Growth and Welfare of African catfish (*Clarias gariepinus* Burchell, 1822) under dietary supplementation of the mixed layer clay mineral montmorillonite–illlite/muscovite (1g557) in commercial aquaculture

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Abstract

Juvenile African catfish (*Clarias gariepinus*) was reared under two commercial recirculation aquaculture conditions (E1, E2). The mixed layer clay mineral montmorillonite–illlite/muscovite (1g557) was applied as feed additive over 70 d. Most efficient growth was observed for the 0.5% 1g557 group, performing 0.8% (E1) and 3.2% (E2) better than the catfish basic diet control. In E1, the leptokurtic distribution with a negative skewness also demonstrated the highest number of larger sized fish per batch. Mortality was similarly low in all treatment groups (E1: 3.6 - 4.9%/ E2: 2.5 - 4.8%). In E1, the number of skin lesions decreased considerably after 29 d in the 0.5% and 2.0% groups (from 1.9/1.5 to 0.8/0.8 lesions per fish, respectively), while it remained nearly constant in the control (from 1.5 to 1.2) ($p<0.05$ between control and 2.0% group). After 70 d, the number of lesions significantly decreased to 0.4 and 0.5 in the 0.5% and 2.0% groups, with minor changes in the control (0.9 lesions per fish). Independent sampling in E2 verified these findings, with the number of lesions decreasing to 0.3 and 0.6 in the 0.5% group and the control. In E1, cortisol and glucose increased strongly in all groups due to induced stress; this was not evident in E2 based on a different sampling procedure. Additional blood parameters were not significant in both experiments, suggesting no negative effects on the African catfish organs and metabolism. Supplementation of 0.5% 1g557 to commercial African catfish diet increases fish growth performance, reduces size variance, and supports fish welfare under different commercial aquaculture conditions.

Key words: African catfish, clay mineral, feed additive, feed supplements, fish welfare, montmorillonite, 1g557.

1. Introduction

African catfish (*Clarias gariepinus* Burchell, 1822) is a warm water aquaculture fish with increasing commercial importance worldwide. The production of *C. gariepinus* in recirculating aquaculture systems (RAS) has increased also in Germany between 2011 (318,575 t year^{-1}) and 2019 (930,246 t year^{-1}) (Destatis, 2012 - 2020). Due to its high tolerance towards adverse water conditions, such as low oxygen (Belão et al., 2011), high ammonium, nitrite, and nitrate concentrations (Schram et al., 2014; Roques et al., 2015; Păpuc et al., 2019), this fish can be reared under high stocking densities, reaching up to 500 kg m^{-3} (Van de Nieuwegiessen et al., 2009).

Clay minerals have been attributed to positive effects in aquaculture, ranging from enhanced water conditioning, detoxification to increased growth, health or well-being in farmed aquatic animals. The physical and chemical properties of clay minerals are determined by their chemical composition and the spatial crystal structure. Ions in this layered structure can be exchanged or easily hydrated. Thus, clay minerals are able to adsorb different ions, such as nitrogen compounds and phosphates (Eturki et al., 2012), but also fatty acids, nucleic acids, or proteins (Edzwald et al., 1976; Heimann, 2010). According to Attramadal et al. (2012), the addition of clay minerals (mainly illite) led to water quality improvements during the breeding of Atlantic cod larvae (*Gadus morhua*), where dissolved organic material was bound, thereby reducing the bacterial load and, in most cases, larval mortality. Montmorillonite in feed adsorbs mycotoxins (toxic metabolites of fungi) (Desheng et al., 2005; Pasha et al., 2008; Hassan et al., 2010; De Mil et al., 2015) or the herbicide glyphosate (Khoury et al., 2010); both posing a growing threat in animal farming, causing neurotoxic or carcinogenic effects, developmental disorders, decreased weight gain, an impaired immune system, or increased mortality (Gill et al., 2018; Marijani et al., 2019; Oliveira & Vasconcelos, 2020; Koletsi et al., 2021). Some mycotoxins might accumulate in tissues and may also reach end consumers (Deng et al., 2010; Anater et al., 2016; Oliveira & Vasconcelos, 2020). Palm et al. (2015, 2021) described an increased survival rate, higher final weights, more efficient feed conversion and reduced size variance of White Leg Shrimp postlarvae (*Litopenaeus vannamei*) under application of feeds containing 2% montmorillonite-illite/muscovite (1g557), or a mixture of 2% of this clay mineral and 2% of the microalgae *Chlorella vulgaris*. Positive influence on growth performance and feed digestibility were also described for Nile tilapia (*Oreochromis niloticus*) fed with supplemented (Cu^{2+}-exchanged) montmorillonite (Hu et al., 2007, 2008). Eya et al. (2008) tested feeds supplemented with 0%, 2.5%, 5%, and 10% bentonite in rainbow trout (*Oncorhynchus mykiss*). After 90 d, 5 and 10% bentonite supplementation significantly improved growth parameters, such as percent weight gain, specific growth rates (SGR), and feed efficiency.

The welfare of African catfish has been assessed by analysing the behaviour, external injuries (skin lesions), cortisol, glucose, lactate, growth and mortality (Martins et al., 2006a, 2006b; Van de Nieuwegiessen et al., 2008, 2009; Baßmann et al., 2017, 2020). Rearing fish under very high stocking densities might affect fish welfare and survival negatively, suggesting application of feed additives to increase fitness and survival in return. Since 2016, montmorillonite-illite has been approved as technological feed additive by the EU-regulation EU 2016/1964 under the abbreviation 1g557 (European Commission, 2016), having positive effects onto survival and growth performance of White Leg shrimps (Palm et al. 2015, 2021). The present study analysed external injuries, growth, mortality, and blood parameters of African catfish under supplementation of 1g557 as a feed additive in order to promote growth and welfare of this species under commercial production conditions in two different aquaculture facilities.

2. Material and methods

2.1 Production systems and maintenance

Two experiments (E1 and E2) were conducted, E1 at the aquaculture research facility 'FishGlassHouse' of the University of Rostock, and E2 at a local catfish farmer (Fischzucht Abtshagen, Mecklenburg-Western Pomerania, NE Germany). Both used recirculation aquaculture systems (RAS) for catfish production at a commercial scale.

The system used in E1 has been previously described by Palm et al. (2018). It consists of nine identical rearing tanks, each measuring (L x W x H) 1.8 x 1.0 x 0.7 m, 1.26 m^3. The process water is cleaned through a settling tank (1.3 m^3, equipped with lamella inserts, specific surface area of 105.00 m^2 m^{-3}) and a trickling filter (total volume: 5.9 m^3, specific surface area: 125 m^2 m^{-3}), subsequently collected in a sump (2.7 m^3), before getting returned to the fish tanks. The RAS contained a total of 15.1 m^3 water. Regular water exchange was done with tap water (approx. 624 L d^{-1} = 4.1% of the total volume). The settling tank was cleaned weekly. The temperature was set to 27°C. The pH was adjusted by adding calcium hydroxide as soon as it dropped below 5.5.

In E2, six identical rearing tanks (L x W x H: 1.37 x 0.94 x 0.9 m, 1.16 m^3) which were part of a larger RAS were used. The RAS was equipped with two settling tanks (each 0.95 m^3, lamella inserts, specific surface area of 125 m^2 m^{-3}) and two biofilters (one trickling filter, approx. 14.1 m^3, specific surface area: 125 m^2 m^{-3} and one moving bed filter, 5.1 m^3, biocarrier volume: approx. 2.75 m^3 with biocarrier >750 m^2/m^3 total surface area). The water was collected in a sump (2.5 m^3). This RAS contained a total of 18.8 m^3 water. Water exchange was done twice a week when the settling tanks were cleaned (each time 1.9 m^3).

In both experiments, temperature, oxygen concentration and saturation, pH, electric conductivity (EC), salinity, and redox potential were recorded daily (each in triplicates) with a portable multimeter (Hach-Lange HQ40D, Germany) at the settling tanks influx, its efflux, and behind the trickling filter. Twice a week, water samples were analyzed in triplicates by using an automatic photo-analyzer (Gallery™, Thermo Fisher Scientific) for ammonium/ ammonia (NH_4^+/ NH_3), nitrite (NO_2^-), nitrate (NO_3^-), and ortho-phosphate (PO_4^{3-}).

2.2 Experimental feeds

The experimental feeds (feed mill Spezialfuttermittelwerk Beeskow GmbH, Germany) were either mixed with 0.5% or with 2.0% 'Friedland clay' 1g557 as a registered technological feed additive under EFSA (2014). The composition of the basic catfish feed is given in Tab. 1, with the ingredients wheat, fish meal 70M, poultry meal, HP-soya extract grist, haemoglobin powder, hydrolyzed feather meal, pea protein, monocalcium phosphate and additional vitamins A, C, D, E. After mixing the respective amount of 1g557 (Palm et al. 2021) to the basic feed, fish oil and water was added according to the manufacturer's recommendation, pelleted (feed pellet laboratory press type 14-175 by Amandus Kahl GmbH & Co., Germany), and deep frozen at -20°C until feeding. Feed processing was repeated five times during the experiments to obtain fresh feeds. The pellet stability in water was tested beforehand and regarded as sufficient.

The clay mineral 1g557 originated from the open cast mine near Friedland in Mecklenburg-Western Pomerania, Northern Germany, and is a mixture of different minerals, dominated by 35 - 53% swellable montmorillonite/illite, about 30% non-swellable illite/muscovite, and < 20% koalinite, and quartz. Siderite, pyrite and other minor constituents (< 1%) are also present (Henning & Kasbohm, 1998; EFSA, 2014; FIM Biotech, 2017). The

empirical formula is $Na_{0.03}Ca_{0.04}K_{0.16}(Al_{1.87}Fe_{0.16}Mg_{0.16})(Si_{3.31}Al_{0.69})O_{10}(OH)_{2-}$ (EFSA, 2014). By definition, Friedland clay is not a true bentonite, although its physical properties are determined by montmorillonite, which is the main component in bentonites. Compared to other bentonites, Friedland clay has a lower swelling capacity and a lower specific surface area (Henning & Kasbohm, 1998).

Table 1: Composition of experimental feeds according to manufacturer's specifications.

Nutrients	**Coppens Special Pro EF 4.5**	**Catfish experimental feed (feed mill Beskow)**		
	adaption period	control group	0.5% group	2.0% group
Crude protein [%]	42.0	45.2	44.974	44.296
Crude fat [%]	13.0	15.0	15.0	15.0
Carbohydrates [%]	not specified	19.6	19.502	19.208
Crude ash [%]	7.8	5.1	5.075	4.998
Crude fiber [%]	1.5	1.4	1.393	1.372
Phosphorus [%]	1.14	1.0	0.995	0.980
Digestible energy [MJ kg^{-1}]	17.1	20.1	20.0	19.7
1g557 [%]	0	0	0.5	2.0

2.3 Fish stocking

In E1, 926 presorted juvenile African catfish were obtained on 1st Feb. 2019 from Fischzucht Abtshagen and stocked randomly into the nine tanks with 103 fish/tank (one tank with 102 fish, appr. 2.5 kg/m^3). Respectively, three tanks were allocated to one of three treatment groups: 0.5%, 2.0%, and control group. The fish from the 0.5% and 2.0% groups had significantly different mean initial weights and body lengths. Also the 2.0% group had a significantly different condition index to both other groups (Tab. 3).

In E2, 618 juvenile African catfish were bred directly at Fischzucht Abtshagen, presorted and stocked on 24th Apr. 2020 into six tanks (103 fish/ tank). Three tanks each were allocated to one of two treatment groups, 0.5% and control. These fish had insignificant mean initial weights, body lengths, and condition indices (Tab. 4).

In both experiments a randomized block design in triplicates was used. During an adaptation period of 31 d, all fish were fed with a regular commercial catfish diet (Coppens Special Pro EF 3 - 4.5 mm, Tab. 1). On 5th Mar. 2019 (E1) and on 26th May 2020 (E2) this diet was changed by switching to the pelleted experimental feeds (Tab. 1). The unsupplemented African catfish feed mixture (feed mill Beskow) was taken as control. In the supplemented feeds, replacement of 0.5% or 2.0% of the regular feed with 1g557 reduced the nutrient content by maximum 0.5% or 2.0%, and the digestible energy by 0.1 - 0.4%. The amount of feed given per day was based on an existing commercial feeding protocol (between 3.9 and 1.5% of fish body weight depending on the growth stage, Fischzucht Abtshagen). Feeding took place every two hours between 07:00 p.m. – 05:00 a.m. by using automatic feeders.

2.4 Sampling

After the adaptation period, samplings were performed every four weeks. In E1, the first sampling (T0) was conducted on 4th Mar. 2019 by measuring body weights, body lengths, the initial levels for plasma cortisol, blood glucose, and external injuries (skin lesions and fin erosions as result of aggressive behavior) as a sub-sample (11 fish per tank = 33 fish per treatment group). The next sub-sample (15 fish per tank = 45 fish per treatment group)

was taken over three days from 1st Apr. – 4th Apr. 2019 (T1), whereby fish growth and welfare parameters were recorded again. A further sub-sample (15 fish per tank = 45 fish per treatment group) followed from 1st May – 3rd May (T2) recording same parameters. The final sampling (T3) was done on 13th May 2019 by taking body weights, body lengths, the number of external injuries and mortality from all remaining fish.

In E2, the first sampling (T0) was conducted on 25th May 2020 by measuring the same parameters as in E1, but using three unstressed fish and three stressed fish per tank (18 fish per treatment group). The next sub-sample followed on 22nd Jun. 2020 (T1) with measuring the same parameters in an equal sample size as before. A further sub-sample followed on 22nd July 2020 (T2). The final sampling was done on 3rd Aug. 2020 (T3) by taking weights, lengths, number of external injuries from all remaining fish and the additional blood parameters (see above).

Since some fish were removed from the experiment after each sampling, feed conversion ratios (FCR) were calculated for each sampling date ($FCR = TFI/W_t - W_0$ with TFI = total feed intake (SI Einheit), W_0 = initial fish weight (SI Einheit), and W_t = final fish weight (SI Einheit)). All remaining fish in the tanks were considered. The condition index (condition index = fish mass [g] * 100/ fish length $[cm]^3$) was determined at stocking, T0, and T3. At stocking and T3 all fish were sampled (E1: at stocking: 309, 308, 309; T3: 166, 164, 171; E2: at stocking: 309 each; T3: 172, 163); at T0 sub-samples of 33 fish from each group were taken in E1 and E2.

2.5 Blood parameters

To compare fish welfare, the mortality, growth performance, number of external injuries and the blood parameters plasma cortisol and blood glucose were analyzed. Prior to samplings in E1, the water level of each rearing tank was reduced to approx. 20 cm and all fish from this tank were removed with nets and transferred into 100 L-tubs (confinement stress). This was done in order to prevent continuous catching stress, with potentially different individual time periods inside the fish tanks until blood sampling, affecting the cortisol response. The transfer and maintenance inside the sorting tubs is a stressor simulating regular aquaculture procedures. Afterwards, 15 randomly chosen fish per tank (45 per group) were stunned via brain percussion, killed by cutting the gills, and blood sampled over their caudal vessels. Blood glucose was measured *in situ* using test stripes (Accu Check Aviva). Approx. 0.5 mL blood was transferred to reaction tubes with a coated coagulation inhibitor (5.4 mg K-EDTA) and stored on ice. The blood samples were centrifuged (1,250 rpm, at 4°C, for 10 min, Hettich Universal 320 R) and the plasma phase was used for cortisol ELISA (Cusabio, fish cortisol, sensitivity: 0.0023 ng mL^{-1}) according to the manufacturer's instructions. The plasma samples were analyzed by using a micro-plate reader at 450 nm (iMark, Bio-Rad).

In E2, nine fish were directly taken from each of the six tanks, stunned, killed, and subsequently blood sampled. This procedure was conducted within 10 min to get a proper indication of the cortisol baseline (reflecting unstressed fish). Cortisol starts to rise within a few minutes after inducing acute stress (Wendelaar Bonga, 1997). Afterwards, all remaining fish were treated as in E1; stress was induced by water level reduction to approx. 20 cm, followed by the catching process and confinement. Then, nine fish per tank were stunned, killed, and blood sampled as described for E1 (reflecting stressed fish, without exactly considering the temporal influence of stress or the stress intensity).

Additional blood samples (approx. 3.0 mL in total) were taken to analyze hematocrit, leucocytes, erythrocytes, aspartate aminotransferase (AST), glutamate dehydrogenase

(GLDH), cholesterol, triglycerides, urea, sodium, potassium, calcium, chloride, phosphate, glucose, total protein. The concentrations of sodium, potassium and chloride were measured by using an ion-selective electrode. All other chemical blood parameters were quantified by photometry/flow cytometry. In E1 three fish were sampled per group (one per tank) at T0, T1 and T3. In E2, two fish per tank (six per group) were sampled at T0, T1 and T3. The sample size differed due to a different amount of blood that could be taken from the fish specimens.

The number of skin injuries on body and fins (not on heads due to the stunning method) were recorded always from the same two persons and independently from treatment groups, excluding bias. Only fresh biting wounds penetrating the epidermal layer or reaching down to the underlying tissue were counted. Multiple skin lesions that were obviously related to a single biting attack were counted as one injury, regardless of their individual size. Skin lesions that could not be assigned to a single attack were counted as multiple wounds. Injury marks (scars) were not documented if they were already in the healing process (visible by regenerated epidermal layer or mucus), because they cause no or only minor pressure onto the immune system and hence do not impact fish welfare any more. Sex, weight and length were recorded from the sampled fish. All remaining fish were weighed as a group, counted, and allocated to their respective rearing tanks.

2.6 Statistics

The resulting data were tested first for distribution. For normal distributed data and three experimental groups, One Way Analyses Of Variance (ANOVA) and post hoc multiple range tests were used; Tukey's-HSD test for variance homogeneity and Dunnett-T3 test for variance inhomogeneity. For not normal distributed data and unequal n, nonparametric Kruskal-Wallis-Test was applied. Parameters of two experimental groups (E2) were analysed by *t*-test if data were normal distributed, otherwise Mann-Whitney test showed significances. All tests were performed with a significance level of $p < 0.05$. In addition, a frequency distribution was performed including range, symmetry, kurtosis, and skewness for fish masses and lengths. These statistical evaluations were conducted by using the SPSS 25 (IBM, 2011) statistical software package. Tests performed are specified in the results section of the respective data.

3. Results

3.1 Water quality

Tab. 2 summarizes the water parameters of E1 and E2. During E1, the water temperature inside the system ranged between 26.6 - 27.8 °C, with highest values at the influxes of the rearing tanks (behind trickling filtration) and lowest at the sedimentation tank influx. The oxygen concentrations were > 7 mg L^{-1} (> 90% saturation) at the inflows of the rearing tanks (after trickling filter) and > 5.5 mg L^{-1} (mostly > 70% saturation) at their effluxes (sedimentation tank influx). During the adaptation phase the pH ranged between 8.3 - 7.1. From T0 - T3, pH-values fluctuated with a general trend towards the low-acid range. The lowest pH of approx. 4 was measured behind trickling filtration. The EC increased slightly, but decreased several times following samplings and resulting water exchanges, before increasing again. EC valued between 855 - 2002 µS cm^{-1}. As a result, an almost identical curve for salinity was recognized; the salinity on average was 0.6 ± 0.1 ‰. The redox potential showed regular fluctuations between 63.3 and 261.2 mV, which reflected the amount of charged molecules absorbed and released into the water. The concentration of

NH_4^+ was mostly between 0.1 - 0.2 mg L^{-1}. A peak of NH_4^+ (up to max. 3.7 mg L^{-1}) in the last third of the experiment was reduced by sampling-related water exchange and the matured biofilter. NO_2^- was mainly below 0.1 mg L^{-1}. The maximal concentration at the influxes of the rearing tanks (after trickling filtration) was measured at 0.4 mg L^{-1}. NO_3^- increased steadily during the experiment from 23.3 up to max. 170.9 mg L^{-1}, but decreased temporary after water exchange. PO_4^{3-} showed a similar trend as NO_3^-; however, reaching 8.7 mg L^{-1} at maximum.

Table 2: Water parameters (mean ± standard deviation) in E1 and E2, measured daily (or 2x weekly*) in the settling tank influx (SI), the settling tank efflux (SE), and the trickling filter (TF). T = temperature, O_2 = oxygen, EC = electric conductivity, RedOx = redox potential, NH_4-N = ammonium-nitrogen, NO_2-N = nitrite-nitrogen, NO_3-N = nitrate-nitrogen, PO_4-P = ortho-phosphate.

	E 1			E 2		
	SI	SE	TF	SI	SE	TF
T (°C)	27.0 ± 0.2	27.1 ± 0.2	27.3 ± 0.2	28.7 ± 1.0	28.8 ± 1.0	28.8 ± 1.0
O_2 (mg L^{-1})	6.5 ± 0.5	6.0 ± 0.7	7.5 ± 0.2	6.1 ± 0.7	5.0 ± 1.0	6.8 ± 0.5
O_2 (%)	81.7 ± 5.9	75.3 ± 9.4	94.2 ± 2.7	78.8 ± 8.9	64.6 ± 11.9	87.7 ± 5.3
pH	6.6 ± 1.1	6.7 ± 1.1	6.8 ± 1.4	7.0 ± 0.7	7.0 ± 0.7	7.1 ± 0.8
EC (µS cm^{-1})	1254.5 ± 260.3	1256.5 ± 259.5	1263.1 ± 263.1	881.2 ± 80.5	881.4 ± 80.8	881.9 ± 81.0
RedOx (mV)	153.8 ± 42.6	157.8 ± 42.0	163.0 ± 47.4	160.4 ± 36.7	156.4 ± 34.0	155.8 ± 32.6
NH_4-N* (mg L^{-1})	0.6 ± 0.9	0.6 ± 1.0	0.5 ± 0.9	0.7 ± 2.0	0.7 ± 2.0	0.7 ± 1.9
NO_2-N* (mg L^{-1})	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.1
NO_3-N* (mg L^{-1})	71.1 ± 36.4	71.8 ± 36.1	72.5 ± 36.1	196.4 ± 50.3	199.9 ± 52.9	195.5 ± 51.5
PO_4-P* (mg L^{-1})	3.8 ± 1.8	3.9 ± 1.9	3.8 ± 1.9	17.2 ± 9.8	16.6 ± 9.8	16.6 ± 9.8

During E2, the water temperature inside the system ranged between 26.0 and 29.9 °C. The oxygen concentration was slightly lower compared with E1, with > 5.3 mg L^{-1} (> 70% saturation) at the inflows of the rearing tanks (after trickling filter) and > 4.5 mg L^{-1} (approx. 60% saturation) at their effluxes (sedimentation tank influx). The pH was in a total range of 8.1 - 4.7. As in E1, EC showed an increasing trend, but did not have a linear elevation due to water changes. Values between 681 - 1169 µS cm^{-1} were measured. The mean salinity was 0.4 ± 0.04‰. The redox potential was between 44.8 - 213.9 mV. The amount of NH_4^+ ranged mostly between 0.002 - 0.7 mg L^{-1}; a peak up to 9.4 mg L^{-1} occurred in the last week of E2. NO_2^- showed a higher range compared with E1, but was < 1.1 mg L^{-1} after sedimentation (before biofiltration) and < 0.6 mg L^{-1} inside the fish tanks (after biofiltration). Similar to E1, NO_3^- increased steadily, from 106.8 up to max. 284.0 mg L^{-1}, but decreased temporary after water exchange. PO_4^{3-} showed a similar trend as NO_3^-; it was mostly < 20 mg L^{-1}, with a maximum of 47.1 mg L^{-1} at the end.

3.2 Fish growth performance

Mean weights, lengths, difference in weight (%), and the condition index of fish at stocking, T0 and T3 are given for E1 and E2 in Tab. 3. At stocking of E1, fish of the control group had insignificantly the same size to those of the 0.5% and 2.0% groups. However, due to the random distribution, fish of the two experimental groups were significantly different from each other, with fish of the 0.5% group weighing on average 1.3 g more than those of the 2.0% group.

Changing to the test feed at beginning of E1 (T0), sub-samples of 33 fish per group weighed 107.8 - 112.6 g with lengths of 24.5 - 25.2 cm ($p > 0.05$). After 70 d (T3), the 0.5% group showed with 484.2 g the highest weight, 0.8% above the control group (480.5 g). The

2.0% group was 2.3% below the growth of the control group (469.5 g). Thus, the 0.5% group tended to show the best average growth performance (insignificant), followed by the control and the 2.0% group. Both, the fish weight and length were insignificant, with a negative trend of 2.3% in the 2.0% group.

During E2, the fish of control and 0.5% group showed no significant difference in their size at stocking. At T0, sub-samples of 33 fish weighed 115.2 - 115.6 g with lengths of 25.3 cm ($p > 0.05$). After 70 d (T3), the 0.5% group tended to show again the highest weight compared with the control group (422.2 g vs. 409.2 g; $p > 0.05$). In E2, the 0.5% group was 3.2% above the weight of the control group (insignificant).

Table 3: Growth performance (mean, ± SD) of African catfish in the treatment groups of E1 and E2 fed by different montmorillonite levels (control: C, 0.5%, 2.0%) with Δ [%] as the difference in weight relative to control (C) and condition index (CI). At T0, a sub-sample of 33 fish was weighed and measured in length; at T3 all remaining fish were weighed and measured in length; at T0: n.g. = not given since sub-sample; $p \leq 0.05$.

	n	group	weight [g]		length [cm]		Δ [%]	CI [g/cm^3]	
Experiment 1 (E1)									
before stocking and adaptation phase	309	C	30.7 ^{a,b}	± 5.8	16.7 ^{a,b}	± 1.1	0	0.652^{a}	0.1
	308	0.5%	31.6 ^{a}	± 6.0	16.9 ^{a}	± 1.1	+2.9	0.652^{a}	0.0
	309	2.0%	30.3 ^{b}	± 6.1	16.6 ^{b}	± 1.1	-1.3	0.661^{b}	0.0
T0 (start of experiment)	33	C	107.8 ^{a}	± 17.2	24.8 ^{a}	± 1.5	n.g.	0.7^{a}	0.1
	33	0.5%	112.6 ^{a}	± 16.2	25.2 ^{a}	± 1.3	n.g.	0.7^{a}	0.0
	33	2.0%	107.9 ^{a}	± 17.1	24.5 ^{a}	± 1.4	n.g.	0.7^{a}	0.0
T3 (after 70 d)	166	C	480.5^{a}	± 89.2	39.2^{a}	± 3.0	0	0.8^{a}	0.1
	164	0.5%	484.2^{a}	± 86.7	39.4^{a}	± 2.8	+0.8	0.8^{a}	0.1
	171	2.0%	469.5^{a}	± 93.3	39.0^{a}	± 2.9	-2.3	0.8^{a}	0.1
Experiment 2 (E2)									
before stocking and adaptation phase	309	C	29.8 ^{a}	± 3.6	17.0 ^{a}	± 0.8	0	0.6^{a}	0.1
	309	0.5%	29.7 ^{a}	± 3.3	17.0 ^{a}	± 0.8	-0.3	0.6^{a}	0.0
T0 (start of experiment)	33	C	115.6 ^{a}	± 20.8	25.3 ^{a}	± 1.6	n.g.	0.7^{a}	0.0
	33	0.5%	115.2 ^{a}	± 16.4	25.3 ^{a}	± 1.4	n.g.	0.7^{a}	0.1
T3 (after 70 d)	172	C	409.2^{a}	± 73.1	37.5^{a}	± 2.4	0	0.8^{a}	0.2
	163	0.5%	422.2^{a}	± 76.1	37.9^{a}	± 2.5	+3.2	0.8^{a}	0.2

In E1, the FCR ranged from 0.66 to 0.97, whereas in E2 it ranged from 0.76 to 1.71 (Tab. 4). A leptokurtic distribution (above 3) with a skewness below 0 represents best batch growth, revealing highest number of larger sized fish per batch. Highest kurtosis (13.8 fish length, 5.5 weight) with a skewness of -2.4 (length) and -1.2 (width) was observed in the 0.5% group at the end of E1 after 103 days, with similar length (7.6, -1.6; 7.4, -1.1) and weigth (2.2, -0.8; 3.2, -1.2) distributions for the 2.0% group and the control. At the end of E2, the batches of the 0.5% group and the control grew similarly slightly platykurtic, with a kurtosis and a skewness around 0 (length 0.0, -0.1; 0.5, -0.6 and weigth -0.2, 0.4; 0.8, -0.1).

Table 4: Feed conversion ratio (FCR) in the treatment groups of E1 (on top) and E2 (below). Since a few fish were removed from the experiment in each sampling, FCR cannot be indicated from initial stocking to T3. FCR is therefore given for each sampling date based on the respective previous sampling weights.

	group	Stocking – T0	T0 – T1	T1 – T2	T2 – T3
E1	C	0.66	0.78	0.86	0.96
	0.5%	0.66	0.76	0.89	0.97
	2.0%	0.65	0.77	0.91	0.93
	C	0.76	0.95	1.06	1.71

E2	0.5%	0.76	0.94	1.04	1.44

3.3 Fish welfare

The mortalities in both experiments are given in Tab. 5. During E1, the mortality inside the control, 0.5%, and 2.0% groups amounted 11, 15, and 9 fish. This result is a percentage mortality of 3.6%, 4.9%, and 2.9%, respectively (Ø 3.8). During E2, the mortality inside the control and 0.5% groups amounted 8 and 15 fish, resulting in a percentage mortality of 2.5% and 4.8%, respectively (Ø 3.7).

Table 5: Mortality within the treatment groups of E1 and E2, both at T3.

	Groups at T3		
	Control (E1/E2)	**0.5% (E1/E2)**	**2.0% (E1)**
Mortality [n]	11/8	15/15	9
Mortality [%]	3.6/2.5	4.9/4.8	2.9

The number of external injuries decreased in all treatment groups over the run of both experiments. In E1, however, there was a significant decrease in the number of external injuries in both experimental groups compared with the control (Fig. 1 a). At T0, the 0.5% group had a relatively high mean value of 1.9 (± 1.8) lesions per individual compared with both other groups (control group: 1.5 ± 1.4; 2.0% group: 1.5 ± 1.5). Due to high standard deviations, there was no significant difference between the groups. At T1, the number of lesions in the 0.5% and 2.0% groups decreased to 0.8 (± 1.3) and 0.8 (± 1.5), respectively, a significant reduction compared with the control (1.2 (± 1.3) ($p < 0.05$) lesions per fish). At T2, the number of lesions continued to decrease in all groups, insignificant between each other. At T3, the numbers of lesions in the 0.5% and 2.0% groups decreased to 0.4 ± 0.8 and 0.5 ± 0.8, significantly lower compared with the control group (0.9 ± 1.0). A similar pattern was observed in E2 (Fig. 1 b). The numbers of external injuries of the control and 0.5% groups were insignificant at T0, with 2.1 (± 1.5) and 1.8 (± 1.8) lesions per fish. At T1 and T2, the groups remained insignificant, with the 0.5% group tending to have fewer lesions than the control. At T3, the number of lesions per fish was significantly different between the control, 0.6 (± 1.2), and the 0.5% group, 0.3 (± 0.8).

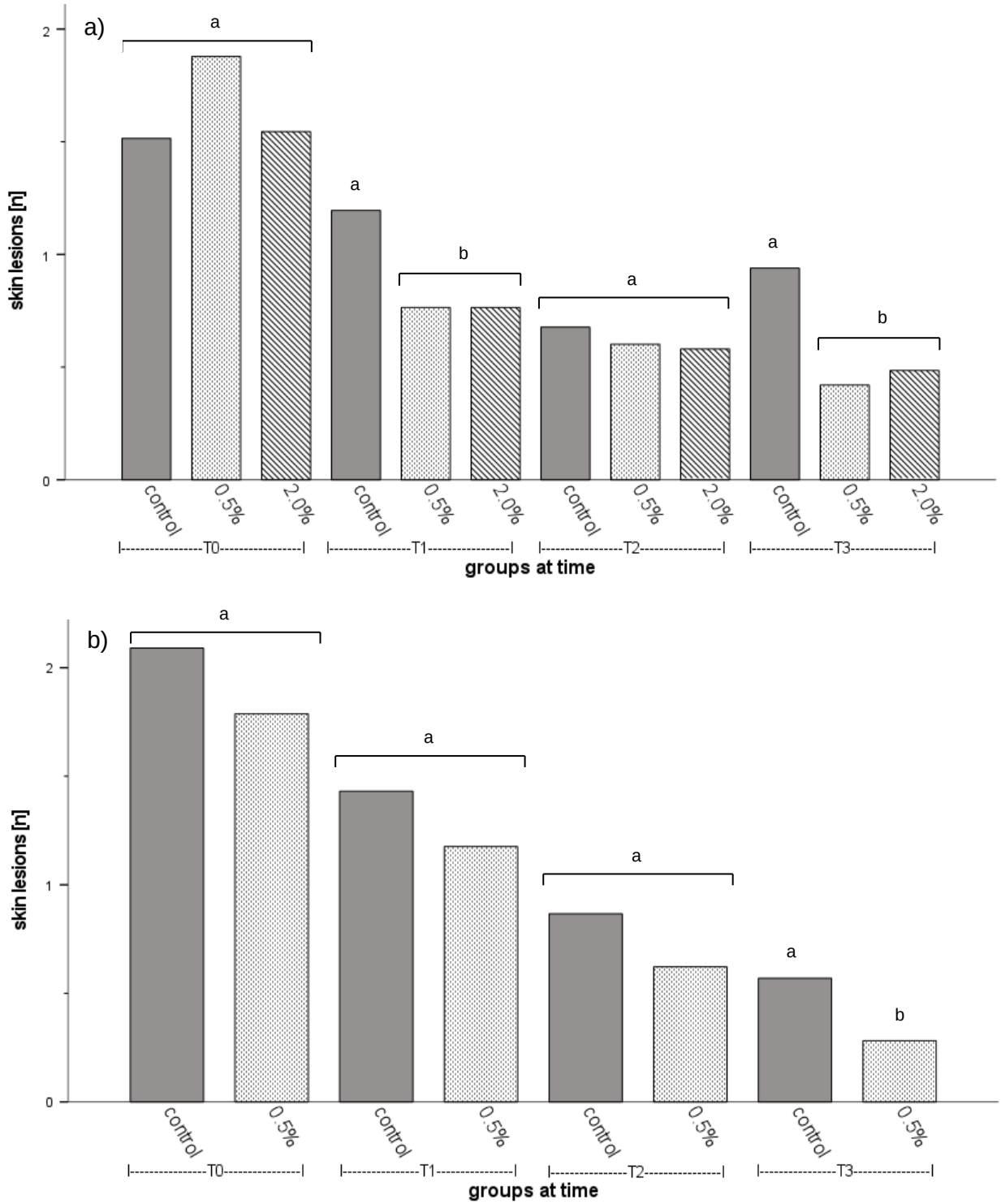

Figure 1 a, b: Average number of external injuries of a) three treatment groups (control, 0.5% and 2.0%) in E1, and b) two treatment groups (control and 0.5%) in E2, each at T0 (baseline prior to feed supplementation), at T1 (after 29 d of feed supplementation), at T2 (after 58 d), and at T3 (after 70 d), respectively. Significance $p \leq 0.05$ (Kruskal-Wallis-ANOVA (a); Mann-Whitney test (b)).

In E1, the mean plasma cortisol prior to feed supplementation (at T0) in the control, 0.5%, and 2.0% groups were 22.7 (± 14.4) ng mL^{-1}, 28.8 (± 15.2) ng mL^{-1}, and 24.7 (± 13.1) ng mL^{-1} ($p > 0.05$). After the diet change (at T1), plasma cortisol in the 0.5% and 2.0% group

increased by approx. 249% or 190% (to 100.5 ± 65.5 ng mL^{-1} and 71.5 ± 68.4 ng mL^{-1}, respectively). However, an increase of approx. 344% was also noted in the control group (to 100.8 ± 68.1 ng mL^{-1}). Significant differences were recorded between the 2.0% group and both others. At T2, plasma cortisol concentrations in the control, 0.5%, and 2.0% groups were at 82.7 ± 43.6 ng mL^{-1}, 140.8 ± 53.2 ng mL^{-1}, and 99.2 ± 76.7 ng mL^{-1} and so approx. 264%, 389% and 302% higher compared with their respective baseline values. Here, the 0.5% group was significantly higher than both other groups (Fig. 2 a). In E2, the mean plasma cortisol baselines (at T0) in the control and 0.5% groups were 19.2 (± 9.0) ng mL^{-1} and 20.8 (± 7.2) ng mL^{-1} in unstressed fish, while they were slightly increased in stressed fish (33.3 ± 17.8 ng mL^{-1} and 32.3 ± 15.3 ng mL^{-1}). After the diet change (at T1), mean levels remained nearly identical with 19.7 (± 9.4) ng mL^{-1} and 19.9 (± 3.8) ng mL^{-1} in unstressed fish, and with 25.2 (± 8.5) ng mL^{-1} and 24.4 (± 8.1) ng mL^{-1} in stressed fish. At T2, a minor elevation occurred in all treatment groups. Unstressed fish in the control and 0.5% group showed 32.7 (± 13.5) ng mL^{-1} and 32.3 (± 15.3) ng mL^{-1}, while stressed fish showed 29.5 (± 12.1) ng mL^{-1} and 33.2 (± 11.8) ng mL^{-1} (Fig. 2 b).

In E1, the mean glucose baseline levels were at 2.4 (± 0.4) mmol L^{-1} in the control group and at 2.5 (± 0.4) mmol L^{-1} in both the 0.5% and 2.0% groups, with no significant difference. After switching to the supplemented diets (at T1), the mean glucose of the control group increased to 3.8 (± 0.9) mmol L^{-1}, the 0.5% group to 3.6 (± 0.8) mmol L^{-1}, and the 2.0% group to 3.4 (± 0.7) mmol L^{-1}, with the 2.0% group being significantly lower than the control group. At T2, the glucose of the control group averaged 4.0 (± 1.0) mmol L^{-1}, the 0.5% group 4.4 (± 1.0) mmol L^{-1}, and the 2.0% group 4.3 (± 0.9) mmol L^{-1}, with no significant differences between the groups (Fig. 3 a). In E2, the mean glucose baseline levels (at T0) in the control and 0.5% groups were 2.7 (± 0.4) mmol L^{-1} and 2.8 (± 0.6) mmol L^{-1} in unstressed fish, while they were elevated to 4.0 (± 0.5) mmol L^{-1} and 4.2 (± 0.8) mmol L^{-1} in stressed fish, respectively. At T1, glucose decreased slightly throughout all groups, with mean levels of 2.0 (± 0.2) mmol L^{-1} and 2.1 (± 0.3) mmol L^{-1} in unstressed fish of the control and 0.5% groups, and 3.5 (± 0.4) mmol L^{-1} and 3.9 (± 0.7) mmol L^{-1} in stressed fish, respectively. At T2, glucose of the control and 0.5% groups averaged 2.4 (± 0.5) mmol L^{-1} and 2.5 (± 0.5) mmol L^{-1} in unstressed fish, while it was regularly increased to 3.5 (± 0.7) mmol L^{-1} and 3.6 (± 0.7) mmol L^{-1} in stressed fish, respectively (Fig. 3 b). There were no significant differences between the groups within the different sampling events.

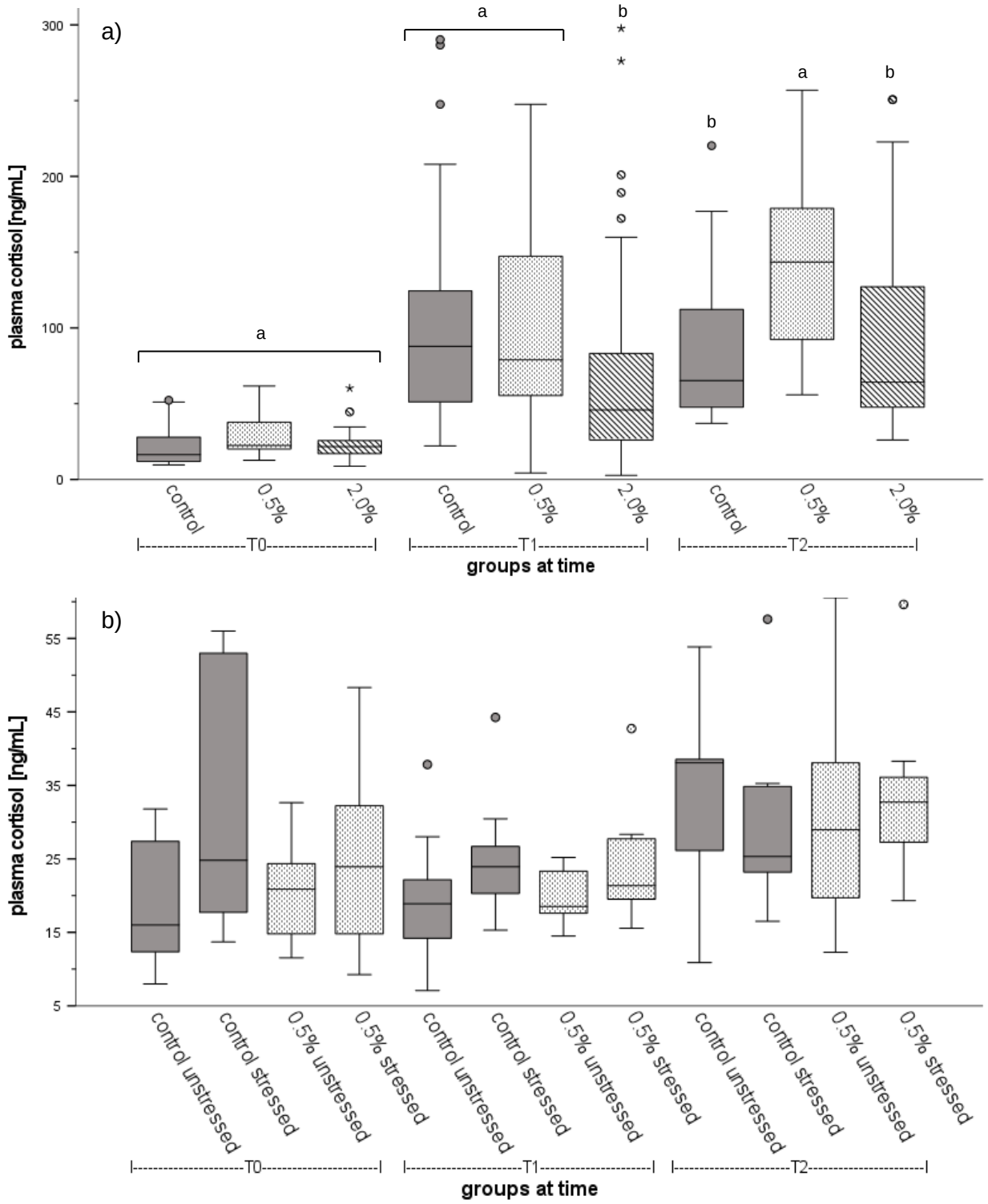

Figure 2 a, b: Plasma cortisol concentrations a) in three treatment groups (control, 0.5% and 2.0%) in E1 after stress, and b) of unstressed and stressed fish in two treatment groups (control and 0.5%) in E2, each at T0 (baseline prior to feed supplementation), at T1 (after 29 d of feed supplementation), and at T2 (after 58 d), respectively. Asterisk (*) = extreme values; circlets (°) = outliers. In a), at T2, an extreme value of 387.7 ng mL^{-1} in the 2% group is not illustrated. Significance $p \leq 0.05$ (Kruskal-Wallis-ANOVA (a)). In b) all data were insignificant (*t*-test or Whitney-Mann test).

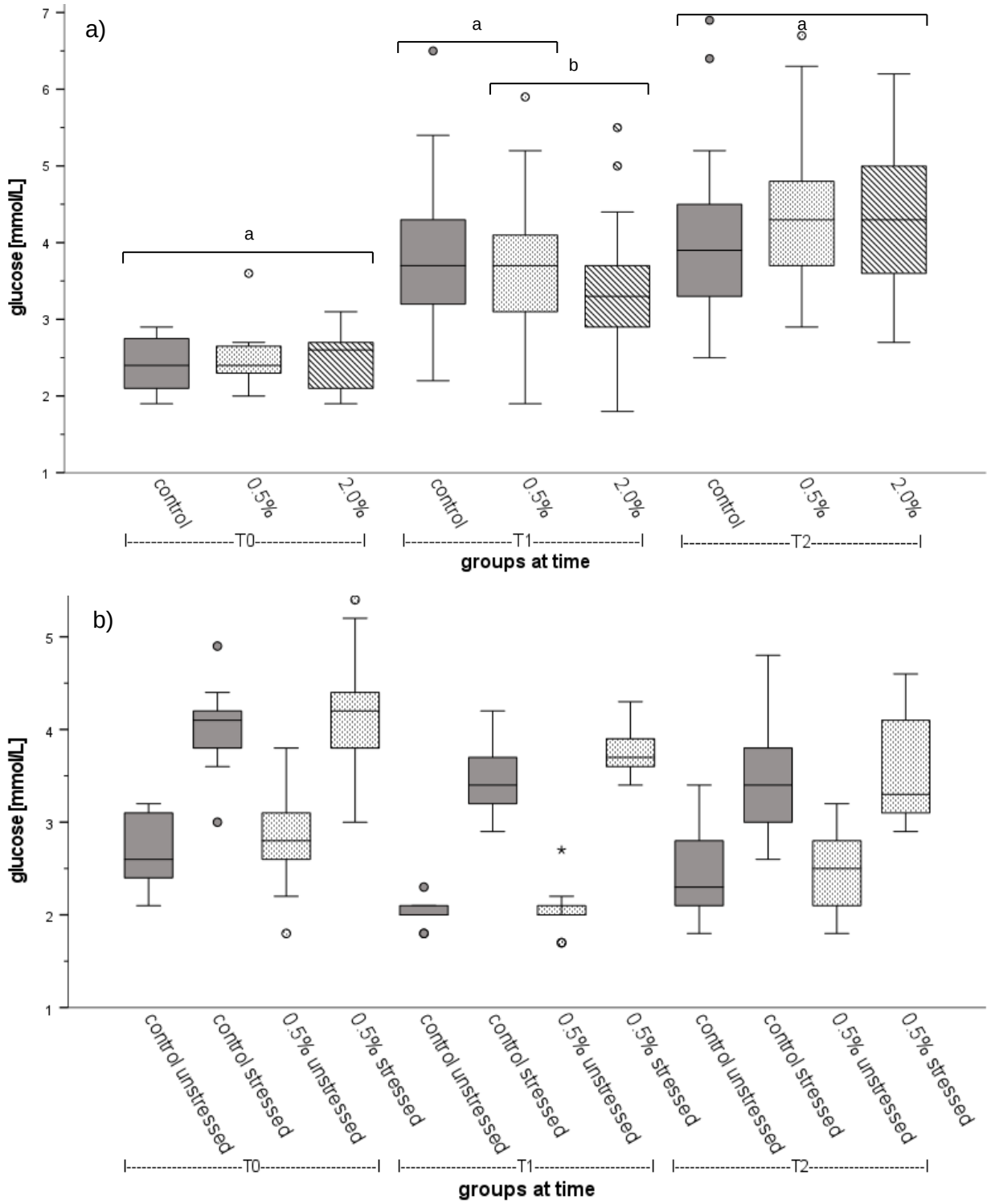

Figure 3 a, b: Blood glucose levels of a) three treatment groups (control, 0.5% and 2.0%) in E1 after stress, and b) of unstressed and stressed fish in two treatment groups (control and 0.5%) in E2, each at T0 (baseline prior to feed supplementation), at T1 (after 29 d of feed supplementation), and at T2 (after 58 d), respectively. Asterisk (*) = extreme values; circlets (°) = outliers. In a), at T2, an extreme value of 8.3 mmol L^{-1} in the 0.5% group is not illustrated. In b), at T1, an extreme value of 5.6 mmol L^{-1} in the stressed 0.5% group is not illustrated. Significance $p \leq 0.05$ (ANOVA, Tukey-HSD, Kruskal-Wallis-ANOVA, (a)). In b) all data were insignificant (*t*-test or Whitney-Mann test).

The blood parameters (Tab. 6) aspartate transaminase (AST)/glutamic oxaloacetic transaminase (GOT), glutamate dehydrogenase (GLDH), total protein, urea, calcium, and phosphate were not significant in both experiments. The cellular blood components (leucocytes, erythrocytes, haematocrit) and the chemical blood parameters (cholesterol, trigycrides, sodium, potassium) (Tab. 7) were also not significant in both experiments.

Table 6: Liver and kidney blood parameters at T0 (baseline) and T3 (end of the experiment) in E1 and E2.

	n	group	AST (GOT) [U/l]		GLDH [U/l]		urea [mmol/l]		calcium [mmol/l]		phosphat [mmol/l]		total protein [g/l]	
Experiment 1 (E1)														
T0 (start of experiment)	9	base line	333.7	± 79.7	29.4	± 3.7	1.1	± 0.1	2.8	± 0.1	3.9	± 0.2	29.9	± 1.4
T3 (after 70 d)	3	C	208.7	± 38.2	28.2	± 3.7	1.1	± 0.1	3.3	± 0.2	3.7	± 0.6	39.3	± 1.2
	3	0.5%	167.3	± 12.2	28.1	± 4.7	1.0	± 0.1	3.3	± 0.3	3.3	± 0.1	38.0	± 4.1
	3	2.0%	179.0	± 5.0	22.7	± 2.1	1.0	± 0.1	3.2	± 0.4	3.2	± 0.4	37.0	± 2.2
Experiment 2 (E2)														
T0 (start of experiment)	12	base line	150.8	± 28.7	14.7	± 2.5	1.3	± 0.2	3.0	± 0.1	3.0	± 0.2	33.7	± 1.2
T3 (after 70 d)	6	C	109.0	± 18.8	18.4	± 2.2	1.1	± 0.1	3.4	± 0.1	2.5	± 0.2	39.5	± 2.4
	6	0.5%	142.7	± 48.8	26.5	± 9.2	1.3	± 0.1	3.5	± 0.2	2.8	± 0.2	41.1	± 2.2

Table 7 Cellular blood components and chemical blood parameters at T0 (baseline) and T3 (end of the experiment) in E1 and E2.

	group	leucocytes [G/l]	erythrocytes [T/l]	hematocrit [%]	cholesterol [mmol/l]	triglycerides [mmol/l]	sodium [mmol/l]	potassium [mmol/l]	chloride [mmol/l]
Experiment 1 (E1)									
T0	base line	3.3 ± 2.7	1.1 ± 0.6	21.5 ± 7.3	2.9 ± 0.2	1.8 ± 0.1	124.6 ± 2.6	13.0 ± 1.7	112.0 ± 2.0
T3	C	0.9 ± 0.3	2.0 ± 0.5	26.2 ± 1.3	3.5 ± 0.1	2.0 ± 0.1	128.3 ± 1.2	9.3 ± 1.6	107.0 ± 1.4
	0.5%	1.0 ± 0.2	1.7 ± 0.3	25.8 ± 0.2	3.4 ± 0.3	2.0 ± 0.4	129.7 ± 0.9	8.4 ± 0.5	107.7 ± 0.5
	2.0%	1.0 ± 0.0	1.3 ± 0.4	24.5 ± 0.7	3.1 ± 0.3	1.7 ± 0.2	128.7 ± 1.2	9.5 ± 2.3	110.7 ± 2.1
Experiment 2 (E2)									
T0	base line	3.7 ± 0.8	1.9 ± 0.4	26.7 ± 2.0	2.8 ± 0.3	1.8 ± 0.1	130.0 ± 1.9	7.6 ± 1.3	111.5 ± 1.5
T3	C	13.0 ± 7.5	2.1 ± 0.2	36.3 ± 3.0	3.4 ± 0.2	2.0 ± 0.1	133.5 ± 1.7	7.3 ± 0.6	110.5 ± 1.6
	0.5 %	7.0 ± 3.3	1.7 ± 0.6	34.7 ± 7.1	3.4 ± 0.4	2.0 ± 0.1	133.7 ± 2.0	7.4 ± 0.3	111.0 ± 1.6

4. Discussion

4.1. Culture conditions

The water parameters (Tab. 2) provide basic information of the rearing conditions for African catfish, having also a determining importance on growth and welfare. Despite the high tolerance of African catfish (Păpuc et al., 2019), it can be assumed that suboptimal or poor water quality might potentially affect the welfare of this species. Therefore, it was crucial to provide optimal culture conditions for both experimental approaches inside the two commercial aquaculture farms.

The water temperature in E1 was close to 27°C. There was max. 1.2 °C variation in the entire system; the temperature variations at the individual sample sites were even closer and deviated by only 0.6 °C. In E2, the mean temperature was slightly higher with 28.8 °C; however, both temperature ranges correspond to the optimum for African catfish (Păpuc et al., 2019). In E1, dissolved oxygen was >90% saturation or >7 mg L^{-1} (after trickling filtration). In E2, this was slightly lower with (>70% saturation or >5.3 mg L^{-1}. Masser et al. (1999)

recommended maintaining dissolved oxygen levels >60% saturation or >5 mg/L for optimal growth of most warm water species. Thus, the oxygen conditions were in fact optimal during both experiments. A consistently high redox potential was found, also indicating a good oxygen supply.

Due to fish respiration, increasing feeding amounts, and bacterial nitrogen metabolism, the pH value usually decreases continuously towards acid conditions (Masser et al., 1999). In E1, the pH started at approx. 8, and dropped temporarily to below 5.5 several times from the fifth week onwards. However, water changes during samplings and regular liming kept the pH mostly above 6.5. A very similar trend was observed in E2. Ndubuisi et al. (2015) reported adequate pH ranges between 5 and 9 for growth and survival of *C. gariepinus*. So, the pH in our study was still in an adequate range. NH_4^+ between 0.1 - 0.2 mg L^{-1} as mainly measured in our study can be tolerated very well by African catfish (Păpuc et al., 2019). Under the given temperature and pH ranges only very minor concentrations of toxic unionized ammonia (NH_3) were present (Losordo et al., 1998; Masser et al., 1999). We calculated the unionized form of NH_3 with a maximum of <0.01 – <0.02 mg L^{-1} at pH 8.2 and 27°C, being distinctly lower with decreasing pH.

Fluctuations of NO_2^- up to 0.4 mg L^{-1} in E1 (0.6 mg L^{-1} in E2) were below or at the recommended maximum (up to 0.6 mg L^{-1}) for African catfish aquaculture, and did not affect growth, well-being, or health (Roques et al., 2015). In E1, NO_3^- (after trickling filter) was on average 72.5 (± 36.1) mg L^{-1}, but tended to increase over the experiment to a maximum of 170.9 mg L^{-1}. In E2, the mean NO_3^- (after trickling filter) was higher with 190.8 (± 56.9) mg L^{-1} and a maximum of 284.0 mg L^{-1}. Schram et al. (2014) recommended not exceeding NO_3^- levels of 140 mg L^{-1}. In E1, nitrate was mostly below, but it exceeded this threshold for about five days and reached 170.9 mg L^{-1} at maximum, which was still considered to be fairly harmless. During E2, the threshold of 140 mg L^{-1} was exceeded for long periods; however, NO_3^- was still relatively low compared to other studies that addressed African catfish in recirculation aquaculture systems. Palm et al. (2018) reported NO_3^- values of 185.5 mg L^{-1} and 125.6 mg L^{-1} at survival rates of 81.4 % and 88.6 % during an entire production periode. Dai et al. (2012) suggested NO_3^- values below 1000 mg L^{-1} and 100% daily water exchange as safe under African catfish production conditions. The survival rates reached 95.1 % - 97.5% in the present study. From this perspective, even the slightly elevated NO_3^- levels during E2 might have caused no major effect on the observed welfare status or growth of the African catfish in the present experiments.

In summary, water quality in both experiments and aquaculture systems was in a similar range and represented appropriate to optimal culture conditions for the African catfish. Because the physicochemical water parameters were within a similar range as reported by Palm et al. (2018) and the survival rates were high, we conclude that the use of 1g557 inside the test feeds did not negatively affected the functionality of both RAS systems.

4.2 Fish growth

At stocking in E1, the fish mean weights in the control, 0.5% and 2.0% groups were 30.7, 31.6, and 30.3 g, respectively ($p < 0.05$ between 0.5% and 2.0% group). At T0, the 0.5% group still showed the highest mean weight with 114.0 g, but was insignificant to both other groups. After 70 days of feeding the supplemented diets (T3), the groups were still insignificant with mean weights of 480.5, 484.2, and 469.5 g, respectively. However, a trend was seen that a 2% lower nutrient content in the 2% group resulted in slightly less weight (2.3%) compared to the control group. So the fact that 2% of the feed was replaced by 2% 1g557 seemed to have a negative effect on fish growth.

A different picture was seen in comparison of the 0.5% group with the control. In E1, there was a slight increase in weight gain of 0.8%, and this was despite the fact that 0.5% of the feed was replaced by 0.5% 1g557. This trend could be verified in E2, where the 0.5% group had an even 3.2% higher weight than the control group ($p > 0.05$). In addition, a lower size variance of African catfish was observed in E1. This is consistent with earlier studies where the addition of montmorillonite or 1g557 achieved good growth performance and lower divergence, even when other species were involved (Hu et al., 2007; 2008; Palm et al., 2015). This suggests that African catfish as well as white-leg shrimps (Palm et al., 2015) can grow more homogeneously under the effect of 1g557 as a feed additive.

The Feed conversion ratio during E1 ranged from 0.66 before and 0.76-0.97 after application of 1g557, constantly increasing with increasing fish size from 30-31 (2.5 kg/m^3) until 469-484 g (21 kg/m^3). Palm et al. (2018) reported an FCR of African catfish inside the same aquaculture system between 0.89 and 1.01 in 5 different production phases, from an initial weight of 40-275 g (2.8-19.3 kg/m3) until the final weight of 1496-1780g (95.5 - 112 kg/m^3). Consequently, the growth performance was as expected during E1, with a slightly better FCR because of smaller sized fish and more extensive production conditions (see Palm et al. 2018) during the entire experiment. During E2, the FCR was higher and not directly comparable because of using a different aquaculture system and cultivation conditions. However, the FCR was already higher during the adaptation phase, indicating that this difference was not caused by the application of the feed additive but originated from the different cultivation system.

4.3 Fish welfare

In the present study 2.9 - 4.9% of the fish did not survive in the experimental groups fed with 0.5% or 2.0% 1g557. Partially, mortalities were slightly lower in the control groups, but in total in a very similar range. So, no negative influence of feed supplementation with 1g557 could be detected between the treatment groups. Other studies using regular fish feeds showed similar mortalities, such as 2.5% (van de Nieuwegiessen et al., 2008) or 6% (Baßmann et al., 2020). Palm et al. (2018) reported survival rates up to 90.2% under stocking densities of 199.2 kg m^{-3}, and increasing survival rates under more extensive conditions. This may be the best comparison, as the same RAS was used here with very similar stocking density, same system maintenance, but under common commercial feeds. So, the mortality in our present study can be considered low.

An initial increase in agonistic behavior and subsequently a higher number of skin lesions after stocking juvenile African catfish can be considered normal. From our experience, after a few weeks, this usually decreases. Other studies reported 1 - 8 skin lesions per fish (van de Nieuwegiessen et al., 2008; 2009; Manuel et al., 2014). In a three-weeks experiment African catfish fingerlings whose feed contained montmorillonite improved their skin quality, and no adverse effect on growth was determined (Ismaila et al., 2011). In both presented experiments, the highest injury values were recorded at T0. At T1, there was a reduction in the number of external injuries, whereby at E1, the two experimental groups fed with 1g557 showed a significantly decreased number of external injuries than the control. In E2, a similar trend was observed. Finally, significant different numbers of external injuries were recorded independently in both experiments at T3, with all groups fed diets supplemented with 1g577 having approx. half as many injuries as the control groups.

Cortisol responses were generally highly diverse, particularly in E1. The mean values ranged between 20 - 140 ng mL^{-1}. Therefore, fewer fish were used for cortisol analyses in E2. In addition, unstressed fish (baseline) and fish after stress induction were used. The

plasma cortisol concentrations of fish in E2 were comparable to those at T0 in E1, but apart from that lower. The plasma cortisol levels of stressed fish in E2 were mostly elevated to those of unstressed fish. Solely at T2 the cortisol response of the stressed control was below that of the unstressed control, which cannot be explained, as it was probably not cortisol suppression. The data were also widely scattered in the unstressed 0.5% group, whereas they were closer together in stressed fish. For comparison, Martins et al. (2006b) reported most cortisol levels for unstressed as well as stressed African catfish to be between approx. 20 and 100 ng mL^{-1}, with cortisol of unstressed being significantly or at least trending lower than that of stressed fish. Largely, this is consistent with our data. It is likely that the sampling method had a strong influence in our experiments, as all fish were caught and removed from the tanks. Thus, temporal differences as well as a changing stressor intensity on individual fish could easily occur and may have led to different cortisol responses. Glucose ranged from 2 to 5 mmol L^{-1} overall in both experiments, with stressed fish tending to have higher values than unstressed fish in E2. This is also consistent with the results of Martins et al. (2006b). However, we found no major differences between our experimental groups despite significant differences in E1 (at T1), indicating that 1g557 had no adverse effect.

The additional blood parameters aspartate transaminase (AST)/glutamic oxaloacetic transaminase (GOT), glutamate dehydrogenase (GLDH), total protein, urea, calcium, and phosphate were not significant in both experiments and indicated regular liver and kidney function. Similarly, the cellular blood components (leucocytes, erythrocytes, haematocrit) and the chemical blood parameters (cholesterol, trigycrides, sodium, potassium) were not significant, suggesting that there were no negative effects of the tested feed additive on the African catfish organs and metabolism.

5. Conclusions

The application of the mixed layer clay mineral montmorillonite–illlite/muscovite (1g557) in RAS for African catfish has the potential to improve both growth and welfare of the fish without negatively affecting their blood parameters and stress responses or the RAS itself. After 70 days of cultivation in each of two independent experiments, fish treated with 0,5 % 1g557 tended to have the highest mean weight and least size variance ($p > 0.05$), while the number of external injuries was significantly ($p < 0.05$) reduced by more than a half when compared with the non-supplemented control. Dietary supplementation of 1g557 showed these beneficial effects for the tested fish sizes between 100 und 500 g when given at a concentration of 0.5%. However, a higher 2.0% 1g557 supplementation reduced the fish growth most probably due to the reduced amount of digestible energy inside the test feed. Further studies need to address why the fish growth performed better and the incidence of external injuries was drastically reduced under supplementation of the tested mixed layer clay mineral, supporting our attempts to improve fish welfare through application of entirely natural products under recirculation aquaculture conditions in future.

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Benedikt Gille: Investigation, Data Curation

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