				44.0 %	±	2.6	27.9 %	±	3.8
	A	48.2 %	±	3.5	46.2 %	±	2.2	27.3 %	±	3.5
	B	47.4 %	±	2.4	42.7 %	±	3.1	24.7 %	±	2.8
	C	49.7 %	±	1.8	41.6 %	±	3.9	26.4 %	±	2.3
	D	53.1 %	±	3.5	48.7 %	±	2.9	30.8 %	±	3.5
Nitric oxide (μM)	CTR	38.45	±	5.00						
	CTR-				**16.27a**	**±**	**1.40**	14.46	±	0.98
	CTR+				**21.82$^{b*\#}$**	**±**	**1.53**	15.54	±	1.21
	A	38.58	±	6.90	**24.95$^{\#}$**	**±**	**2.87**	16.92	±	1.06
	B	30.78	±	3.62	**18.98$^{*\#}$**	**±**	**2.56**	17.96	±	1.90
	C	37.72	±	9.33	**16.30***	**±**	**1.47**	12.99	±	0.75
	D	37.14	±	4.23	**17.63$^{*\#}$**	**±**	**1.05**	16.52	±	0.93

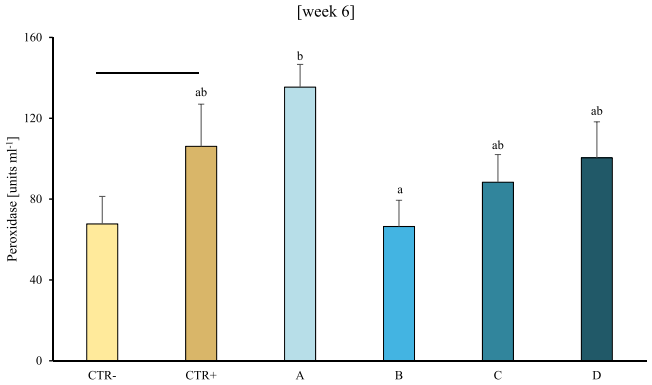

Fig. 4. Peroxidase activity (units ml^{-1}) in week 6, expressed as average ± S.E. Letters indicate significant differences found between dietary treatments, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

1. Lys and BCAAs
   Lys and Leu were significantly higher in group D than in all the other stressed groups (CTR+, A, B and C).Ile and Val were higher in fish fed diet D than group CTR+ and A (Fig. 5).
2. Phenylalanine [Phe]
   Phe was highest in CTR+ than groups A, C and D. B and C also had significantly higher levels of Phe than group D (Fig. 6.).
3. Aspartic acid
   Highest levels of Asp were found in group A, being significantly higher than in all other stressed groups (CTR+, B, C and D). C also had significantly lower levels of Asp than groups CTR+ , B and D.
4. Glycine [Gly] and serine [Ser]
   Group A had significantly higher levels of Gly when compared to group D. Ser was highest in fish fed diet A, being significantly higher than groups B, C and D. Ser was also significantly higher in CTR+ when compared to group D.
5. γ-amino-n-butyric acid [GABA]
   GABA levels were significantly higher in group CTR+ than in groups A, C and D.
6. CTR- vs CTR+

There were no significant differences in free plasma AA levels found between the control groups CTR- and CTR+ .

Week 10 (final sampling)

Table 3
Plasma free amino acids (expressed as mean ± S.E.). Letters indicate significant differences found between dietary treatments, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, with symbols indicating the significant differences.

Amino acids	week 2					week 6						week 10					
(μg ml^{-1} plasma)	**CTR**	**A**	**B**	**C**	**D**	**CTR-**	**CTR+**	**A**	**B**	**C**	**D**	**CTR-**	**CTR+**	**A**	**B**	**C**	**D**
Arginine	5.6 ± 0.1	5.5 ± 0.2	5.5 ± 0.2	5.0 ± 0.3	5.2 ± 0.5	6.4 ± 0.5	7.1 ± 0.3	6.8 ± 0.8	7.0 ± 0.5	6.8 ± 0.5	8.2 ± 0.3	5.8 ± 0.5	6.8 ± 0.7	6.2 ± 0.1	6.7 ± 0.6	7.1 ± 0.4	6.9 ± 0.4
Histidine	5.5 ± 0.1	5.1 ± 0.1	5.0 ± 0.2	4.6 ± 0.3	4.6 ± 0.4	5.1 ± 0.3	5.5 ± 0.2	5.2 ± 0.4	4.9 ± 0.1	4.8 ± 0.2	5.5 ± 0.2	5.9 ± 0.4	5.9 ± 0.4	5.9 ± 0.4	6.0 ± 0.6	5.1 ± 0.2	5.6 ± 0.2
Lysine	6.4 ± 0.5	5.7 ± 0.4	7.7 ± 1.1	8.2 ± 2.4	10.5 ± 3.3	6.7 ± 0.4	6.7 ± 0.6a	5.5 ± 0.4a	8.1 ± 0.6a	10.5 ± 0.8a	17.6 ± 2.1b	5.9 ± 0.5	6.6 ± 0.2a	6.1 ± 0.0a	7.2 ± 0.2a	10.2 ± 0.8b	11.2 ± 0.3b
Threonine	3.9 ± 0.1b	3.6 ± 0.1ab	3.6 ± 0.2ab	3.1 ± 0.3a	2.8 ± 0.2a	4.5 ± 0.5	4.6 ± 0.4	4.1 ± 0.2	4.8 ± 0.1	4.7 ± 0.4	4.8 ± 0.3	4.3 ± 0.1	4.70 ± 0.4	4.4 ± 0.0	5.0 ± 0.4	5.1 ± 0.3	4.8 ± 0.2
Isoleucine	5.1 ± 0.1	4.6 ± 0.1	4.7 ± 0.3	6.3 ± 1.7	8.2 ± 2.3	4.8 ± 0.3	4.8 ± 0.1a	4.4 ± 0.3a	5.0 ± 0.3ab	5.2 ± 0.1ab	6.0 ± 0.2b	5.5 ± 0.1	5.2 ± 0.1ab	4.9 ± 0.1a	5.0 ± 0.1ab	5.6 ± 0.1b	6.4 ± 0.1c
Leucine	7.1 ± 0.4	6.2 ± 0.2	6.7 ± 0.9	11.6 ± 5.3	17.8 ± 6.8	6.8 ± 0.6	6.7 ± 0.3a	6.2 ± 0.4a	7.3 ± 0.4a	7.6 ± 0.3a	11.2 ± 1.1b	7.4 ± 0.2	7.3 ± 0.1ab	6.8 ± 0.0a	6.9 ± 0.2a	7.9 ± 0.3b	10.6 ± 0.2c
Valine	7.3 ± 0.3	6.3 ± 0.2	6.9 ± 0.4	10.0 ± 3.2	13.7 ± 4.3	7.3 ± 0.4	7.1 ± 0.3a	6.3 ± 0.4a	7.5 ± 0.4ab	7.5 ± 0.3ab	9.1 ± 0.6b	**8.3 ± 0.1#**	**7.6 ± 0.2a***	7.0 ± 0.2a	7.1 ± 0.5a	8.1 ± 0.3a	9.6 ± 0.2b
Tryptophan	3.5 ± 0.0	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.2 ± 0.1	3.8 ± 0.2	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.0	4.4 ± 0.0	4.2 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.2
Methionine	4.6 ± 0.1	4.4 ± 0.3	4.6 ± 0.3	4.4 ± 0.4	4.3 ± 0.2	5 ± 0.2	4.8 ± 0.1	5.5 ± 0.6	4.6 ± 0.1	4.5 ± 0.2	5.2 ± 0.3	7.3 ± 0.5	6.0 ± 0.4	6.5 ± 0.3	7.3 ± 0.2	6.2 ± 0.4	7.1 ± 0.6
Phenylalanine	160.2 ± 12.2	128.9 ± 19.2	160.4 ± 18.9	153 ± 28.9	129.3 ± 32.7	186.3 ± 7.0	205.5 ± 25.2c	123.6 ± 11.1ab	170.0 ± 2.3bc	135.7 ± 5.5b	68.4 ± 8.7a	**190.8 ± 9.4#**	**129.4 ± 17.4***	**[excluded]**	169.7 ± 18.1	121.4 ± 25.6	86.6 ± 9.3
Cysteine	1.5 ± 0.0	1.6 ± 0.1	1.5 ± 0.0	1.6 ± 0.0	1.5 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	1.6 ± 0.0	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	1.7 ± 0.0	1.7 ± 0.0a	2.0 ± 0.1b	1.7 ± 0.0a	1.7 ± 0.0a	1.6 ± 0.1a
Tyrosine	8.2 ± 0.3ab	9.4 ± 0.6b	8.1 ± 0.6ab	6.2 ± 1.1a	6.3 ± 1.1ab	10.1 ± 1.0	10.6 ± 0.2b	10.5 ± 1.2b	9.1 ± 0.5ab	8.7 ± 0.3ab	7.4 ± 0.7a	11.3 ± 0.4	11.2 ± 0.8b	11.4 ± 1.1b	9.6 ± 0.8ab	9.0 ± 0.3ab	7.3 ± 0.7a
Aspartic acid	12.2 ± 1.0ab	15.5 ± 2.5b	10.6 ± 1.2ab	8.7 ± 0.9a	10.0 ± 0.9ab	12.6 ± 0.3	12.4 ± 0.2b	23.1 ± 1.4c	11.7 ± 0.4b	7.8 ± 0.4a	9.9 ± 0.7b	10.0 ± 0.9	8.8 ± 0.6a	35.3 ± 4.1c	13.7 ± 0.2b	9.0 ± 1.2a	9.1 ± 0.7a
Asparagine	5.4 ± 0.2	5.3 ± 0.3	5.4 ± 0.3	4.4 ± 0.4	5.1 ± 0.3	5.8 ± 0.4	5.8 ± 0.3	6.1 ± 0.7	5.2 ± 0.4	5.1 ± 0.3	5.9 ± 0.2	5.7 ± 0.2	6.1 ± 0.3	5.5 ± 0.1	5.7 ± 0.6	5.5 ± 0.4	5.7 ± 0.3
Glutamic acid	8.5 ± 0.4	8.3 ± 0.1	9.5 ± 0.5	10.1 ± 0.9	10.8 ± 1.0	6.5 ± 0.3	6.7 ± 0.3	7.4 ± 0.8	6.7 ± 0.3	6.9 ± 0.3	8.4 ± 0.6	7.4 ± 0.2	7.0 ± 0.2a	7.1 ± 0.3ab	8.0 ± 0.2ab	7.1 ± 0.2ab	8.4 ± 0.5b
Glutamine	5.4 ± 0.1	5.3 ± 0.2	5.3 ± 0.2	4.8 ± 0.3	5.0 ± 0.5	6.1 ± 0.4	6.8 ± 0.3	6.5 ± 0.8	6.7 ± 0.5	6.5 ± 0.4	7.8 ± 0.3	5.5 ± 0.4	6.5 ± 0.6	5.9 ± 0.1	6.3 ± 0.6	6.7 ± 0.3	6.5 ± 0.4
Alanine	8.4 ± 0.3	8.4 ± 0.6	8.1 ± 0.8	6.5 ± 0.6	6.7 ± 0.4	9.9 ± 1.0	12.2 ± 0.5	11.0 ± 1.3	10.0 ± 1.0	9.2 ± 0.1	9.7 ± 0.5	8.8 ± 0.6	11.9 ± 1.6	10.5 ± 1.3	9.8 ± 0.8	9.8 ± 0.5	9.1 ± 0.7
Glycine	5.0 ± 0.4	5.3 ± 0.2	4.8 ± 0.8	4.2 ± 0.9	3.9 ± 0.9	3.8 ± 0.2	4.7 ± 0.3ab	5.7 ± 0.8b	3.8 ± 0.1ab	3.8 ± 0.1ab	3.6 ± 0.3a	3.9 ± 0.7	3.2 ± 0.1	3.5 ± 0.4	4.0 ± 0.3	3.2 ± 0.5	2.8 ± 0.5
Proline	1.5 ± 0.0	1.5 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.2	1.5 ± 0.4	1.4 ± 0.1b	1.3 ± 0.1ab	1.3 ± 0.0ab	1.2 ± 0.0ab	1.1 ± 0.1a	1.6 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Serine	4.4 ± 0.1	4.5 ± 0.3	4.2 ± 0.6	3.2 ± 0.6	2.8 ± 0.5	4.4 ± 0.4	4.9 ± 0.2bc	5.6 ± 0.3c	4.3 ± 0.3ab	4.0 ± 0.1ab	3.7 ± 0.2a	4.9 ± 0.3	4.5 ± 0.1	5.8 ± 0.1	4.8 ± 0.3	4.7 ± 0.7	3.7 ± 0.3
Taurine	5.0 ± 0.3	4.7 ± 0.5	5.0 ± 1.0	5.2 ± 0.1	6.1 ± 1.0	4.2 ± 0.1	6.3 ± 1.3	8.1 ± 3.0	4.5 ± 0.3	4.2 ± 0.4	5.5 ± 1.0	5.5 ± 1.4	4.8 ± 0.2ab	4.4 ± 0.3ab	7.7 ± 0.8b	4.1 ± 0.7a	5.2 ± 0.9ab
Ornithine	10.3 ± 0.7	9.2 ± 1.0	11 ± 0.8	10.1 ± 0.3	11 ± 0.5	9.4 ± 0.5	9.1 ± 0.1b	6.4 ± 1.1a	9.5 ± 0.3b	8.5 ± 0.2ab	8.1 ± 0.2ab	8.9 ± 0.4	8.3 ± 0.5	6.8 ± 0.1	8.0 ± 0.4	8.1 ± 0.9	8.1 ± 1.0
γ-Amino-n-butyric acid	4.7 ± 0.6	4.2 ± 0.5	4.3 ± 0.7	3.5 ± 1.0	3.2 ± 0.9	4.9 ± 0.6	5.5 ± 0.4b	3.1 ± 0.1a	3.8 ± 0.3ab	3.0 ± 0.2a	3.1 ± 0.4a	5.5 ± 0.8	5.4 ± 0.9b	4.2 ± 0.3ab	4.2 ± 0.3ab	3.6 ± 0.4ab	3.0 ± 0.2a
Hydroxyproline	3.1 ± 0.1	3.2 ± 0.1	3.0 ± 0.2	2.7 ± 0.1	3.0 ± 0.1	3.0 ± 0.2	3.4 ± 0.2	4.2 ± 0.5	3.3 ± 0.3	3.3 ±.2	4.1 ± 0.3	3.9 ± 0.3	4.5 ± 0.5	4.9 ± 0.2	4.1 ± 0.6	3.8 ± 0.3	3.7 ± 0.1
β-Alanine	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.1	1.1 ± 0.0	1.4 ± 0.1	1.7 ± 0.3	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	1.3 ± 0.1

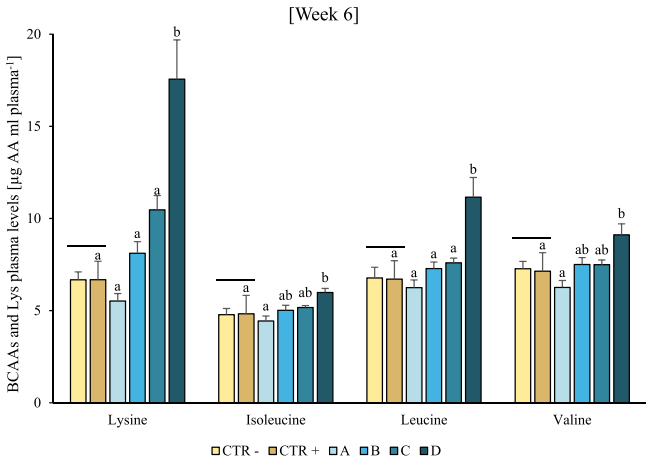

Fig. 5. Plasma free BCAAs and Lys (µg AA ml plasma^{-1}) in week 6. Expressed as average ± S.E. Letters indicate significant differences found between dietary treatments, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

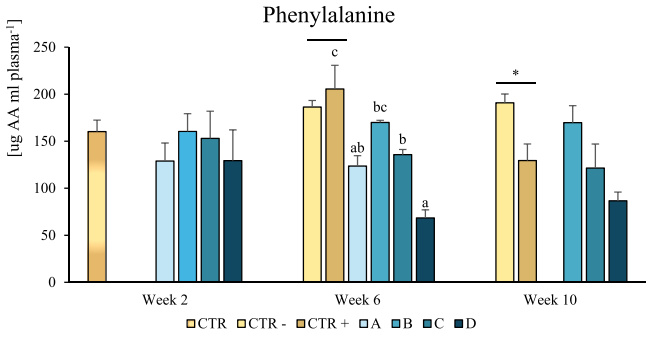

Fig. 6. Plasma free phenylalanine levels (µg AA ml plasma^{-1}) in weeks 2, 6 and 10. Expressed as average ± S.E. Letters indicate significant differences found between diets, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$). Phe levels of group A were excluded from the comparisons.

1. Lys and BCAAs
   Lys was higher in groups C and D when compared to groups CTR+ , A and B. Fish fed diet D also had the highest levels of BCAAs. Ile, Leu and Val were significantly higher in group D compared to all other stressed groups. C also had significantly higher levels of Ile than group A. Leu was significantly higher in group C compared to groups A and B (Fig. 7).
2. Phenylalanine
   Phe levels were similar between the stressed groups. Group A was excluded from Phe statistical analysis in week 10 due to the loss of the third replicate and the substantial divergence between the remaining two replicates.
3. Aspartic acid
   Asp levels were significantly higher in group A compared to all other groups. Group B had significantly higher levels of Asp compared to groups CTR+ , C and D.
4. Glutamic acid
   Significantly higher in group D compared to CTR+ .
5. GABA
   CTR+ had significantly higher levels of GABA than group D.

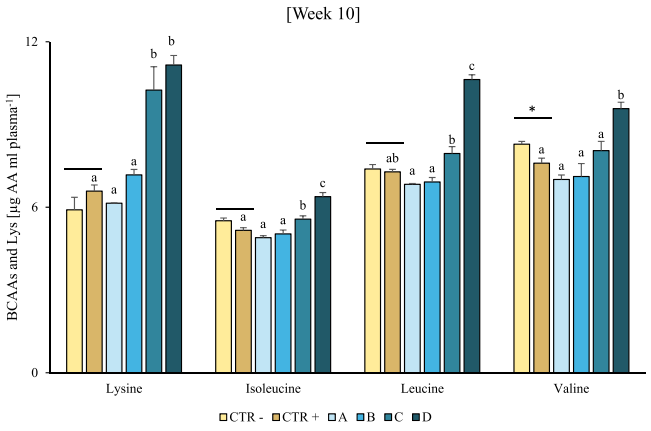

Fig. 7. Plasma free BCAAs and Lys (µg AA ml plasma^{-1}) in week 10. Expressed as average ± S.E. Letters indicate significant differences found between diets, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

6. CTR- vs CTR+

CTR- had significantly higher levels of Val and Phe than control group exposed to stress CTR+ .

3.4. Growth and health indicators

Comparison of SGR and weight measurements between the control groups on week 6 and week 10 (end of the experiment) revealed significant differences. On week 6, there were significant differences in SGR and mean weight between the different dietary treatments, with fish fed diet A having significantly lower SGR (1.8 ± 0.1 % day^{-1}) and mean weight (101.8 ± 6.7 g) than fish fed diet B (2.2 ± 0.0 % day^{-1} and 126.8 ± 6.3 g). By week 10, stressed groups did not show significant growth differences between them (Fig. 8). SGR and mean weight were significantly higher in group CTR- (2.1 ± 0.0 % day^{-1}; 210.2 ± 13.0 g) compared to the stressed control group CTR+ (1.9 ± 0.1 % day^{-1}; 173.6 ± 6.4 g)

On the transfer day, the initial health screening revealed a cataract incidence of 40 %. By the first sampling, on week 2, the cataract incidence was above 89 % for all groups, and by week 10 was 100 % in control groups and group A, and 78 ± 11 % in group fed diet D (Fig. 9). Regarding cataract size scores, by the end of the experiment (week 10), groups CTR-, C and D had an incidence of 4-scored cataracts of 33 %, while the incidence for group CTR+ was 67 % (Table 4)

On week 10, the incidence of fin erosion in control group CTR+ was 44 %, and 6 % in group fed diets C and D (Fig. 10).

4. Discussion

The posing threat of chronic stress on the welfare of animals is not new to the research community. The performance – and thus, economic - downside of maintaining animals under poor welfare is also well established. Extending the category of sentiency to species other than our own carries important discussions and challenges to historically – and presently – accepted practices (Birch, 2017; Brown, 2015; Fraser et al., 1997; Haynes, 2011). Lumpfish's role as a cleaner fish has come under scrutiny due to high mortalities and disease outbreaks (Stien et al., 2020). Acknowledging teleosts' awareness of pain brings more urgency to the welfare situation that farmed lumpfish face.

Health is an integral part of the overall welfare of an animal, as compromised health translates to compromised welfare (Segner et al., 2012; Southgate, 2010). Besides health, the stress status of a species is

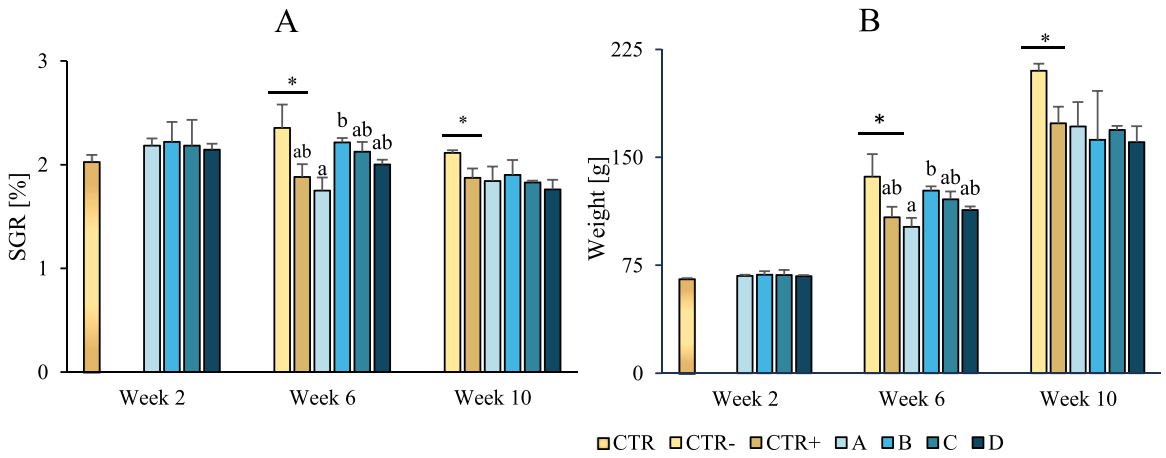

Fig. 8. Specific growth [A] and mean weight [B] in weeks 2, 6 and 10. Expressed as average ± S.E. Letters indicate significant differences found between dietary treatments, using a one-way ANOVA ($p < 0.05$) excluding CTR- from the comparison. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

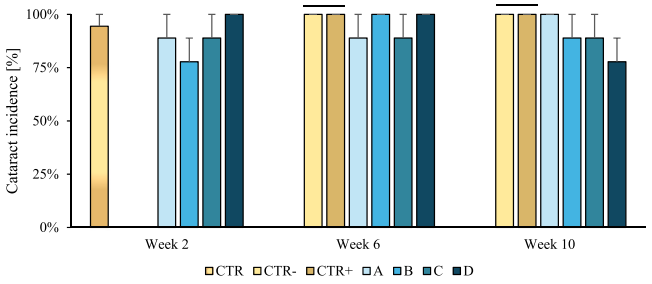

Fig. 9. Incidence of cataracts (% of fish with detected cataracts within each group) on all groups on weeks 2, 6 and 10, expressed as % ± S.E. A one-way ANOVA ($p < 0.05$) revealed no significant differences between groups. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

telling of its welfare status. In the context of lumpfish aquaculture, where stressors are prevalent, providing tailored feeds that bolster immunity and help maintain homeostasis under stress, could mitigate the burden of poor welfare to which they are subject to. In the case of cleaner fish, a tailored diet that confers health benefits, could potentially translate to increased grazing performance, as appetite and foraging behaviour are affected by the health status. In our previous work, we showed how stressed lumpfish alter their energy metabolism, fuelling specific amino acids into the stress coping mechanisms (da Santa Lopes et al., 2024). So, what could be the influence on lumpfish's health, when a tailored AA supplementation is provided? That was what we explored in this study, and our findings are discussed below.

4.1. Stress

Stressors can take many shapes, and not always are visible or even perceived. Furthermore, stress does not need to evoke a significant increase in stress hormones – such as cortisol and catecholamines - to impact metabolic and other physiological processes (Balasch and Tort, 2019; Petitjean et al., 2019; Wendelaar Bonga, 1997).

To navigate the potential impact of stress on the lumpfish, regardless of diet, this study had a control group (fed a commercial diet) left undisturbed (CTR-) and a group also fed a commercial diet but stressed four times per week (CTR+). We saw no statistically significant differences in cortisol – the main stress hormone - between disturbed and undisturbed lumpfish fed the commercial diet, at any given timepoint. Although this is not expected, it is also not surprising. There are several factors that can lead to a lack of expressive cortisol levels, such as habituation, time of sampling and even exhaustion (Aschbacher et al., 2013; Staven et al., 2019). Another telling aspect of these results is that the differences found between dietary treatments regarding the triglyceride levels, are attributed to the adjustment of AAs in the diets. As seen by the triglyceride levels of group A on week 6. Group A had the lowest triglyceride levels, compared to fish fed diet C and D – richer in Lys and BCAAs. This result suggests that the lack of sufficient Lys and BCAAs, could be pushing the fish fed diet A towards a metabolic compensation mechanism, with increased reliance on other energy reserves such as lipids. Mobilization of triglycerides and compensatory metabolic shifts to meet up energy demands under stressful situations have been documented in fish (Costas et al., 2011; Gracey et al., 2011). Quite possibly, fish in group A were scrambling to find resources to meet up energy demands.

4.2. Amino acids and Immunonutrition

Using or knowledge on nutrition – and nutrients- to safeguard and boost health in fish is not a recent endeavour. In fact, many nutrients and additives have been explored for their role in health promotion (Kiron, 2012; Waagbø and Remø, 2020). Of these functional nutrients, amino acids are being increasingly recognized as important modulators of fish immunity and stress response. For instance, in a study with 1.03% dietary inclusion of threonine fed to olive flounder (*Paralichthys olivaceus*) promoted optimal growth and immunity – with increased lysozyme and antiprotease activity (Hasanthi et al., 2023). Additionally, dietary inclusion of methionine and tryptophan affected the stress response and immune functions in European seabass (*Dicentrarchus labrax*) (Azeredo et al., 2017). Tryptophan has also been shown to attenuate stress HPI-axis activation in European seabass during inflammation (Peixoto et al., 2024). Nevertheless, the full scope of AAs and their role in immunity and stress is yet to be fully explored. BCAAs and Lys have traditionally been considered for their protein-building function, less so for their functionality in health and immunity. Our previous work in chronically stressed lumpfish revealed an increase in the use of BCAAs and Lys, confirmed by significantly lowered levels of these AAs circulating in plasma (da Santa Lopes et al., 2024). This allowed us to target the potential immunonutrition role of these AAs in the present study.

The dietary treatments in this study consisted of varying inclusion levels of BCAAs and Lys. These AAs have been demonstrated to act as a buffer for stress inflammatory effects. Indeed, in an *in vitro* study, Lee et al. (2017) showed the anti-inflammatory effects of BCAAs, which reduced proinflammatory cytokines in macrophages and protected from H_2O_2 damage (Lee et al., 2017). In our experiment, the different

Table 4
Incidence of the different health and cataract size scores for each group on weeks 2. 6 and 10. Scores according to LHSS. Expressed as % mean ± S.E.

	Incidence of each health score					
Week 2	CTR		A	B	C	D
<1	0.0% ± 0.0		0.0% ± 0.0	22.2% ± 22.2	0.0% ± 0.0	22.2% ± 22.2
1<2	55.6% ± 14.1		78.0% ± 11.1	22.2% ± 11.1	56.0% ± 22.2	44.0% ± 11.1
2<3	44.4% ± 14.1		22.0% ± 11.1	56.0% ± 11.1	44.0% ± 22.2	22.2% ± 11.1
>3	0.0% ± 0.0		0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0	11.0% ± 11.1
Week 6	CTR-	CTR+				
<1	11.1% ± 11.1	33.3% ± 22.2	22.2% ± 11.1	11.1% ± 11.1	33.3% ± 19.2	44.4% ± 22.2
1<2	55.6% ± 11.1	44.4% ± 11.1	55.6% ± 11.1	55.6% ± 11.1	33.3% ± 19.2	22.2% ± 11.1
2<3	11.1% ± 11.1	22.2% ± 11.1	22.2% ± 11.1	33.3% ± 0.0	22.2% ± 22.2	11.1% ± 11.1
>3	22.2% ± 11.1	0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0	11.1% ± 11.1	22.2% ± 11.1
Week 10						
<1	11.1% ± 11.1	33.3% ± 20.0	22.2% ± 11.1	0.0% ± 0.0	11.1% ± 11.1	33.3% ± 19.2
1<2	66.7% ± 19.2	22.2% ± 11.1	44.4% ± 29.4	66.7% ± 19.2	77.8% ± 22.2	55.6% ± 29.4
2<3	22.2% ± 22.2	33.3% ± 22.2	33.3% ± 19.2	22.2% ± 22.2	0.0% ± 0.0	11.1% ± 11.1
>3	0.0% ± 0.0	11.1% ± 11.1	0.0% ± 0.0	0.0% ± 0.0	11.1% ± 11.1	0.0% ± 0.0
	Cataract incidence for each size score					
Week 2	CTR		A	B	C	D
0	5.56% ± 5.56		11.1% ± 11.1	11.1% ± 11.1	0.0% ± 0.0	0.0% ± 0.0
1	27.8% ± 18.1		11.1% ± 11.1	22.2% ± 11.1	0.0% ± 0.0	33.3% ± 33.3
2	5.6% ± 5.6		33.3% ± 19.2	22.2% ± 11.1	33.3% ± 33.3	0.0% ± 0.0
3	61.1% ± 15.9		44.4% ± 29.4	44.4% ± 29.4	66.7% ± 33.3	66.7% ± 33.3
4	0.0% ± 0.0		0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0
Week 6	CTR-	CTR+				
0	0.0% ± 0.0	11.1% ± 11.1	33.3% ± 33.3	11.1% ± 11.1	22.2% ± 22.2	44.4% ± 29.4
1	11.1% ± 11.1	22.2% ± 22.2	11.1% ± 11.1	0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0
2	11.1% ± 11.1	33.3% ± 19.2	11.1% ± 11.1	0.0% ± 0.0	11.1% ± 11.1	11.1% ± 11.1
3	11.1% ± 11.1	22.2% ± 11.1	22.2% ± 22.2	0.0% ± 0.0	22.2% ± 22.2	0.0% ± 0.0
4	66.7% ± 19.2	16.7% ± 11.1	22.2% ± 22.2	88.9% ± 11.1	44.4% ± 29.4	44.4% ± 29.4
Week 10						
0	0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0	11.1% ± 11.1	22.2% ± 11.1
1	11.1% ± 11.1	22.2% ± 11.1	22.2% ± 11.1	0.0% ± 0.0	0.0% ± 0.0	0.0% ± 0.0
2	11.1% ± 11.1	11.1% ± 11.1	0.0% ± 0.0	11.1% ± 11.1	0.0% ± 0.0	22.2% ± 22.2
3	44.4% ± 11.1	0.0% ± 0.0	22.2% ± 11.1	22.2% ± 11.1	55.6% ± 29.4	22.2% ± 11.1
4	33.3% ± 19.2	66.7% ± 19.2	55.6% ± 11.1	55.6% ± 11.1	33.3% ± 19.2	33.3% ± 19.2

inclusions of BCAAs and Lys produced changes in the plasma AAs profiles of lumpfish under chronic stress. Along the several weeks of experiment, three key patterns emerged: the varying levels of BCAAs and Lys; the shift in glucogenic AAs; and the fluctuations in stress-related AAs.

Across the study, the plasma levels of BCAAs and Lys mirrored the dietary inclusion, with groups C and D having consistently higher levels compared to the other stressed groups. While unsurprising, this dose-response relationship helps to validate that BCAAs and Lys supplementation effectively increases the availability of these AAs in the plasma of stressed lumpfish.

In fish, the energetic cost of stress is reflected in increased glycogenolysis and gluconeogenesis, regulated by stress hormones. On a first stage, the rapid release of catecholamines drive hepatic glycogen breakdown to achieve “fast” energy. Subsequently, cortisol shifts the energy expenditure to a more sustained glucose release, mobilizing amino acids, prompting protein breakdown and altering fatty acid metabolism. Indeed, cortisol influence on amino acid requirements has been described previously in fish (Aragão et al., 2008). During the entire trial, group A had consistently the highest levels of Asp. Aspartate is not

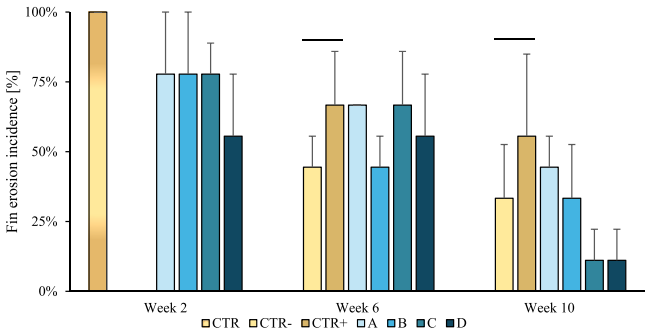

Fig. 10. Incidence of fin erosion (% of fish with detected fin erosion within each group) on weeks 2, 6 and10, expressed in % ± S.E. A one-way ANOVA ($p < 0.05$) revealed no significant differences between groups. A one-way ANOVA was also employed between CTR+ and CTR-, pointed by the line above both bars. The presence of a symbol "*" above the line in both bars, indicates significant differences between the control groups ($p < 0.05$).

an essential AA but is however, a major glucogenic precursor, with the majority of circulating Asp being sourced in the skeletal muscle. Additionally, group A also held higher levels of Gly and Ser, which are two well described glucogenic AAs. Gly can be converted into Ser, via serine hydroxymethyltransferase, which can then be metabolized into pyruvate, entering the gluconeogenesis route. While our study does not provide direct evidence of this, the elevated levels of Gly and Ser could be suggestive of altered gluconeogenic metabolism. Indeed, Gly and Ser have been demonstrated to be important glucogenic substrates in fish during stress, helping maintain glucose levels (Suehs et al., 2024; Walton and Cowey, 1979).

It is possible that group A started to reflect the state of strain imposed by chronic stress. The depleted levels of triglycerides show a rearrangement on the preferred energy substrates and might have pushed the fish towards other sources of energy. Indeed, the increased glucose levels in group A reinforce the explanation of increased glucogenic activity.

In our previous work, we demonstrated that chronic exposure to stress lowered the levels of free BCAAs and Lys in lumpfish plasma (da Santa Lopes et al., 2024). Similarly, the direct impact of chronic stress on the AA profile in plasma was visible during this experiment. Val levels decreased, potentially due to higher utilisation of Val by the CTR+ group, as chronic stress exerts a broad pressure on the metabolic resources. Phe and Tyr are considered precursors for catecholamine production, and Phe can be converted to Tyr (Salamanca et al., 2020). When comparing the control groups, stress vs undisturbed, we saw lower Phe in the stressed group. Hence, CTR+ fish were likely using their Phe reserves to produce these stress hormones. As fish are exposed to stress, there is a need to ramp up catecholamine production. Higher availability of Tyr can signal an attempt to support this increase in catecholamine production. It is plausible that the simultaneous lower levels of Phe and high levels of Tyr in group A could reflect an increased demand for catecholamine production, where Phe was redirected to Tyr production. In contrast, group D had consistently the lower levels of Tyr. The low Orn levels in group A are also revealing, as this AA is involved in nitrogen metabolism, polyamine production and can indirectly contribute to energy production. The deficit in BCAAs and Lys in group A could be affecting Orn metabolism, redirecting it to the production of polyamines – important molecules involved in tissue integrity, cell proliferation and stress adaptation. It could also be diverting Orn for glucogenic purposes.

Group A saw an increase in circulating levels of Cys. Being fundamental for glutathione synthesis, a disruption of this pathway could lead to the underutilization of Cys (Yu and Long, 2016). In addition, stress exposure elevates the need for antioxidant agents such as glutathione to counter oxidative damage, increasing the flow of Cys into circulation (Espinosa-Diez et al., 2015; Fu et al., 2019; Lushchak and Bagnyukova, 2007).

During the stress phase group D had the lowest levels of GABA, being higher on CTR+ . GABA is a primary inhibitory neurotransmitter, and when fish are under stress, increased levels can reflect an attempt to counteract stress signals and maintain balance. In essence, a metabolic coping mechanism (Ruenkoed et al., 2023; Wang et al., 2023; Bae et al., 2024a). In light of this, it is possible that the fish fed diets C and D did not need to upregulate GABA, as they were more effective in maintaining metabolic homeostasis. The surplus of BCAAs and Lys on groups C and D could support alternative stress-coping mechanisms such as immune functions, energy production and protein synthesis, and be acting as a stress buffer. Meanwhile, fish fed diet A could not allocate resources towards GABA production as efficiently because, metabolically, their resources were stretched in other directions. Interestingly, glutamic acid is necessary to produce GABA, and the higher glutamic acid levels on group D reinforce the idea that this group was able to manoeuvre the chronic stress exposure without needing to resort to GABA coping mechanisms. In other words, less glutamic acid was diverted to GABA production, allowing it to remain high in circulation, which indicates a more stable response to stress. GABA signalling is a recognized modulator if fish stress responses, and dietary inclusion of GABA has been shown to attenuate stress response in teleosts. It is conceivable that the lower GABA levels modulated by the BCAAs/Lys enriched diets could imply a reduced neuroendocrine strain and increased tolerance to stress. However, this requires further investigation to fully comprehend the mechanisms at play, and the scope of GABA's stress mitigation potential in chronically stressed lumpfish. To this end, a potential immunonutrition study including GABA supplementation could be useful. Indeed, GABA dietary inclusion was shown to mitigate stress adverse effects in olive flounder exposed to crowding stress, with increased immunity and survival (Bae et al., 2024b). Dietary GABA has also been documented to improve stress tolerance in tawny puffer fish (*Takifugu flavidus*) during transport (Yu et al., 2024).

4.3. Immunity and health

While prolonged stress can impact the teleost's immune response, the effects of short-term exposure to stress are not so straightforward. In fact, stress can exert immunoenhancing properties (Yada and Tort, 2016). In our previous study, lumpfish exposed to air had significantly higher levels of NO (da Santa Lopes et al., 2024). Accordingly, in the present study, we found that the control group exposed to stress (CTR+) had significantly higher NO levels than their undisturbed counterparts (CTR-). NO is an intriguing and versatile molecule, with several biological functions. It acts as a neurotransmitter, modulating and signalling neuronal processes, and is a potent inflammatory mediator, increasing vasodilation and leukocyte migration (Cioni et al., 2019; Peter et al., 2022). It also carries immune functions as in when released by neutrophils and macrophages during respiratory burst, effectively killing bacteria. Interestingly, this pro-inflammatory molecule was also increased on group A during week 6. Another very relevant immune mechanism are the antiproteases. Antiproteases not only help counter pathogens' proteolytic weaponry but also assist in containing host's own proteases. This important safety net allows the organism's own proteases to remove damaged tissue without causing collateral damage on healthy cells (Ellis, 1990b; Doumas et al., 2005; Nugteren and Samsom, 2021). Unsurprisingly, this mechanism was found significantly higher in group D than in CTR+ and A, likely because there was a good nutritional support, offered by the increased dietary levels of BCAAs and Lys. It is possible that BCAAs/Lys-enriched diets modulated immune cell function and populations, which can affect the antiprotease release by immune cells. Val supplementation has been shown to improve non-specific immune mechanisms in golden pompano and rainbow trout (Ahmad, Ahmed, and Dar, 2021; Z. Huang et al., 2018). The lack of expressive differences in more immune parameters might be explained by the absence of a pathogenic infection, which would likely exacerbate hidden

variations on immune competence. There was, however, an increase in peroxidase activity in group A. While peroxidase is an important immune mechanism, it can also signal an ongoing mild inflammatory status (e.g. when an organism is containing damage while trying to repair tissue on a small lesion such as fin erosion). The influence of dietary AA alterations on infection was not, however, the aim of our study. Regarding specific health measurements on week 10, while fin erosion and cataract incidences lacked statistical significance, it is worthy to point out that only 11 % of fish in groups C and D had signs of fin erosion, as opposed to 44 % in group A and 56 % in group CTR+ . It is well accepted that better nutritional support, assists in tissue maintenance and repair. Lys is a component of collagen, integral for tissue structure, and similarly, BCAAs are crucial for the maintenance of healthy tissues (Ramakrishnan and Sulochana, 1993; Yamauchi and Sricholpech, 2012; Ahmad et al., 2021; Huang et al., 2021). Hence, the inadequate supply of Lys and other structurally important AAs can contribute to the erosion of tissues in fish (Lall, 2010). A chronic stress study with a more severe stressor could reveal other insights in the potential benefit of increased nutritional support. In this study, the goal was not to subject the fish to a severe stressor, which could have otherwise, profoundly affected these welfare indicators.

4.4. Growth

By the end of the experiment, lumpfish fed the different diets did not differ in mean weight. Having in mind that lumpfish is produced for sea lice grazing, fast growth is not targeted in the production of lumpfish (Imsland et al., 2021). In fact, it is more favourable that the fish use the diet to grow better than faster. The stress did however affect the growth, as seen by the significantly lower average weight found in group CTR+ compared to CTR-. This is a well-documented outcome of chronic stress, which diverts energy from growth to other physiological processes such as the struggle to adapt and regain homeostasis (Beyers and Rice, 2002; Costas et al., 2011). These results show that in contrast to the control diet, BCAAs/Lys-enriched diets can support resilience under chronic air exposure, while not affecting growth. For practical aquaculture implications, this information means that BCAAs/Lys supplementation can maintain growth while offering enhanced stress resilience. It also expands knowledge on the impact of chronic air exposure on growth, information that can be used by farmers for growth projection.

4.5. Synopsis

This study was organized in two stages, the undisturbed phase, and the stress exposure phase. During the undisturbed phase, all groups seem somewhat even, there were no signs of strain. No challenges rose, and thus, metabolites were stable, and the immune markers unaltered. The fish, having no major increase in energy demands, remain with their AA stockpile relatively stable.

As the study progresses into the stress phase, the distinctions crystallize. The sudden shift from undisturbedness to stress caused visible metabolic alterations. The fish fed a diet with lower levels of Lys and BCAAs (group A) struggle to keep energy demand, tapping into lipid reserves – as seen by the drop in triglyceride levels and favouring glucogenic activity. In addition, the immune system kicks in the high gear. This is visible by the increased NO and peroxidase activity. The increased NO can also indicate an inflammatory state, which together with the elevated glucose, can indicate that the struggle for maintaining homeostasis is real. NO was also elevated in CTR+ against their undisturbed counterpart (CTR-), showing that the stress exposure was subtle, yet inflammatory. In contrast, the fish fed higher levels of Lys and BCAAs cruised the stress exposure with slightly more ease, explained in part for the anti-inflammatory effects of BCAAs and the stabilization of amino acid reserves, serving as buffer (Lee et al., 2017). Triglyceride levels remained higher, showing that the energy flow was not significantly impacted, and glucose levels remained unaltered. Group A, depleting their lipid reserves, scrambled to find spare parts, resulting in an increase in certain glucogenic AAs in circulation, supporting the significant glucose increase. The inadequate supply of specific AAs in diet A might have increased the challenge on keeping the integrity of certain structures, such as the eye lens. By week 10, fish seem to be able to adapt to some extent, and lipid reserves stabilise. In essence, the nutritional support provided by the increased availability of Lys and BCAAs helped to mitigate the strain caused by chronic stress on metabolism and immunity. In contrast, the sub-optimal inclusion of dietary BCAAs and Lys in stressed fish leads to increased glucogenic activity - sourced by glucogenic AAs – and an increase in the reliance on other stress coping mechanisms such as GABA. The immune support provided by diets that address nutritional requirements under stress can be a deciding factor in the health outcome of lumpfish under commercial settings. While these results are promising, the full extent of the impact of key amino acid dietary support on lumpfish health and survival in sea pens should be assessed.

4.6. Study opportunities and limitations

4.6.1. FAAs profiling

The plasma free amino acid profiling provided mechanistic insights on the influence of chronic air exposure's nutritional and metabolic strain – and how dietary manipulations affect it. However, at the moment, FAA profiling is not practical for wide adoption as a routine biomarker by the industry. In our study, we used this tool as concept, to inform and guide on dietary manipulations. The opportunity arises to establish non-invasive nutritional biomarkers correlated to plasma free amino acid profiles.

4.6.2. Diets cost-effectiveness

A first step towards improving diets in aquaculture is proof of concept. It is important to understand how a conceptualized feed influences the physiological status of the animal. However, it is likewise relevant to know the feasibility of such diet. An excellent diet, if not commercially feasible, will remain precisely that, a concept. The opposite is also true, as an inexpensive diet that undermines health has no commercial value. Striking a balance between commercial feasibility and nutritional value is essential. Our study served as a first step proof of concept, obtaining the different dietary amino acid profiles through adjustments in ingredient proportions of the diets rather than adding crystalline amino acids. This approach is cost-conscious and aligned with realistic feed formulation practices, although further work is required to determine costs and scalability.

4.6.3. Nutrient interactions

Altering the amino acid profile of a diet can introduce unexpected outcomes, such as interactions with other nutrients (e.g. vitamins, fatty acids; minerals). This is a limitation of the present study, since amino acid metabolism depends on other nutrient cofactors. Our approach of manipulating AA profile via ingredient proportion partly mitigates this uncertainty, as amino acids remained protein-bound, following a more natural digestive and absorption course. In contrast, crystalline amino acids are rapidly absorbed in the gut, often faster than other nutrients, leading to asynchrony between these amino acid's appearance in plasma and the availability of cofactors required for their metabolism. For example, vitamin B6 is an essential co-factor in amino acid metabolism, while Lys interacts with vitamin C for collagen synthesis(Yamauchi and Sricholpech, 2012). Other vitamins such as C and E have antioxidant properties, which can help spare amino acids from catabolism. Lysine is also directly tied to fatty acid metabolism, being a precursor to carnitine, while BCAAs can also modulate lipid metabolism (Li et al., 2017; Newgard, 2012). Exploring the interplay between other nutrients and amino acids on stressed lumpfish would be a natural step forward in lumpfish aquafeed research.

4.6.4. Behavioural data

Behaviour is a key non-invasive indicator of fish welfare that can reveal subclinical stress. Behaviour was observed daily and abnormalities (e.g. erratic swimming; loss of equilibrium; lack of appetite) were registered but none were detected. The absence of a detailed ethogram is a limiting aspect of our study, as it would strengthen the stress characterization. Future work should integrate behavioural data, especially in realistic sea-pens farming condition, where stressors are more diverse and unpredictable.

4.6.5. Ethical considerations

While this study did not dive into the sea of ethical and philosophical debate, scientific responsibility compels us to acknowledge the ethical dimension of lumpfish's concerning welfare. Lumpfish are not merely economic assets; they are sentient animals with complex biological needs. The precautionary principle prompts us, in the case of uncertainty, to take the cautionary side: when evidence suggests potential for suffering, and compromised welfare – even in the absence of full scientific certainty – measures must be taken to halt harm (World Commission on the Ethics of Scientific Knowledge and Technology, 2005). This principle of precaution is enshrined in international legislation (Commission of the European Communities, 2000). From this perspective, the aggravated welfare conditions are not acceptable and thus, the use of lumpfish as is, should be reconsidered. Lumpfish are part of the salmon aquaculture industry, which provides animal products for human consumption. Continuous assessment of humane alternatives is vital to ensure that food production remains sustainable, nutritious and ethically responsible.

5. Conclusion

This study demonstrates that chronic air exposure effects can be elusive when assessing just traditional stress biomarkers. Yet, under the surface, the effects of stress can be observed, namely in metabolic shifts, immunity and amino acid profiles. The decreased supply of dietary BCAAs and Lys in Group A pushed the fish towards increased glucogenic activity – as seen by the increase in glucogenic AAs and confirmed by the higher glucose levels. The dietary effects were also made visible with the decreased triglycerides, indicating a shift in the preference for energy sources. In contrast, the nutritional support provided by Lys and BCAAs helped safeguard metabolic homeostasis and lower demand for stress coping mechanisms such as GABA. High inclusion of Lys and BCAAs in diets also seem to have promoted specific immune mechanisms such as antiproteases. Future work should address the efficacy of targeted immunonutrition under commercial scale, since better nutritional support can help lumpfish be more stress resilient and healthier, an important requisite for an improved delousing performance.

As a final remark, this is the first study to demonstrate that adjusting dietary branched chain amino acids and lysine can influence metabolic and immune activity on lumpfish, which can be a useful strategy in aquaculture settings. However, improving conditions with tools such as immunonutrition is but one step, which alone does not solve all welfare issues.

CRediT authorship contribution statement

Christopher Pimentel: Writing – review & editing, Methodology, Formal analysis. **Francisco Pinto-Cunha:** Writing – review & editing, Methodology, Formal analysis. **Patrick Reynolds:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Imsland Albert K. D.:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Formal analysis. **Cláudia Aragão:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Formal analysis. **Fernandes Jorge:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Tiago da Santa Lopes:** Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Benjamin Costas:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Lourenço Ramos-Pinto:** Writing – review & editing, Methodology, Investigation, Formal analysis.

Ethics statement

This study was approved by the Norwegian Animal Research Authority (FOTS nr. 30399). All animal handling procedures were performed in accordance with national and European guidelines, including the EU Directive 2010/63 on the use of animals for scientific purposes.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tiago da Santa Lopes reports financial support was provided by Gildeskål Forskningsstasjon AS. Patrick Reynolds reports financial support was provided by Gildeskål Forskningsstasjon AS. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Afonso, L.O.B., 2020. Identifying and managing maladaptive physiological responses to aquaculture stressors. Fish. Physiol. 38, 163–191. https://doi.org/10.1016/BS.FP.2020.10.002.

Ahmad, I., Ahmed, I., Dar, N.A., 2021. Dietary valine improved growth, immunity, enzymatic activities and expression of TOR signaling cascade genes in rainbow trout, *Oncorhynchus mykiss* fingerlings. Sci. Rep. 11 (1), 1–17. https://doi.org/10.1038/S41598-021-01142-4;SUBJMETA=250,2722,443,45,601,631;KWRD=BIOCHEMISTRY,ICHTHYOLOGY,IMMUNOLOGY,PHYSIOLOGY,ZOOLOGY.

Ahmad, I., Ahmed, I., Fatma, S., Peres, H., 2021. Role of branched-chain amino acids on growth, physiology and metabolism of different fish species: a review. Aquac. Nutr. 27 (5), 1270–1289. https://doi.org/10.1111/ANU.13267.

Andersen, S.M., Waagbø, R., Espe, M., 2016. Functional amino acids in fish health and welfare. Front. Biosci. Elite 8 (1), 143–169. https://doi.org/10.2741/757/PDF.

Aragão, C., Corte-Real, J., Costas, B., Dinis, M.T., Conceição, L.E.C., 2008. Stress response and changes in amino acid requirements in Senegalese sole (*Solea senegalensis* Kaup 1858). Amino Acids 34 (1), 143–148. https://doi.org/10.1007/s00726-007-0495-2.

Aschbacher, K., O'donovan, A., Wolkowitz, O.M., Dhabhar, F.S., Su, Y., Epel, E., 2013. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. Psychoneuroendocrinology 38 (9), 1698–1708. https://doi.org/10.1016/j.psyneuen.2013.02.004.

Azeredo, R., Machado, M., Afonso, A., Fierro-Castro, C., Reyes-López, F.E., Tort, L., Gesto, M., Conde-Sieira, M., Míguez, J.M., Soengas, J.L., Kreuz, E., Wuertz, S., Peres, H., Oliva-Teles, A., Costas, B., 2017. Neuroendocrine and immune responses undertake different fates following tryptophan or methionine dietary treatment: tales from a teleost model. Front. Immunol. 8 (SEP), 1226. https://doi.org/10.3389/FIMMU.2017.01226/BIBTEX.

Bae, J., Moniruzzaman, M., Je, H.W., Lee, S., Choi, W., Min, T., Kim, K.W., Bai, S.C., 2024a. Evaluation of gamma-aminobutyric acid (GABA) as a functional feed ingredient on growth performance, immune enhancement, and disease resistance in olive flounder (*Paralichthys olivaceus*) under high stocking density. Antioxidants 13 (6), 647. https://doi.org/10.3390/ANTIOX13060647.

Bae, J., Moniruzzaman, M., Je, H.W., Lee, S., Choi, W., Min, T., Kim, K.W., Bai, S.C., 2024b. Evaluation of gamma-aminobutyric acid (GABA) as a functional feed ingredient on growth performance, immune enhancement, and disease resistance in olive flounder (*Paralichthys olivaceus*) under high stocking density. Antioxidants 13 (6), 647. https://doi.org/10.3390/ANTIOX13060647.

Balasch, J.C., Tort, L., 2019. Netting the stress responses in fish. Front. Endocrinol. 0 (FEB), 62. https://doi.org/10.3389/FENDO.2019.00062.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42 (3), 517–525. https://doi.org/10.1093/icb/42.3.517.

Beyers, D.W., Rice, J.A., 2002. Evaluating stress in fish using bioenergetics-based stressor-response models. Biol. Indic. Aquat. Ecosyst. Stress 289–320.

Birch, J., 2017. Animal sentience and the precautionary principle. Anim. Sentience 16 (1). https://doi.org/10.51291/2377-7478.1200.

Bjerkås, E., Breck, O., Waagbø, R., 2006. The role of nutrition in cataract formation in farmed fish. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 1 (033). https://doi.org/10.1079/PAVSNNR20061033, 16–16.

Broom, D.M., Johnson, K.G., 2019. One Welfare, One Health, One Stress: Humans and Other Animals 1–13. https://doi.org/10.1007/978-3-030-32153-6_1.

Brown, C., 2015. Fish intelligence, sentience and ethics. Anim. Cogn. 18 (1), 1–17. https://doi.org/10.1007/S10071-014-0761-0/METRICS.

Bshary, R., Brown, C., 2014. Fish cognition. Curr. Biol. 24 (19), R947–R950. https://doi.org/10.1016/j.cub.2014.08.043.

Bshary, R., Wickler, W., Fricke, H., 2002. Fish cognition: a primate's eye view. Anim. Cogn. 5 (1), 1–13. https://doi.org/10.1007/S10071-001-0116-5.

Castillo, S., Gatlin, D.M., 2018. Dietary requirements for leucine, isoleucine and valine (branched-chain amino acids) by juvenile red drum Sciaenops ocellatus. Aquac. Nutr. 24 (3), 1056–1065. https://doi.org/10.1111/ANU.12644.

Ciji, A., Akhtar, M.S., 2021. Stress management in aquaculture: a review of dietary interventions. Rev. Aquac. 13 (4), 2190–2247. https://doi.org/10.1111/RAQ.12565.

Cioni, C., Angiulli, E., Toni, M., 2019. Nitric oxide and the neuroendocrine control of the osmotic stress response in teleosts. Int. J. Mol. Sci. 20 (3), 489. https://doi.org/10.3390/IJMS20030489.

Commission of the European Communities. (2000). *Communication from the Comission on the precautionary principle*.

Costas, B., Conceição, L.E.C., Aragão, C., Martos, J.A., Ruiz-Jarabo, I., Mancera, J.M., Afonso, A., 2011. Physiological responses of Senegalese sole (*Solea senegalensis* Kaup, 1858) after stress challenge: effects on non-specific immune parameters, plasma free amino acids and energy metabolism. Aquaculture 316 (1–4), 68–76. https://doi.org/10.1016/j.aquaculture.2011.03.011.

Davenport, 1985. Synopsis of biological data on the lumpsucker *Cyclopterus lumpus* (Linnaeus, 1758) FAO Food and Agriculture Organization of the United Nations. FAO Fish. Synop. 147 (31), 1–27.

Dhabhar, F.S., 2008. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection versus immunopathology. Allergy Asthma Clin. Immunol. 4 (1), 2. https://doi.org/10.1186/1710-1492-4-1-2.

Doumas, S., Kolokotronis, A., Stefanopoulos, P., 2005. Anti-inflammatory and antimicrobial roles of secretory leukocyte protease inhibitor. Infect. Immun. 73 (3), 1271–1274. https://doi.org/10.1128/IAI.73.3.1271-1274.2005.

Ellis, A.E., 1990b. Serum antiproteases in fish. Tech. Fish. Immunol. 95–99. ⟨https://cir.nii.ac.jp/crid/1573950399902395392.bib?lang=en⟩.

Espinosa-Diez, C., Miguel, V., Mennerich, D., Kietzmann, T., Sánchez-Pérez, P., Cadenas, S., Lamas, S., 2015. Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol. 6, 183–197. https://doi.org/10.1016/J.REDOX.2015.07.008.

Fiskeridirektoratet, 2023. Aquaculture statistics: Use of cleanerfish 1998-2023. Norwegian Directorate of Fisheries. ⟨https://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse⟩.

Fraser, D., Weary, D.M., Pajor, E.A., Milligan, B.N., 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. Welf. 6 (3), 187–205. https://doi.org/10.1017/S0962728600019795.

Fu, X., Cate, S.A., Dominguez, M., Osborn, W., Özpolat, T., Konkle, B.A., Chen, J., López, J.A., 2019. Cysteine disulfides (Cys-ss-X) as sensitive plasma biomarkers of oxidative stress. Sci. Rep. 9 (1), 1–9. https://doi.org/10.1038/s41598-018-35566-2.

Garcia de Leaniz, C., Gutierrez Rabadan, C., Barrento, S.I., Stringwell, R., Howes, P.N., Whittaker, B.A., Minett, J.F., Smith, R.G., Pooley, C.L., Overland, B.J., Biddiscombe, L., Lloyd, R., Consuegra, S., Maddocks, J.K., Deacon, P.T.J., Jennings, B.T., Rey Planellas, S., Deakin, A., Moore, A.I., Pavlidis, M., 2022. Addressing the welfare needs of farmed lumpfish: Knowledge gaps, challenges and solutions. Rev. Aquac. 14 (1), 139–155. https://doi.org/10.1111/raq.12589. John Wiley and Sons Inc.

Gorissen, M., Flik, G., 2016. The endocrinology of the stress response in fish: an adaptation-physiological view. Fish. Physiol. 35, 75–111. https://doi.org/10.1016/B978-0-12-802728-8.00003-5.

Gracey, A.Y., Lee, T.H., Higashi, R.M., Fan, T., 2011. Hypoxia-induced mobilization of stored triglycerides in the euryoxic goby *Gillichthys mirabilis*. J. Exp. Biol. 214 (18), 3005–3012. https://doi.org/10.1242/JEB.059907.

Graham, S., Secombes, C.J., 1988. The production of a macrophage-activating factor from rainbow trout *Salmo gairdneri* leucocytes. Immunology 65 (2), 293. /pmc/articles/PMC1384927/?report=abstract.

Guo, H., Dixon, B., 2021. Understanding acute stress-mediated immunity in teleost fish. Fish. Shellfish Immunol. Rep. 2, 100010. https://doi.org/10.1016/J.FSIREP.2021.100010.

Hardy, R.W., 2012. The Nutritional Pathology of Teleosts. Fish Pathology. Wiley-Blackwell, pp. 402–424. https://doi.org/10.1002/9781118222942.ch10.

Hasanthi, M., Kim, M.G., Lim, H., Lim, J., Hur, S.W., Lee, S., Lee, B.J., Kim, K.W., Lee, K. J., 2023. The dietary requirement for threonine in juvenile olive flounder (*Paralichthys olivaceus*). Fish. Aquat. Sci. 26 (1), 58–68. https://doi.org/10.47853/FAS.2023.E5.

Haynes, R.P., 2011. Competing conceptions of animal welfare and their ethical implications for the treatment of non-human animals. Acta Biotheor. 59 (2), 105–120. https://doi.org/10.1007/S10441-011-9124-2/METRICS.

Herrera, M., Fernández-Alacid, L., Sanahuja, I., Ibarz, A., Salamanca, N., Morales, E., Giráldez, I., 2020. Physiological and metabolic effects of a tryptophan-enriched diet to face up chronic stress in meagre (*Argyrosomus regius*). Aquaculture 522, 735102. https://doi.org/10.1016/J.AQUACULTURE.2020.735102.

Hoseini, S.M., Reverter, M., 2022. Health-promoting effects of amino acids in fish. Biotechnol. Adv. Aquac. Health Manag. 493–533. https://doi.org/10.1007/978-981-16-5195-3_23/COVER.

Hu, Y., Feng, L., Jiang, W., Wu, P., Liu, Y., Kuang, S., Tang, L., Zhou, X., 2021. Lysine deficiency impaired growth performance and immune response and aggravated inflammatory response of the skin, spleen and head kidney in grown-up grass carp (*Ctenopharyngodon idella*). Anim. Nutr. 7 (2), 556. https://doi.org/10.1016/J.ANINU.2020.07.009.

Huang, D., Maulu, S., Ren, M., Liang, H., Ge, X., Ji, K., Yu, H., Pence, B.D., Qu, F., Hossein Hoseinifar, S., Ren renmc, M., Hualiang Liang, ffrccn, 2021. Dietary Lysine Levels Improved Antioxidant Capacity and Immunity via the TOR and p38 MAPK Signaling Pathways in Grass Carp, Ctenopharyngodon idellus Fry. Front. Immunol. 12, 635015. https://doi.org/10.3389/FIMMU.2021.635015.

Huang, Z., Tan, X.H., Zhou, C.P., Yang, Y.K., Qi, C.L., Zhao, S.Y., Lin, H.Z., 2018. Effect of dietary valine levels on the growth performance, feed utilization and immune function of juvenile golden pompano, *Trachinotus ovatus*. Aquac. Nutr. 24 (1), 74–82. https://doi.org/10.1111/ANU.12535.

Imsland, A.K.D., Reynolds, P., Hangstad, T.A., Kapari, L., Maduna, S.N., Hagen, S.B., Jónsdóttir, Ó.D.B., Spetland, F., Lindberg, K.S., 2021. Quantification of grazing efficacy, growth and health score of different lumpfish (*Cyclopterus lumpus* L.) families: possible size and gender effects. Aquaculture 530, 735925. https://doi.org/10.1016/j.aquaculture.2020.735925.

Jena, N.P., 2017. Animal welfare and animal rights: an examination of some ethical problems. J. Acad. Ethics 15 (4), 377–395. https://doi.org/10.1007/S10805-017-9282-1.

Kiron, V., 2012. Fish immune system and its nutritional modulation for preventive health care. Anim. Feed Sci. Technol. 173 (1–2), 111–133. https://doi.org/10.1016/j.anifeedsci.2011.12.015.

Lall, S.P., 2010. Disorders of nutrition and metabolism. In: Fish diseases and disorders, Volume 2. CABI, pp. 202–237. https://doi.org/10.1079/9781845935535.0202.

Lee, J.H., Park, E., Jin, H.J., Lee, Y., Choi, S.J., Lee, G.W., Chang, P.S., Paik, H.D., 2017. Anti-inflammatory and anti-genotoxic activity of branched chain amino acids (BCAA) in lipopolysaccharide (LPS) stimulated RAW 264.7 macrophages. Food Sci. Biotechnol. 26 (5), 1371. https://doi.org/10.1007/S10068-017-0165-4.

Li, J.M., Li, L.Y., Qin, X., Ning, L.J., Lu, D.L., Li, D.L., Zhang, M.L., Wang, X., Du, Z.Y., 2017. Systemic regulation of L-carnitine in nutritional metabolism in zebrafish, Danio rerio. Sci. Rep. 7 (1), 1–14. https://doi.org/10.1038/SREP40815;TECHMETA=116,64;SUBJMETA=1737,2723,319,443,601,631;KWRD=ANIMAL+PHYSIOLOGY,FAT+METABOLISM.

Li, P., Mai, K., Trushenski, J., Wu, G., 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37 (1), 43–53. https://doi.org/10.1007/s00726-008-0171-1.

Lushchak, V.I., Bagnyukova, T.V., 2007. Hypoxia induces oxidative stress in tissues of a goby, the rotan *Perccottus glenii*. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 148 (4), 390–397. https://doi.org/10.1016/J.CBPB.2007.07.007.

Machado, M., Azeredo, R., Díaz-Rosales, P., Afonso, A., Peres, H., Oliva-Teles, A., Costas, B., 2015. Dietary tryptophan and methionine as modulators of European seabass (*Dicentrarchus labrax*) immune status and inflammatory response. Fish. Shellfish Immunol. 42 (2), 353–362. https://doi.org/10.1016/j.fsi.2014.11.024.

Machado, M., Arenas, F., Svendsen, J.C., Azeredo, R., Pfeifer, L.J., Wilson, J.M., Costas, B., 2020. Effects of water acidification on senegalese sole *Solea senegalensis* health status and metabolic rate: implications for immune responses and energy Use. Front. Physiol. 11, 497078. https://doi.org/10.3389/FPHYS.2020.00026/BIBTEX.

Martos-Sitcha, J.A., Mancera, J.M., Prunet, P., Magnoni, L.J., 2020. Editorial: welfare and stressors in fish: challenges facing aquaculture. Front. Physiol. 11, 162. https://doi.org/10.3389/fphys.2020.00162.

Mattilsynet. (2020, February 24). *Nasjonal tilsynskampanje 2018/2019 Velferd Hos Rensefisk*. The Norwegian Food Safety Authority's National Inspection Projects. https://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/rensefisk/mattilsynet_sluttrapport_rensefiskkampanje_2018_2019.37769/binary/Mattilsynet sluttrapport rensefiskkampanje 2018 - 2019%0Ahttps://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/rensefi.

Mellor, D.J., Beausoleil, N.J., Littlewood, K.E., McLean, A.N., McGreevy, P.D., Jones, B., Wilkins, C., 2020. The 2020 five domains model: including human–animal

interactions in assessments of animal welfare. Anim. Open Access J. MDPI 10 (10), 1–24. https://doi.org/10.3390/ANI10101870.
Midtlyng, P.J., Ahrend, M., Bjerkås, E., Waagbø, R., Wall, T., 1999. Current research on cataracts in fish. Bull. Eur. Assoc. Fish. Pathol. 19 (6), 299–301. https://eafp.org/download/1999-Volume19/Issue6/196299-301.pdf.
Moldal, T., Wiik-Nielsen, J., Henrique Silva de Oliveira, V., Christine Svendsen, J., Vhs, O., & Norwegian Fish Health, S.I. (2025). *The Norwegian Veterinary Institute's annual review of the fish health in Norway Editors*. ⟨www.jensendesign.no⟩.
Newgard, C.B., 2012. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab. 15 (5), 606. https://doi.org/10.1016/J.CMET.2012.01.024.
Nugteren, S., Samsom, J.N., 2021. Secretory leukocyte protease inhibitor (SLPI) in mucosal tissues: protects against inflammation, but promotes cancer. Cytokine Growth Factor Rev. 59, 22–35. https://doi.org/10.1016/J.CYTOGFR.2021.01.005.
Ogata, H.Y., 2002. Muscle buffering capacity of yellowtail fed diets supplemented with crystalline histidine. J. Fish. Biol. 61 (6), 1504–1512. https://doi.org/10.1111/j.1095-8649.2002.tb02493.x.
Peixoto, D., Carvalho, I., Machado, M., Aragão, C., Costas, B., Azeredo, R., 2024. Dietary tryptophan intervention counteracts stress-induced transcriptional changes in a teleost fish HPI axis during inflammation. Sci. Rep. 14 (1). https://doi.org/10.1038/S41598-024-57761-0.
Peter, M.C.S., Gayathry, R., Peter, V.S., 2022. Inducible nitric oxide synthase/nitric oxide system as a biomarker for stress and ease response in fish: implication on Na+ homeostasis during hypoxia. Front. Physiol. 13, 795. https://doi.org/10.3389/fphys.2022.821300.
Petitjean, Q., Jean, S., Gandar, A., Côte, J., Laffaille, P., Jacquin, L., 2019. Stress responses in fish: from molecular to evolutionary processes. Sci. Total Environ. 684, 371–380. https://doi.org/10.1016/J.SCITOTENV.2019.05.357.
Pickering, A.D., Pottinger, T.G., 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish. Physiol. Biochem. 7 (1–6), 253–258. https://doi.org/10.1007/BF00004714/METRICS.
Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol. 58 (3–4), 239–248. https://doi.org/10.1016/S0165-2427(97)00048-2.
Ramakrishnan, S., Sulochana, K.N., 1993. Decrease in glycation of lens proteins by lysine and glycine by scavenging of glucose and possible mitigation of cataractogenesis. Exp. Eye Res. 57 (5), 623–628. https://doi.org/10.1006/EXER.1993.1167.
Remø, S.C., Hevrøy, E.M., Breck, O., Olsvik, P.A., Waagbø, R., 2017. Lens metabolomic profiling as a tool to understand cataractogenesis in Atlantic salmon and rainbow trout reared at optimum and high temperature. PLoS ONE 12 (4). https://doi.org/10.1371/JOURNAL.PONE.0175491.
Reynolds, P., Imsland, A.K.D., Boissonnot, L., 2022. Causes of mortality and loss of lumpfish *Cyclopterus lumpus*. Fishes 7 (6), 328. https://doi.org/10.3390/FISHES7060328.
Ruenkoed, S., Nontasan, S., Phudkliang, J., Phudinsai, P., Pongtanalert, P., Panprommin, D., Mongkolwit, K., Wangkahart, E., 2023. Effect of dietary gamma aminobutyric acid (GABA) modulated the growth performance, immune and antioxidant capacity, digestive enzymes, intestinal histology and gene expression of Nile tilapia (*Oreochromis niloticus*). Fish. Shellfish Immunol. 141, 109056. https://doi.org/10.1016/J.FSI.2023.109056.
Salamanca, N., Giráldez, I., Morales, E., Rosa, I. de La, Herrera, M., 2020. Phenylalanine and tyrosine as feed additives for reducing stress and enhancing welfare in gilthead seabream and meagre. Animals 11 (1), 45. https://doi.org/10.3390/ANI11010045.
da Santa Lopes, T., Costas, B., Ramos-Pinto, L., Reynolds, P., Imsland, A.K.D., Fernandes, J.M.O., 2023. Exploring the effects of acute stress exposure on lumpfish plasma and liver biomarkers. Animals 13 (23). https://doi.org/10.3390/ani13233623.
da Santa Lopes, T., Costas, B., Ramos-Pinto, L., Reynolds, P., Imsland, A.K.D., Cláudia, A., Fernandes, J.M.O., 2024. Lumpfish physiological response to chronic stress. Front. Mar. Sci. 11, 1443710. https://doi.org/10.3389/FMARS.2024.1443710.
Segner, H., Sundh, H., Buchmann, K., Douxfils, J., Snuttan Sundell, K., Mathieu, C., Ruane, N., Jutfelt, F., Toften, H., Vaughan, L., Segner, H., Sundh, Á.S., Sundell, Á.F., Jutfelt, H.K., Douxfils, Á., Mathieu, J.C., Toften Nofima, H., Vaughan, N.L., 2012. Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. Fish. Physiol. Biochem. 38, 85–105. https://doi.org/10.1007/s10695-011-9517-9.
Singer, Peter, Harari, Y.N., 2023. Animal liberation now: the definitive classic renewed. Harper, an imprint of HarperCollins Publishers.
Skår, M.W., Haugland, G.T., Powell, M.D., Wergeland, H.I., Samuelsen, O.B., 2017. Development of anaesthetic protocols for lumpfish (*Cyclopterus lumpus* L.): effect of anaesthetic concentrations, sea water temperature and body weight. PLOS ONE 12 (7), e0179344. https://doi.org/10.1371/journal.pone.0179344.
Southgate, P., 2010. Welfare and farmed fish. In: Fish diseases and disorders, Volume 2. CABI, pp. 357–370. https://doi.org/10.1079/9781845935535.0357.
Staven, F.R., Nordeide, J.T., Imsland, A.K., Andersen, P., Iversen, N.S., Kristensen, T., 2019. Is habituation measurable in lumpfish *Cyclopterus lumpus* when used as cleaner fish in atlantic salmon *Salmo salar* aquaculture? Front. Vet. Sci. 6 (JUL), 227. https://doi.org/10.3389/FVETS.2019.00227/BIBTEX.
Stien, L.H., Størkersen, K.V., & Gåsnes, S.K. (2020). *Analyse Av Dødelighetsdata Fra Spørreundersøkelse Om Velferd Hos Rensefisk*. https://www.mattilsynet.no/fisk_og_akvakultur/akvakultur/rensefisk/analyse_av_dodelighetsdata_fra_sporreundersokelse_om_velferd_hos_rensefisk.37771/binary/Analyse av dødelighetsdata fra spørreundersøkelse om velferd hos rensefisk.
Suehs, B.A., Gatlin, D.M., Wu, G., 2024. Glycine nutrition and biochemistry from an aquaculture perspective. Anim. Front. Rev. Mag. Anim. Agric. 14 (4), 17. https://doi.org/10.1093/AF/VFAE014.
Treasurer, J., Noble, C., Puvanendran, V., Planellas, S.R., Iversen, M., 2018. Cleaner fish welfare. In: Treasurer, J. (Ed.), Cleaner fish biology and aquaculture applications. 5M Publishing Ltd, pp. 281–312. ⟨https://lovdata.no/dokument/NL/lov/2009-06-19-97⟩.
Vijayan, M.M., Aluru, N., Leatherland, J.F., 2010. Stress response and the role of cortisol. In: Fish Diseases and Disorders, 2. CABI, pp. 182–201. https://doi.org/10.1079/9781845935535.0182.
Waagbø, R., Remø, S.C., 2020. Functional diets in fish health management. Aquac. Health Manag. 187–234. https://doi.org/10.1016/B978-0-12-813359-0.00007-5.
Waagbø, R., Tröße, C., Koppe, W., Fontanillas, R., Breck, O., 2010. Dietary histidine supplementation prevents cataract development in adult Atlantic salmon, *Salmo salar* L., in seawater. Br. J. Nutr. 104 (10), 1460–1470. https://doi.org/10.1017/S0007114510002485.
Wagner, T., & Congleton, J.L. (2004). Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile chinook salmon (*Oncorhynchus tshawytscha*). Https://Doi.Org/10.1139/F04-050, 61(7), 1066–1074. https://doi.org/10.1139/F04-050.
Walton, M.J., Cowey, C.B., 1979. Gluconeogenesis from serine in rainbow trout *Salmo gairdneri* liver. Comp. Biochem. Physiol. Part B Comp. Biochem. 62 (4), 497–499. https://doi.org/10.1016/0305-0491(79)90123-8.
Wang, Z., Zhou, Q., Tu, J., Wang, Y., Song, R., Chu, Z., Li, S., Li, J., Zhang, H., Zhang, M., Shao, J., 2023. Ameliorative effect of gamma-aminobutyric acid on the antioxidant status and ammonia stress resistance of *Micropterus salmoides*. Aquac. Rep. 32, 101734. https://doi.org/10.1016/J.AQREP.2023.101734.
Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77 (3), 591–625. https://doi.org/10.1152/physrev.1997.77.3.591.
World Commission on the Ethics of Scientific Knowledge and Technology, 2005. The Precautionary Principle. COMEST. ⟨https://unesdoc.unesco.org/ark:/48223/pf0000139578⟩.
Yada, T., Tort, L., 2016. Stress and disease resistance: immune system and immunoendocrine interactions. Fish. Physiol. 35, 365–403. https://doi.org/10.1016/B978-0-12-802728-8.00010-2.
Yamauchi, M., Sricholpech, M., 2012. Lysine post-translational modifications of collagen. Essays Biochem. 52 (1), 113. https://doi.org/10.1042/BSE0520113.
Yu, X., Long, Y.C., 2016. Crosstalk between cystine and glutathione is critical for the regulation of amino acid signaling pathways and ferroptosis. Sci. Rep. 6 (1), 1–11. https://doi.org/10.1038/srep30033.
Yu, X., Hou, W., Xiao, L., 2024. Gamma-aminobutyric acid (GABA) avoids deterioration of transport water quality, regulates plasma biochemical indices, energy metabolism, and antioxidant capacity of tawny puffer (*Takifugui flavidus*) under transport stress. Biology 13 (7), 474. https://doi.org/10.3390/BIOLOGY13070474.
Zar, J.H., 1984. Biostatistical analysis, 2nd ed. Prentice-Hall.