



# Article Effects of Dissolved Potassium on Growth Performance, Body Composition, and Welfare of Juvenile African Catfish (*Clarias gariepinus*)

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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). **Abstract:** Optimal crop production in aquaponics is influenced by water pH and potassium concentrations. The addition of potassium hydroxide (KOH) into the recirculating aquaculture system (RAS) may benefit aquaponics by increasing the water pH for better biofilter activity and supplementing K for better plant growth and quality. We investigated the growth, feed conversion, body composition and welfare indicators of juvenile African catfish (*Clarias gariepinus*) treated with four concentrations of K (K0 = 2, K200 = 218, K400 = 418, and K600 = 671 mg L<sup>-1</sup>). While growth, feed conversion and final body composition were unaffected, the feeding time and individual resting significantly increased with increasing K<sup>+</sup>. The swimming activity and agonistic behavior were reduced significantly under increased concentrations of K<sup>+</sup>. Leftover feed and the highest number of skin lesions were observed under K600. We suggest that K<sup>+</sup> concentrations between 200 and 400 mg L<sup>-1</sup> can improve the welfare status of juvenile African catfish. This enables the application of KOH in RAS to supply alkalinity to achieve optimum nitrification at minimum water exchange and improve the nutritional profile of the process water with benefits for the welfare status of African catfish and aquaponics plant production and quality.

**Keywords:** African catfish; *Clarias gariepinus*; aquaponics; nutrient management; potassium; plant growth; plant quality; animal welfare; water quality

## 1. Introduction

Potassium (K) is vital to plant and animal nutrition, as it is involved in many different physiological processes. In plants, K takes part in osmoregulation [1,2], influences enzyme activities [3,4] and is involved in energy metabolism [5]. In animals, K plays a crucial role in nerve functioning [6,7], also influences enzyme activities [8–12] and affects the acid–base balance [13]. To fulfill the needs of fish in freshwater aquaculture, commercial diets for the African catfish (*Clarias gariepinus*) contain about 0.94–0.97% of K [14].

Recommended values of K<sup>+</sup> concentrations in hydroponic solutions range between 156 and 300 mg L<sup>-1</sup> [15]. K<sup>+</sup> concentrations inside the process water of African catfish (*C. gariepinus*) in RAS are 2–13 times lower [14,16], requiring fertilization before its use for hydroponics. In coupled aquaponics [17], however, high K<sup>+</sup> concentrations benefit the plants but may impair the fish. In terrestrial animals, overdose symptoms of K<sup>+</sup> have been well investigated [18–20], leading, e.g., to excessive salivation, muscular tremor, and even to collapse [20]. In aquatic animals, the 50% mortality (TL<sub>m</sub>) after 24 h KCl exposure occurred in snail eggs (*Lymnaea* sp.) at 1941 mg L<sup>-1</sup>, water fleas (*Daphnia magna*) at 343 mg L<sup>-1</sup> and

the bluegill (*Lepomis macrochirus*) at 5500 mg L<sup>-1</sup> [21]. The latter showed no mortality for 96 h at 871 mg L<sup>-1</sup> of K [22], demonstrating high tolerance levels. The Channel catfish (*Ictalurus punctatus*) had a dietary K requirement of 2.6 g K per kg feed if the fish gets the K solely through the feed [23]. At water K<sup>+</sup> levels of 4 mg L<sup>-1</sup>, the Channel catfish had no need for dietary K due to the ability of uptake from the rearing water. On the other hand, Shearer [24] observed anorexia, tetany, and death under deficient dietary K supply for Chinook salmon (*Oncorhynchus tshawytscha*) fry in freshwater, indicating optimum feed K concentrations of 0.6–1.2% for this species. The effects of elevated K<sup>+</sup> water concentrations (K<sup>+</sup> > 200 mg L<sup>-1</sup>) on commercial aquaculture fish species have not yet been described.

Water pH is critical in aquaculture and is often controlled to optimize the water quality for the fish and the nitrifying biofilter. In commercial African catfish RAS, usually with high stocking densities and feed input, pH regulation can reduce freshwater usage due to better microbial nitrification. In hydroponics, inadequate water pH negatively affects nutrient availability. Consequently, for African catfish aquaponics, pH control is recommended. Alkalinity agents for aquaculture contain sodium (NaHCO<sub>3</sub>, NaOH) or calcium (CaCO<sub>3</sub>, Ca(OH)<sub>2</sub>, CaO) [25] with the disadvantage of Na<sup>+</sup> and Ca<sup>2+</sup> accumulation. In hydroponics (and can be assumed for aquaponics), Na<sup>+</sup> interferes with the uptake of the essential plant nutrients K<sup>+</sup>, H<sup>+</sup> and NH<sub>4</sub><sup>+</sup>-N, although high Ca<sup>2+</sup> concentrations can reduce these negative effects [26]. Too high Ca<sup>2+</sup> concentrations, especially combined with a high pH, can lead to unwanted precipitation with PO<sub>4</sub><sup>3-</sup> [27,28], making this macronutrient unavailable for the plants. This suggests the use of KOH for pH regulation in aquaponics [29,30].

Commercial RAS-production of African catfish in Germany is practiced since 2007, with production volumes around 1000 tons per year since 2015 [31]. First studies on its application in coupled and decoupled aquaponics are described in the literature [17]. However, water K<sup>+</sup> concentrations in aquaponics do not meet plant requirements for optimal growth and quality if the only source of  $K^+$  in the aquaponics system is fish feed [14]. Rakocy et al. [32] suggested the addition of KOH in coupled aquaponics to control water pH, improve biofilter efficiency while promoting plant growth. However, no information is available on the effects of the elevated K<sup>+</sup> concentrations on the performance and welfare of African catfish. While the toxicity of the nitrogenous compounds NH<sub>3</sub> [33],  $NO_2^{-}$  [34] and  $NO_3^{-}$  [35] and the safe use of  $PO_4^{3-}$  [36] with African catfish have been assessed, the effects of elevated K<sup>+</sup> concentrations are still unknown. The purpose of the present study was to better understand the effects of high  $K^+$  concentrations in the rearing water on the growth and welfare of African catfish. Chemical analyses of the proximate body composition have been done to identify possible effects of elevated K<sup>+</sup> concentrations in the rearing water on fish. Future potential applications of the KOH addition in aquaponics are discussed.

## 2. Results

#### 2.1. Water Quality

The physicochemical water parameters (mean  $\pm$  CV) over the run of the experiment are given in Table 1. Significant differences (p < 0.05) were detected for DO, temperature, conductivity, NO<sub>2</sub><sup>-</sup>-N and K<sup>+</sup>. No differences ( $p \ge 0.05$ ) were detected for pH, TAN (total ammonia nitrogen), TON (total oxidized nitrogen), NO<sub>3</sub><sup>-</sup>-N, TDN (total dissolved nitrogen), PO<sub>4</sub><sup>3-</sup>-P and Mg<sup>2+</sup>. In K200, the mean DO (dissolved oxygen) was lower than in the other groups. The temperature was lowest in K200 and highest in K600. Conductivity was lowest in K200 and highest in K0 and K400. NO<sub>2</sub><sup>-</sup>-N concentrations were highest in K400 and lowest in K200. The mean values of the physic-ochemical parameters were DO = 6.3–6.5 mg L<sup>-1</sup>, temperature = 29.1–29.8 °C, pH ~ 6.8, conductivity = 2344–2411 µS cm<sup>-1</sup>, TAN = 0.66–1.61 mg L<sup>-1</sup>, NO<sup>2–</sup>-N = 0.35–0.59 mg L<sup>-1</sup>, TON = 29.5–32.9 mg L<sup>-1</sup>, NO<sub>3</sub><sup>-</sup>-N = 29.1–32.3 mg L<sup>-1</sup>, TDN = 30.4–34.5 mg L<sup>-1</sup>, PO<sub>4</sub><sup>3-</sup>-P = 3.3–3.6 mg L<sup>-1</sup> and of Mg<sup>2+</sup> = 9.6–10.8 mg L<sup>-1</sup>.

	Unit -	Group				
Parameter		К0	K200	K400	K600	
DO	$(mg L^{-1})$	$6.5\ ^{\mathrm{a}}\pm4.6$	$6.3^{b} \pm 5.1$	$6.4~^{\mathrm{a}}\pm7.2$	$6.2~^{a} \pm 5.0$	
DO	(%)	$84.9~^{\mathrm{a}}\pm5.8$	81.6 $^{\rm b}$ $\pm$ 5.3	84.1 $^{\mathrm{a}}\pm6.9$	$84.8~^{a}\pm4.9$	
Temperature	(°C)	29.5 $^{\mathrm{ab}}\pm$ 2.4	29.1 $^{ m c}$ $\pm$ 2.3	29.5 $^{\mathrm{a}}\pm2.5$	29.8 $^{ m b}$ $\pm$ 2.4	
pH		$6.8\pm9.6$	$6.8\pm7.9$	$6.8\pm9.0$	$6.8\pm7.1$	
Conductivity	$(\mu S \text{ cm}^{-1})$	$2382~^{a}\pm4.7$	$2344 {\ b} \pm 4.3$	2411 $^{\mathrm{a}}\pm4.6$	2376 $^{\mathrm{ab}}\pm3.6$	
TAN	$(mg L^{-1})$	$1.48 \pm 128.9$	$0.98 \pm 122.2$	$1.61 \pm 133.9$	$0.66 \pm 106.2$	
$NO_2^{-}-N$	$(mg L^{-1})$	$0.48~^{ m ab}\pm56.9$	$0.35 \ ^{ m b} \pm 44.4$	$0.59~^{\mathrm{a}}\pm 63.0$	$0.52~^{\mathrm{a}}\pm49.1$	
TON	$(mg L^{-1})$	$30.0\pm46.3$	$29.5\pm43.9$	$32.9\pm43.4$	$31.3\pm40.6$	
$NO_3^{-}-N$	$(mg L^{-1})$	$29.6\pm47.0$	$29.1\pm44.4$	$32.3\pm44.2$	$30.8\pm41.4$	
TDN	$(mg L^{-1})$	$31.5\pm48.4$	$30.4\pm45.00$	$34.5\pm46.3$	$32.0\pm40.9$	
$K^+$	$(mg L^{-1})$	11.7 $^{\mathrm{a}}$ $\pm$ 34.7	217.7 $^{\mathrm{b}}$ $\pm$ 27.4	418.5 $^{\rm c}$ $\pm$ 30.6	$671.0^{\text{ d}} \pm 23.5$	
PO4 <sup>3–</sup> -P	$(mg L^{-1})$	$3.4\pm50.1$	$3.3\pm61.1$	$3.6\pm51.6$	$3.4\pm50.8$	
Mg <sup>2+</sup>	$(\operatorname{mg} L^{-1})$	$9.6\pm34.9$	$10.1\pm29.5$	$10.2\pm29.8$	$10.8\pm22.7$	

**Table 1.** Physicochemical water parameters (mean  $\pm$  CV).

Superscript letters indicate significant differences between the experimental groups (p < 0.05).

# 2.2. Fish Performance and Welfare Indicators

Fish growth and feed efficiency parameters (mean  $\pm$  CV) from stocking to final sampling are given in Table 2. No significant differences were detected for initial weight, final weight, final total length, final standard length, growth, SGR, FCR and TFI. No significant mortality occurred.

Table 2. Fish growth and feed efficiency of C. gariepinus (mean  $\pm$  CV). Final sampling was performed on day 42.

Description	Unit —	Group				
Parameter		K0	K200	K400	K600	
Initial weight (W <sub>0</sub> )	$(g fish^{-1})$	$28.6\pm20.5$	$28.3\pm22.0$	$29.6\pm24.8$	$30.5\pm23.6$	
Final weight (W <sub>t</sub> )	$(g fish^{-1})$	$135.0\pm22.3$	$138.7\pm28.3$	$145.6\pm32.7$	$140.7\pm28.5$	
Final total length	(cm)	$26.4\pm8.3$	$26.5\pm9.1$	$26.6\pm10.2$	$26.7\pm8.4$	
Final standard length	(cm)	$23.7\pm8.5$	$23.8\pm9.1$	$23.9\pm10.4$	$24.0\pm8.5$	
Growth (G)	$(g fish^{-1})$	$106.6\pm 6.8$	$110.4\pm2.1$	$116.0\pm4.5$	$110.3\pm13.5$	
SGR	$(\% BW d^{-1})$	$3.4\pm3.0$	$3.5\pm0.6$	$3.5\pm1.3$	$3.3\pm5.6$	
FCR	-	$0.80\pm5.91$	$0.76 \pm 1.28$	$0.75\pm2.50$	$0.80\pm7.36$	
TFI	$(g fish^{-1})$	$84.5\pm1.2$	$83.7\pm1.3$	$87.4\pm2.0$	$87.6\pm7.5$	

Table 3 shows the welfare indicators of the fish from the different groups (mean  $\pm$  CV). Significant differences (p < 0.05) were observed for the number of skin lesions, with the highest numbers in K600 and the fewest numbers in K200. Significant differences for swimming (individual), agonistic behavior (individual), and fight events (group) were determined, which were most frequent in K0 and least frequent in K600. Significant differences were observed for resting (individual) and stock resting (group), most frequent in K600 and least frequent in K0. No differences ( $p \ge 0.05$ ) were detected for air-breathing (individual), stereotypic behavior (individual), and aggregation behavior (group). Feeding time showed significant differences and was longest in K600 and shortest in K0. Additionally, K600 was the only group with uneaten feed during the 30 min period of feeding. The total amount of uneaten feed in K600 over the run of the experiment was 124 g.

$n = 6, \dots, n = 51;$ means $\pm CV$ .					
Parameter	Unit	К0	K200	K400	K600
Skin lesions (biting wounds) *	(n fish <sup>-1</sup> )	$3.3~^{\mathrm{ab}}\pm 68.7$	$3.0^{\text{ b}}\pm78.9$	$4.1~^{\mathrm{ab}}\pm77.7$	$4.7~^{\rm a}\pm 66.9$
Swimming (individual) **	(%)	64.5 $^{\rm a}$ $\pm$ 7.4	$33.9~^{\rm ab}\pm 20.6$	53.8 $^{\rm a}\pm15.9$	$23.1 ^{\mathrm{b}} \pm 3.3$
Resting (individual) **	(%)	24.2 $^{\rm a}\pm19.6$	$62.4 ^{\mathrm{bc}} \pm 12.4$	42.5 $^{\mathrm{ab}}\pm$ 26.4	74.2 $^{\rm c}\pm3.6$
Agonistic behavior (individual) **	(%)	7.5 $^{\rm a} \pm 10.1$	$1.1~^{ m ab}\pm70.7$	$1.1~^{\mathrm{ab}}\pm141.4$	$0.0$ <sup>b</sup> $\pm$ N/A
Air breathing (individual) **	$(n \text{ fish}^{-1} h^{-1})$	$30\pm28.3$	$8\pm93.5$	$10\pm102.0$	$16\pm77.1$
Stereotypic behavior (individual) **	(%)	$11.3\pm141.4$	$0.0 \pm N/A$	$1.6\pm141.4$	$0.0 \pm N/A$
Stock resting (group) **	(%)	15.1 $^{\rm a}\pm52.7$	69.4 $^{ m b} \pm 11.6$	$39.3 \ ^{ab} \pm 36.8$	71.0 $^{ m b} \pm 21.9$
Fight event (group) **	(%)	7.5 $^{\rm a}\pm20.2$	$1.1$ <sup>b</sup> $\pm$ 70.7	$3.2~^{ m ab}\pm40.8$	$0.0$ <sup>b</sup> $\pm$ N/A
Aggregation behavior (group) **	(%)	$43.6\pm15.1$	$87.6\pm5.3$	$45.2\pm44.0$	$78.5\pm17.0$
Feeding time ***	(min)	$2.8~^{a}\pm27.9$	$4.1$ <sup>b</sup> $\pm$ 56.1	$3.7 \ ^{\mathrm{b}} \pm 28.5$	15.1 $^{\rm c}$ $\pm$ 78.7

**Table 3.** Welfare indicators of *C. gariepinus* challenged with different amounts of dissolved potassium (\*: n = 48 (-mortalities); \*\*: n = 6; \*\*\*: n = 51; means  $\pm$  CV).

Superscript letters indicate significant differences between the experimental groups (p < 0.05).

## 2.3. Proximate Body Composition

The proximate body compositions (mean  $\pm$  CV) of the whole fish are given in Table 4. No significant differences ( $p \ge 0.05$ ) in moisture, protein, fat, ash, calcium, phosphorous, sodium, magnesium, and potassium were observed between the groups.

**Table 4.** Final proximate compositions and mineral contents in whole fish of *C. gariepinus* (n = 3, each sample of 8 pooled fish) (mean  $\pm$  CV). Final sampling was performed on day 42.

D	Unit –	Group				
Parameter		К0	K200	K400	K600	
Moisture	(%, ww)	$73.4\pm1.6$	$74.0\pm0.6$	$75.2\pm2.2$	$73.3\pm0.2$	
Protein	(%, ww)	$14.8\pm16.3$	$14.9\pm14.6$	$14.7\pm11.9$	$15.0\pm12.9$	
Fat	(%, ww)	$5.8\pm5.1$	$5.2\pm8.7$	$5.2\pm13.1$	$5.7\pm3.8$	
Ash	(%, ww)	$3.7\pm5.8$	$3.6\pm11.3$	$4.1\pm10.9$	$3.6\pm 6.0$	
Calcium	(g kg <sup>-1</sup> , dm)	$37.0\pm6.3$	$39.7\pm27.6$	$47.1\pm24.0$	$42.7\pm6.9$	
Phosphorus	(g kg <sup>-1</sup> , dm)	$24.4\pm5.7$	$26.8\pm16.3$	$29.6\pm19.0$	$27.5\pm4.7$	
Sodium	$(g kg^{-1}, dm)$	$4.4\pm10.5$	$5.2\pm36.7$	$4.7\pm2.2$	$5.0\pm18.8$	
Magnesium	$(g kg^{-1}, dm)$	$1.5\pm1.4$	$1.5\pm9.0$	$1.6\pm9.8$	$1.5\pm3.2$	
Potassium	$(g kg^{-1}, dm)$	$12.2\pm7.1$	$13.2\pm18.5$	$13.1\pm5.1$	$11.5\pm0.5$	

ww: wet weight, dm: dry matter.

The apparent net nutrient utilization (ANNU) of protein, P and K by the fish showed no significant differences between the treatments (Table 5). The ANNU of protein and K increased with a trend from K0 to K200, and the ANNU of P increased from the K0 to K400.

**Table 5.** Apparent net nutrient utilization (ANNU; %) of *C. gariepinus* (n = 3; mean  $\pm$  CV).

Parameter	К0	K200	K400	K600
Protein	$35.6\pm21.9$	$37.6 \pm 18.4$	$37.3 \pm 17.0$	$36.2\pm16.0$
Phosphorus	$63.4\pm4.3$	$71.5 \pm 16.0$	$76.2 \pm 22.2$	$72.9\pm7.7$
Potassium	$50.0 \pm 18.4$	$55.6\pm20.7$	$52.1\pm9.7$	$46.1\pm 6.8$

# 3. Discussion

This study demonstrates that in African catfish RAS, the addition of K<sup>+</sup> to the process water reaching a concentration of 600 mg L<sup>-1</sup> has no negative effects on growth performance and proximate body composition of the juvenile *C. gariepinus*. The best apparent animal welfare was reached at concentrations of 200–400 mg L<sup>-1</sup>, with lower concentrations resulting in more fight events and higher concentrations reducing feed intake and increasing skin lesions (biting wounds). Consequently, K<sup>+</sup> concentrations inside the process

water up to 400 mg  $L^{-1}$  are tolerated and even beneficial for African catfish under RAS and possibly aquaponics production conditions.

## 3.1. Water Quality

To ensure that any differences between the treatment groups are exclusively attributable to the applied K<sup>+</sup> concentrations, all other influencing parameters were kept constant during the run of the experiment. The experiment was carried out in four identical RAS, positioned in the same room at a constant temperature, all treated in the same way (stocking procedure, feed input, water exchange and data acquisition/sampling) and using same-sized fish from the same batch and producer.

The water pH values, TAN, NO<sub>3</sub><sup>-</sup>-N and PO<sub>4</sub><sup>3-</sup>-P water concentrations, showed no significant differences between the treatment groups and were within the recommended range for African catfish (pH: 5.2–8.5 [16];  $NO_3^-$ -N: <140 mg L<sup>-1</sup> [35]; TAN: toxic, unionized form NH<sub>3</sub> nearly absent at pH values <7 [37,38]). Still, except for the intended different K<sup>+</sup> concentrations, minor differences in DO, temperature, conductivity, and NO<sub>2</sub><sup>--</sup>N concentrations were observed. However, these differences were small and within the tolerance level of African catfish (DO: >3.0–4.5 mg L<sup>-1</sup> [16,39]; temperature: ~25–30 °C [40,41]; conductivity: <2.5 ppm [42] (<5 mS cm<sup>-1</sup> at 29 °C); NO<sub>2</sub><sup>-</sup>-N: <0.6 mg L<sup>-1</sup> [34]). According to the observed values, in the case of DO, no differences in air-breathing events [36,39] and in the case of temperature [40] and  $NO_2^{-}-N$  [34], no difference in growth can be expected. In all treatment groups, the  $NO_3^-$ -N concentrations constantly increased over the run of the experiment, while the  $NO_2^{-}$ -N concentrations remained low around 0.5 mg L<sup>-1</sup>, confirming a regular functioning of the nitrifying biofilter. The TAN concentrations also remained low  $< 1 \text{ mg L}^{-1}$  for the most part of the experiment and started to increase (max. 3–8 mg  $L^{-1}$ ) only during the last two weeks, indicating a beginning overload of the biofilter. Consequently, the four test systems performed as intended during the run of the experiment, revealing comparable water parameters, with the main difference between the treatment groups remaining the four differently applied levels of potassium (K<sup>+</sup>).

## 3.2. Fish Performance and Welfare Indicators

The evaluation of fish performance is usually straightforward and reliable, as it is based on clearly measurable and calculable parameters, such as weight, length, growth, FCR, SGR and TFI. The determination of the actual welfare status of the animal is rather difficult, as the interpretation is based on indirect parameters. Therefore, welfare indication in fish is usually evaluated by a combination of different behavioral [43], performance [44] or immunological parameters [45].

The present study assessed the performance indicators final weight, final total length, final standard length, growth, SGR, FCR, TFI, and the behavioral welfare indicators skin lesions (biting wounds, proof of aggressive behavior), swimming (individual), resting (individual), agonistic behavior (individual), air-breathing (individual), stereotypic behavior (individual), stock resting (group), fight events (group) and feeding time (group) in African catfish reared under different water K<sup>+</sup> concentrations.

Considering weight, length, growth, SGR, FCR, and TFI, the fish performance in this study was not significantly different under the four K<sup>+</sup> water concentrations. The SGRs and FCRs in the present study were in accordance with Strauch et al. [36], who worked with similar-sized fish. The slightly higher FCR's and SGRs in the present study, when compared with the results by Strauch et al. [36], may be caused by differently applied feeding rates and feed.

The behavioral welfare indicators air-breathing rates, stereotypic behavior and aggregation behavior, did not differ between the groups in the present study. Van de Nieuwegiessen et al. [43] observed a greater variation of air-breathing rates (mean values 21–109 breaths fish<sup>-1</sup> h<sup>-1</sup>) depending on stocking density (max. 50–300 kg m<sup>-3</sup>). Compared to these frequencies, the breathing rates in the present study were rather low in relation to much lower stocking densities (present study: max. 17 kg m<sup>-3</sup>). Obviously, the much higher biomass in the study by Van de Nieuwegiessen [43] resulted in much lower DO levels, which resulted in higher air-breathing frequencies when compared with the present study.

It was conspicuous that the fish in K0 had the highest occurrence of agonistic behavior (individual) and fight events (group), while group K600 had the highest number of skin lesions and were least active at the same time. Neither agonistic behavior nor fighting were observed in K600. The lower activity in K600 was also reflected in a significantly elevated feeding time of  $\geq$ 3.7-fold higher than in the other groups.

It seems as if there is a positive influence at K200 and K400 and a negative influence on the fish at highly elevated water K<sup>+</sup> concentrations under K600.

As discussed previously [36], growth is the assimilation of energy in the form of feed-derived fat and protein. To be available for growth, the feed must be digestible, metabolizable, and not be required for maintenance metabolism. The costs for swimming and maintenance are significant, accounting for 15–30% per gross energy intake [46]. In freshwater fish, the internal ion concentration is higher than the external, resulting in the passive uptake of water and the loss of internal ions due to diffusion. The fish control internal homeostasis by producing dilute urine and the active uptake of ions from the surrounding water via the gills [47]. Located in the mitochondria-rich cells of the gills, Na<sup>+</sup>/K<sup>+</sup> ATPase [47], renal outer medullary K<sup>+</sup> channel (ROMKa) [48,49] and NKCC1a (Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> cotransporter) [49] are involved in the K<sup>+</sup> regulation mechanisms. Furukava et al. [48] and Horng et al. [49] showed an upregulation of ROMKa and NKCC1a mRNA's at elevated water K<sup>+</sup> concentrations (10 mM KCl [48] and 4.32 mM KCl [49]), resulting in higher energy consumption to maintain homeostasis.

It appears possible that in K200 and K400, the ion concentrations in the surrounding water were close to the physiological concentrations in the fish, hence close to a physiological-environmental equilibrium. Consequently, the energetic costs for maintaining osmotic homeostasis were lower, resulting in a better energy balance. Although not significantly, this was also reflected in the apparently slightly better FCR's and SGRs of these groups. It seems as if the fish in K600 use more energy for osmoregulation, struggling with the high K<sup>+</sup> concentrations. Consequently, less energy would be available for feed intake, swimming, agonistic behavior, and growth.

## 3.3. Body Composition

The proximate body composition of the fish did not reveal significant differences between the treatment groups, indicating efficient osmoregulation under reduced (K0) and highly elevated (K600) K<sup>+</sup>-concentrations. The body composition data in the present study were comparable with literature references for catfish [50–52]. The potassium, magnesium, sodium and phosphorus concentrations in the fish were also comparable with those reported by Strauch et al. [36]. In contrast to their study, where the phosphorus concentration inside the process water of *C. gariepinus* was elevated, the protein content in the fish of the present study was lower, while the fat and calcium content in the fish were higher. The ANNU for phosphorous and potassium were around 10% higher when compared with the results in their study [36], but at the same time for protein around 10% lower. These differences could be a result of different feed and feeding ratios that result in different nutrient uptake and storage. Though we could not detect any significant differences between the sampled treatment groups, there was a tendency that the ANNU of protein and potassium was slightly better in K200 and K400 and for phosphorous in the K400 group.

#### 3.4. Potential KOH Application

 $K^+$  is a vital macronutrient in commercial plant production. For hydroponic solutions, recommended  $K^+$  concentrations lie between 156 and 300 mg L<sup>-1</sup> [15]. African catfish aquaculture results in much lower levels of dissolved  $K^+$ , ranging between 9 and 122 mg L<sup>-1</sup> [14,16], much less than required to achieve optimal plant growth, especially in  $K^+$  demanding plants, such as tomatoes. Consequently, to achieve better plant performance

in African catfish aquaponics,  $K^+$  water concentrations should be adjusted to reach optimal plant requirements. This would allow improved plant growth, increased harvest and thus profitability of the system. Our results suggest that it is possible to increase  $K^+$  concentrations in African catfish process water up to 400 mg L<sup>-1</sup>, high enough to reach suggested optimal plant growth conditions (see above). Consequently, for a better performance of coupled and decoupled African catfish aquaponics, pH adjustment with KOH might be an optimal solution to reach both optimal pH adjustment and K<sup>+</sup> concentrations that optimize plant growth and aquaponics yield.

## 4. Materials and Methods

## 4.1. Experimental Design

The experiment was carried out in the aquaculture research facilities at the University of Rostock (Justus-von-Liebig-Weg 2, 18059 Rostock). The experimental setup consisted of four identical recirculating aquaculture systems (total water volume:  $500 \text{ L RAS}^{-1}$ ), each containing three aquaria (water volume per aquarium: 135 L), one sump with a pump, a heater, an integrated biofilter and a solid separation unit (further specifications see: [36]). Each system represented one experimental unit, only differing in potassium concentrations (for details, see Section 4.3). The total flow through in each system was set to 54 L min<sup>-1</sup> and adjusted to a flow-through of 18 L min<sup>-1</sup> in each aquarium.

#### 4.2. Fish and Feeding

The aquaria of each RAS and one additional aquarium were stocked with 16 juveniles of African catfish (*Clarias gariepinus*) (Fischzucht Abtshagen, Abtshagen, Germany), with a mean weight of 29.5 g fish<sup>-1</sup>. To allow the fish to acclimate to the experimental conditions, they were stocked seven days before the experiment started. After this acclimation period, the fish from the extra aquarium were used as reference (baseline) for proximate whole fish composition.

All fish were hand-fed with a commercial diet (Skretting Meerval ME-2, 2.0 mm) at 2% body weight per day during acclimation (7 days) and 3% of body weight during the experimental period (42 days) (calculations for fish biomass and daily feeding ration see (1) and (2). The fish were fed once a day between 9:00–10:00 am after recording the water parameters (Section 4.3). After offering feed to the fish for 30 min, the uneaten feed was removed, counted, and the dry mass of uneaten feed was determined by multiplying the number of uneaten pellets with the mean weight of one dry pellet (0.12 g pellet<sup>-1</sup>, mean value out of 75 pellets). The values were recorded and adjusted in the feeding table for further feeding calculations. Dead fish were removed, and the feeding table was adjusted to the current number of fish in the aquaria. During the experiment's run, two fish died in total, one in K0 and one in K600. Additionally, on 17 days (day 19, days 21–31 and days 36–40) of the experiment, the feeding time of fish for each aquarium was determined. This was done by measuring the time between the start (the first pellet touching the water surface) and the end (the last pellet was eaten, or the 30 min are over) of feeding.

Fish biomass per aquarium:

$$\mathbf{m}_{t} = \mathbf{m}_{0} \times \mathbf{e}^{(\text{SGR} \times t)} \tag{1}$$

Feed ration per aquarium and day:

$$m_F = m_t \times FR/100\% \tag{2}$$

where  $m_t$  = fish biomass in one aquarium at day t in g,  $m_0$  = fish biomass in one aquarium at stocking in g, SGR = specific growth rate (assumed value = 0.03), t = time since stocking in days,  $m_F$  = feeding ration of one day in g aquarium<sup>-1</sup>, FR = feeding ratio (2 or 3%).

According to our analysis (see Section 4.4), feed dry matter content was 926.8 g kg<sup>-1</sup> wet weight (ww) containing 514.8 g kg<sup>-1</sup> ww crude protein, 85.9 g kg<sup>-1</sup> ww crude fat, 110.2 g kg<sup>-1</sup> ww crude ash, 8.3 g kg<sup>-1</sup> ww K<sup>+</sup> and 14.3 g kg<sup>-1</sup> ww P, complemented

by manufacturer's specifications resulted in 14 g kg<sup>-1</sup> ww fiber, 4 g kg<sup>-1</sup> ww sodium, 25 g kg<sup>-1</sup> ww Ca<sup>+</sup>, 42 mg kg<sup>-1</sup> ww Fe, 2.1 mg kg<sup>-1</sup> ww iodine, 5 mg kg<sup>-1</sup> ww Cu, 16 mg kg<sup>-1</sup> ww Mn and 110 mg kg<sup>-1</sup> ww Zn. Before each sampling of the fish (baseline and final body composition), the animals were held without feeding for 2 days.

## 4.3. Experimental Units and Water Quality

During the acclimation period, the four RAS were treated equally. With the start of the experiment, the K<sup>+</sup> concentrations were targeted at four levels, with group 1 (control: K0, without addition of KCl): K<sup>+</sup> < 15 mg L<sup>-1</sup>, group 2 (K200): K<sup>+</sup> = 200 mg L<sup>-1</sup>, group 3 (K400): K<sup>+</sup> = 400 mg L<sup>-1</sup> and group 4 (K600): K<sup>+</sup> = 600 mg L<sup>-1</sup>. The final K<sup>+</sup> concentration in control resulted from feed digestion and excretion of the fish inside the tanks. The adjustment of the treatment concentrations was performed by solving KCl in pre-tempered tap water in separate "conditioning tanks" (HD-polyethylene, one per RAS). To adjust the same conductivity between groups (2400  $\mu$ S cm<sup>-1</sup>) in each RAS, NaCl was added to the water in the conditioning tanks from groups K0 to K400. The pretreated water was brought into the RAS as exchange water, which replaced approx. 30% per total RAS volume three times a week, on Mondays, Wednesdays, and Fridays.

The water quality parameters pH-value, water temperature (T), dissolved oxygen concentration (DO) and electric conductivity (EC), describing the general experimental conditions, were measured in triplicates with a multimeter (HACH<sup>®</sup> Multimeter HQ40d) daily, between 8:00–9:00 a.m. (before feeding), in each aquarium.

To determine the water concentrations of total ammonia nitrogen (TAN), total oxidized nitrogen (TON) (NO<sub>2</sub><sup>-</sup>-N + NO<sub>3</sub><sup>-</sup>-N), NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N, total dissolved nitrogen (TDN), ortho-phosphate phosphorus (PO<sub>4</sub><sup>3-</sup>-P), Mg<sup>2+</sup> and K<sup>+</sup>, water samples were taken from each sump, one before, and a second two hours after water exchange on Mondays, Wednesdays, and Fridays. The samples were stored in 100 mL plastic centrifuge tubes at -18 °C. The analysis were performed with an automated discrete analyzer (Thermo Fisher Scientific<sup>TM</sup> Gallery<sup>TM</sup>), according to the manufacturer's protocols. With the aid of the measured results (NH<sub>4</sub><sup>+</sup> (analytical method includes NH<sub>4</sub><sup>+</sup> + NH<sub>3</sub>), NO<sub>3</sub><sup>-</sup> (analytical method includes NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>), NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>3+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>), TAN (3), TON (4), NO<sub>2</sub><sup>-</sup>-N (5), NO<sub>3</sub><sup>-</sup>-N (6), TDN (7) and PO<sub>4</sub><sup>3-</sup>-P (8) concentrations were calculated.

Total ammonia nitrogen (TAN):

$$c_i (TAN) = c_i (NH_4^+) / M (NH_4^+) \times M (N)$$
 (3)

Total oxidized nitrogen (TON):

$$c_i (TON) = c_i (NO_3^{-})/M (NO_3^{-}) \times M (N)$$
 (4)

Nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N):

$$c_i (NO_2^- - N) = c_i (NO_2^-) / M (NO_2^-) \times M (N)$$
 (5)

Nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N):

$$c_i (NO_3^- - N) = c_i (TON) - c_i (NO_2^- - N)$$
 (6)

Total dissolved nitrogen (TDN):

$$c_{i} (TDN) = c_{i} (TON) + c_{i} (TAN)$$
(7)

Ortho-phosphate phosphorus ( $PO_4^{3-}-P$ ):

$$c_i (PO_4^{3-}-P) = c_i (PO_4^{3-}) / M (PO_4^{3-}) \times M (P)$$
 (8)

where  $c_i$  = concentration, M = molar mass, N = nitrogen, TAN = total ammonia nitrogen, NH<sub>4</sub><sup>+</sup> = ammonia nitrogen, NO<sub>3</sub><sup>-</sup> = nitrate, TON = total oxidized nitrogen, NO<sub>2</sub><sup>-</sup> = nitrite, TDN = total dissolved nitrogen, P = phosphorus, PO<sub>4</sub><sup>3-</sup> = orthophosphate.

#### 4.4. Analysis of Feed and Fish

At the end of the experiment, all fish were counted, weighed, and measured (total length and standard length). Skin lesions were counted. Whole fish samples for chemical analysis were taken pooled from 8 fish of each group. The fish were weighed, stunned by a percussion onto the head, afterwards they were immediately decapitated and frozen at -20 °C. The frozen fish samples (baseline and final sampling) were then homogenized with a meat mincing machine (Bosch ProPower MFW67440) before they were again stored at -20 °C until further analysis. Above that, a composite feed sample was taken by collecting three times 150 g (day 1, day 21 and day 42). The feed was mixed and homogenized by a knife mill (Retsch Grindomix GM 300) and stored at -18 °C until further analysis. Feed and fish samples were then analyzed for dry matter, ash, protein, fat, and the elements Ca, P, Na, Mg and K content at LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt der LMS Agrarberatung GmbH, Rostock, Germany) according to standard methods (VDLUFA Ill 3.1, VDLUFA Ill 8.1, VDLUFA Ill 4.1.1, VDLUFA Ill 5.1.1 (B), VDLUFA Ill 10.8.2).

#### 4.5. Performance Calculations

The total feed intake (TFI) (9), growth (10), feed conversion ratio (FCR) (11), specific growth rate (SGR) (12), mortality (13), and apparent net nutrient utilization (ANNU) (14) were calculated.

Total feed intake (TFI):

TFI = feed eaten by fish over the experimental period (g fish<sup>-1</sup>) (9)

Growth (G) (g):

$$G = E_t - W_0 \tag{10}$$

Feed conversion ratio (FCR):

$$FCR = TFI/W_t - W_0 \tag{11}$$

Specific growth rate (SGR):

SGR 
$$(\% d^{-1}) = ((Ln (W_t) - Ln (W_0))/d) \times 100$$
 (12)

Mortality (Mo):

Mo (%) = (number of dead fish/initial number of fish) 
$$\times$$
 100 (13)

Apparent net nutrient utilization (ANNU):

$$ANNU = ((W_t \times X_t - W_0 \times X_0) / (TFI \times X_F)) \times 100$$
(14)

where  $W_t$  = final fish weight,  $W_0$  = initial fish weight, d = days,  $X_0$  = initial nutrient concentration of the fish,  $X_t$  = final nutrient concentration of the fish, XF = nutrient concentration of the feed.

#### 4.6. Ethology

To evaluate fish ethology, the fish of each aquarium were observed for five minutes, once in the middle of the experiment (day 23) and once at the end of the experiment (day 42). At the beginning of the experiment, no observations were made to avoid the assessment of stocking-influenced data. All observations were made at 14:30 and on days where no water exchange was conducted to exclude postprandial somnolence or influence

of stress. During observations, the fish behavior was analyzed according to the ethogram in Table 6 (adapted from [43]). To evaluate individual behavior, one randomly chosen fish was observed for five minutes in each aquarium. If the sight of this fish was lost (e.g., due to murky water or fish swimming in front of the observed fish), it was replaced by the fish closest to where it disappeared. To assess group behavior, all visible fish in one aquarium were considered for evaluation.

Behavior		Definition		
	Swimming	Active displacement of the body while browsing, moving, and eating.		
	Resting	Moving passively through the water or lying still at the bottom of the tank.		
Individual	Agonistic behavior	Chasing or biting a fish or being chased upon or bitten by another.		
	Air-breathing	The animal moves to the water surface and takes a gulp of air. Air from the gills of the fish escapes when it swims back to the bottom of the tank.		
	Stereotypic behavior	Continuous and compulsive swimming under a fixed, repetitive pattern for at least 10 s.		
Group	Stock resting	More than 60% of the fishes in the stock show the behavior pattern "resting".		
	Fight event	Fight events between fishes that are not being individually observed.		
	Aggregation behavior	Gathering of more than 30% of the fishes of the stock in a small area, generally touching each other.		

Table 6. Ethogram—behavioral patterns and their definition, adapted from [43].

Therefore, the recordings (except for air-breathing) were divided into 10 s sections, and it was counted with "1" if the fish showed the respective behavior in a certain time frame ("0" if not). Afterward, the percentage proportion of the five minutes in which the fish showed the respective behavior was calculated (15). For air-breathing, the breaths of air within the 5 min were counted and multiplied by 12 to obtain the breathing rate in n fish<sup>-1</sup> h<sup>-1</sup>.

Percentage proportion of the shown behavior within the 5 min of observation:

Behavior (%) = 
$$(x \times 10 \text{ s}/300 \text{ s}) \times 100$$
 (15)

where x = sum of counted "1".

# 4.7. Statistics

The physicochemical water parameters, the growth and feed efficiency parameters, the fish composition, the apparent net nutrient utilization (ANNU) and the welfare indicators were tested for significant differences between the treatment groups (p < 0.05). All statistical analyses were conducted with "IBM SPSS Statistics 25". All data first were tested for normal distribution (Shapiro–Wilk test). If the data were normally distributed, the data were tested for homogeneity of variance (Levene's test). In the case of homogeneity of variance, the mean values were compared with ANOVA and Tukey-HSD as post hoc test (FCR; temperature; total length; standard length; whole fish composition: moisture, ash, fat and phosphorous; ANNU: P; fish ethology: resting (individual), aggregation behavior (group)). If the data showed no homogeneity of variance, the mean values were compared with the Welch's test and Dunnett's T3 test as post hoc test (growth; NO<sub>2</sub><sup>-</sup>-N). In the case of not normally distributed data, the Kruskal–Wallis test was performed using a Bonferroni-correction (SGR; TFI; DO; pH; conductivity; TAN; TON; NO<sub>3</sub><sup>-</sup>-N; TDN;

K<sup>+</sup>;  $PO_4^{3-}$ -P;  $Mg^{2+}$ ; total weight; whole fish composition: Na, Mg, K; ANNU: protein, potassium; feeding time; skin lesions; fish ethology: swimming (individual), agonistic behavior (individual), air-breathing (individual), fighting events (group), stock resting (group), stereotypic behavior (individual)).

## 5. Conclusions

Modern African catfish recirculating aquaculture production systems and integrated aquaponics have high investment and running costs. To be profitable, stock-, feed-, and water-management, and aspects of animal welfare, must be optimized. Many plants in aquaponics require adjusted nutrient solutions to achieve optimal growth and quality. This study demonstrates that K<sup>+</sup> can be added to the rearing water of African catfish juveniles up to 400 mg L<sup>-1</sup> without impairing the welfare status, productivity, or product quality. This suggests the safe use of potassium-containing alkalinity agents, such as KOH, to control the pH of the rearing water, with positive effects on nitrifying-biofilter activity, fish growth and welfare, but also on plant growth and quality in aquaponics.

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