Quantifying the dietary potassium requirement of juvenile hybrid tilapia (*Oreochromis niloticus*×*O. aureus*)

Shi-Yen Shiau* and Jia-Fen Hsieh

Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan 202, ROC

An 8 week feeding trial was conducted to determine the dietary K requirement for juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Purified diets with eight levels (0, 1, 2, 3, 4, 5, 7, 10 g/kg diet) of supplemental K were fed to tilapia. Each diet was fed to three replicate groups of fish initially weighing a mean value of 0·77 (SE 0·01) g/fish in a closed, recirculating rearing system. Weight gain was higher ($P < 0.05$) in fish fed the diets supplemented with 2, 3 and 4 g K/kg diet than in fish fed diet with 10 g K/kg diet and the unsupplemented control diet. Gill Na^+-K^+ ATPase activity was highest in fish fed the diets supplemented with 1–3 g K/kg diet, followed by fish fed the diet with 5 g K/kg diet and lowest in fish fed the diet with 10 g K/kg diet. Whole-body K content in fish were generally increased as the dietary K supplementation level increased. Analysis by polynomial regression of weight gain and gill Na^+-K^+ ATPase activity and by linear regression of whole-body K retention of the fish indicated that the adequate dietary K concentration for tilapia is about 2–3 g/kg diet.

Potassium: Fish: Tilapia

Na, K and chloride are essential minerals in animals because of their role in electrolyte and acid–base balance. Na and chloride are the principal extracellular cation and anion respectively, whereas K is the principal intracellular cation in animal tissues. Dietary requirements for these three minerals have been reported for several land animals; however, fish are know to readily exchange these minerals across their gills in order to maintain acid–base balance and osmotic pressure with their aquatic environment. It has been demonstrated that Na and chloride are not required in the diet of channel catfish (Bentley, 1990). However, it is generally accepted that K is required by fish (National Research Council, 1983). The quantitative requirement of dietary K for growth has been studied in only two species of fish but the results vary. Based on growth-response data, a dietary level of 8 g K/kg diet was reported to be required to produce maximum growth of chinook salmon (*Oncorhynchus tshawytscha*; Shearer, 1988). However, weight gain of channel catfish (*Ictalurus punctatus*) was reported not to respond quantitatively to the dietary K supplementation and a requirement of 2·6 g K/kg diet was established for this species based on the whole-body K retention (Wilson & El Naggar, 1992).

Tilapia are mainly lacustrine fish which are well adapted to enclosed water; they produce high yields and thus are an important human protein source. Production of tilapia has been increasing throughout the world, and a future increase in production has been projected (New, 1999). The purpose of the present study was to estimate the dietary K requirement of juvenile tilapia (*Oreochromis niloticus* × *O. aureus*) using growth indices supported by measurement of gill Na^+-K^+ ATPase activity and the whole-body K retention.

Materials and methods

Diet preparation

The experimental diet formulation is given in Table 1. The formulation is similar to that used by Shiau & Lo (2000), which has been shown to be adequate for tilapia. Vitamin-free casein (Sigma Chemical, St Louis, MO, USA) was used as the protein source. The mineral mixture was similar to that used by Shiau & Chin (1999), except that it did not contain K.

KCl (Merck Co, Darmstadt, Germany) was added to the test diets at the expense of cellulose to provide concentrations of 0, 1, 2, 3, 4, 5, 7 and 10 g/kg diet (DM basis). The K concentrations of the eight diets were determined by flame photometry after dry ashing according to the method

* **Corresponding author:** Dr Shi-Yen Shiau, fax +886 2 2462 1684, email syshiau@mail.ntou.edu.tw

Table 1. Composition of the basal diet (g/kg diet)*

Ingredient	Amount
Casein (vitamin free)	380
Corn starch	380
Corn oil	70
Fish oil	40
Carboxymethylcellulose	20
Vitamin mixture†	20
Mineral mixture‡	40
α-Cellulose	50

* Proximate analysis of basal diet (g/kg): moisture 130·1, crude protein 336·4, diethyl ether extract 103·3, ash 31·7, crude fibre 69·1.

† Vitamin mixture (mg/g mixture): thiamin hydrochloride 5, riboflavin 5, calcium pantothenate 10, nicotinic acid 6·05, pyridoxine hydrochloride 0·825, folic acid 1·5, inositol 200, L-ascorbyl-2-monophosphate-Mg 2·025, choline chloride 44, menadione 4, α-tocopherol acetate 40, para-aminobenzoic acid 5, retinol acetate 0·4, cholecalciferol 0·4685 μg, biotin 0·003. All ingredients were diluted with α-cellulose to 1 g.

‡ Mineral mixture (mg/g mixture): calcium biphosphate 135·8, calcium lactate 327, ferric citrate 29·7, $MgSO_4 \cdot 7H_2O$ 137, sodium biphosphate 87·2, NaCl 43·5, $AlCl_3 \cdot 6H_2O$ 0·15, KI 0·15, $CuCl_2$ 0·1, $MnSO_4 \cdot H_2O$ 0·8, $CoCl_2 \cdot 6H_2O$ 1, $ZnSO_4 \cdot 7H_2O$ 3. All ingredients were diluted with α-cellulose to 1 g.

of the Association of Official Analytical Chemists (1995) and found to be 0·5 (unsupplemented control), 1·4 (1 g/kg), 2·5 (2 g/kg), 3·5 (3 g/kg), 4·6 (4 g/kg), 5·5 (5 g/kg), and 9·7 (10 g/kg). The diets were prepared by thoroughly mixing the dry ingredients with oil and then adding cold water until a stiff dough resulted. This was then passed through a mincer with a die and the resulting strings were dried using an electrical fan at 28°C. After drying, the diets were broken up and sieved into pellets and stored at −20°C.

Experimental procedure

Male hybrid tilapia were supplied from the Far East Hatchery (Cha-Yi, Taiwan). Upon arrival, they were acclimated to laboratory conditions for 4 weeks in a plastic tank (74 cm wide, 95 cm long, 45 cm high) and fed a commercial diet (Hung Kuo Industrial, Taipei, Taiwan). At the beginning of the experiment, fifteen fish (mean weight 0·77 (SE 0·01) g) were stocked in each aquarium (30·5 cm wide, 61·0 cm long, 55·5 cm high). There were eight treatments. Each experimental diet was fed to fish in three

aquaria. The fish chosen for the experiment and the diets assigned to groups of fish randomly. Each aquarium was part of a closed-recirculated system with a common water reservoir maintained at 26 ± 1°C. The water was circulated at 2 l/min through two separate biofilters to remove impurities and reduce NH_3 concentrations.

The fish were fed 50 g/kg body weight per day. This amount was close to the maximum daily rations consumed by the tilapia during the acclimation period. The daily ration was subdivided into two equal feedings and fed at 09.00 and 17.00 hours. Fish were weighed once every 2 weeks and the daily ration adjusted accordingly. A photoperiod of 12 h light–dark (light 08.00–20.00 hours) was used. The fish were fed the test diets for an 8 week period.

At the end of the feeding trial, the fish were weighed. Weight gain (as measured by the percentage of body-weight gain), feed efficiency and protein efficiency ratio were calculated as described previously (Shiau & Liu, 1994; Chou & Shiau, 1999). After the final weighing, four fish were randomly removed from each aquarium, gill samples were collected and pooled for Na⁺-K⁺ ATPase activity determination (Hwang *et al.* 1988). Four other fish were then taken randomly from each aquarium and pooled for body K determination (Association of Official Analytical Chemists, 1995).

Statistical analysis

Results were analysed by one-way ANOVA. When the ANOVA identified differences among groups, multiple comparisons among means were made with Duncan's new multiple range test. Statistical significance was determined by setting the aggregate type I error at 5 % ($P < 0.05$) for each set of comparisons. Dietary K requirements for juvenile tilapia were estimated by the polynomial regression method (Zeitoun *et al.* 1976) based on weight gain and gill Na⁺-K⁺ ATPase activity of the fish and by the linear regression analysis of the dietary K levels *v.* whole-body K balance values.

Table 2. Weight gain, feed efficiency (FE), protein efficiency ratio (PER), survival and gill Na⁺-K⁺ ATPase activity of tilapia fed diets containing various levels of potassium for 8 weeks*

Potassium (g/kg diet)	Weight gain (%)†	FE‡	PER§	Survival (%)	Na⁺-K⁺ ATPase activity (μmol inorganic phosphate/mg protein per h)
0	299·08ᵃ	0·56ᵃ	2·39ᵃᵇ	83·33	4·91ᵇᶜ
1	336·39ᵃᵇ	0·62ᵃᵇ	2·68ᵇ	94·45	5·44ᶜ
2	358·96ᵇ	0·64ᵇ	2·77ᵇ	88·89	5·72ᶜ
3	356·60ᵇ	0·64ᵇ	2·70ᵇ	83·33	5·61ᶜ
4	345·76ᵇ	0·63ᵇ	2·65ᵇ	83·33	5·25ᵇᶜ
5	333·76ᵃᵇ	0·63ᵇ	2·44ᵇ	88·89	4·86ᵇ
7	316·89ᵃᵇ	0·61ᵃᵇ	2·29ᵃᵇ	81·29	4·78ᵃᵇ
10	282·58ᵃ	0·57ᵃ	1·94ᵃ	80·56	4·35ᵃ
Pooled SEM	16·63	0·03	0·25	8·11	0·24

a,b,cMean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

* Values of weight gain, FE, PER and survival are means of three groups of fish, with fifteen fish per group (*n* 3). Values of Na⁺-K⁺ ATPase activity are means of three groups of fish, with four fish per group (*n* 3). For details of diets, see Table 1.

† Weight gain (%) = 100 × (final weight (g) − initial weight (g))/initial weight (g).

‡ Feed efficiency = (final weight (g) − initial weight (g))/total feed intake (g).

§ Protein efficiency ratio = (final weight (g) − initial weight (g))/total protein intake (g).

Results

The weight gains were significantly ($P < 0.05$) higher in fish fed the diets supplemented with 2, 3 and 4 g K/kg diet than in fish fed the diet supplemented with 10 g K/kg diet and the unsupplemented control diet (Table 2). Patterns of feed efficiency and protein efficiency ratio were similar to those of the weight gain. Survival of fish fed the control diet and K-supplemented diets were 81–94 %. Gill Na$^+$-K$^+$ ATPase activity was highest in fish fed the diets supplemented with 1–3 g K/kg diet, followed by fish fed the diet with 5 g K/kg diet and lowest in fish fed the diet with 10 g K/kg diet.

When the polynomial regression model (Zeitoun et al. 1976) was employed to express the relationship between weight gain, gill Na$^+$-K$^+$ ATPase activity and dietary K concentration, a growth peak and maximum enzyme activity was reached when dietary K concentrations were 3·0 and 2·4 g/kg diet respectively (Fig. 1).

Whole-body K content in fish generally increased as the dietary K supplementation level increased ($y = 7.89x + 6.84$, r 0·93, Table 3). When these data were used to calculate the whole-body K retention for each group of fish, linear regression analysis of the dietary K levels v. whole-body K retention values (Fig. 2) indicated a K requirement of 1·9 g/kg diet ($y = -17.04x + 3.27$, r 0·99).
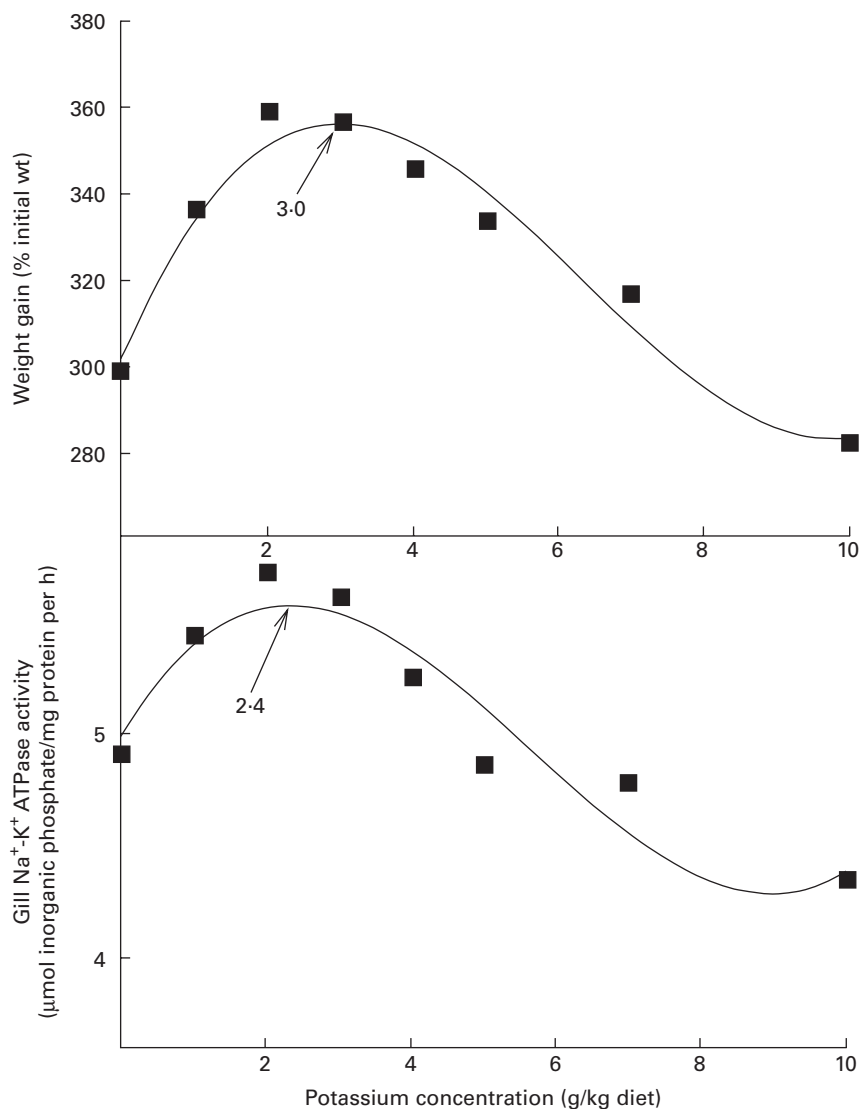
Fig. 1. The effect of dietary potassium on relative weight gain and gill Na$^+$-K$^+$ ATPase activity of tilapia. For details of diet, see Table 1 and for procedures, see p. 214. Each point represents the mean of three groups of fish (n 3), with remains of the fifteen fish (weight gain) and four fish (Na$^+$-K$^+$ ATPase activity) per group. Requirements derived with the polynomial regression method for weight gain, gill Na$^+$-K$^+$ ATPase activity are 3·0 and 2·4 g/kg diet respectively. For weight gain $y = 466.98x^3 - 888.59x^2 + 403.00x + 302.61$, r 0·96. For gill Na$^+$-K$^+$ ATPase activity $y = 8.75x^3 - 14.81x^2 + 5.45x + 4.99$, r 0·89.

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Table 3. Whole-body potassium balance of tilapia fed diets containing various levels of potassium for 8 weeks*

(Mean values for three groups of fish, with four fish per group (n 3))

| Potassium (g/kg diet)	Initial whole-body potassium content (mg)	Total potassium fed (mg)	Final whole-body potassium content (mg)	Final − initial whole-body potassium content (mg)	Whole-body potassium retention (mg)†
0	0·3	1·8	6·3	6·0	4·2
1	0·3	5·0	6·9	6·6	1·6
2	0·3	8·0	8·3	8·0	0
3	0·3	11·7	9·6	9·3	−2·4
4	0·4	14·4	10·8	10·4	−4·0
5	0·4	17·3	11·8	11·4	−5·9
7	0·4	21·1	12·5	12·1	−9·0
10	0·4	26·3	13·8	13·4	−12·9

* For details of diets, see Table 1.
† Final − initial body potassium concentration − total potassium fed.

Discussion

The results of this study indicate that tilapia have a requirement of K that can not met by K in the rearing water. A dietary supplementation is necessary. The K content in the rearing water of this study ranged from 1·08 μg/g (at the beginning of the experiment) to 3·62 μg/g (at the end of the experiment). K uptake directly from the water was observed in the present study as fish fed diets with lower K supplementation (i.e. 0, 1 and 2 g K/kg diet) were able to accumulate considerably more K than they were fed (Table 3).

Reduced growth in fish was associated with dietary K deficiency. Zeitoun et al. (1976) have suggested the use of polynomial regression analysis as a means of estimating the relationship between weight gain and essential nutrient intake. As indicated by Zeitoun, the value corresponding to maximal gain estimated by cubic regression is defined as the maximum concentration of dietary nutrient that produces optimal growth, and beyond which growth is
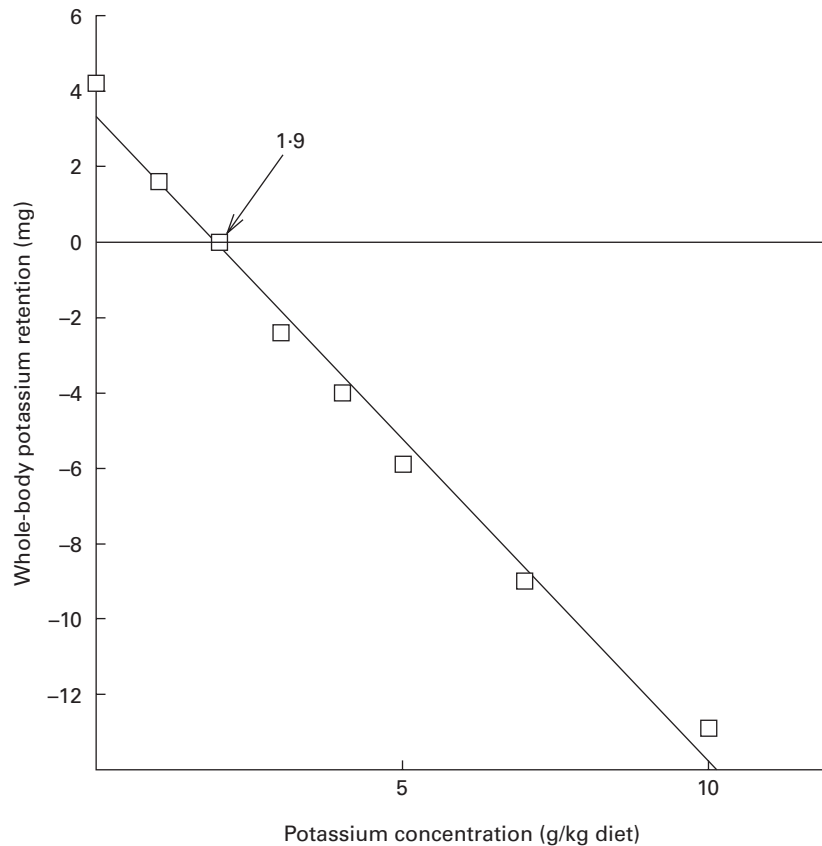
Fig. 2. The relationship between dietary potassium concentration and whole-body potassium retention in tilapia. For details of diet, see Table 1 and for procedures, see p. 214. Each point represents the mean of three groups of fish (n 3) with four fish per group. Requirement derived with the linear regression method is 1·9 g/kg diet ($y = -17.04x + 3.27$, r 0·99).

depressed. In the present study, the weight gain of fish reached a maximum at 2 g K supplementation/kg diet and decreased thereafter. Thus, the requirement was estimated by a polynomial regression analysis (Zeitoun *et al.* 1976). Total body K content of fish K-supplemented diets generally increased as the K supplementation level increased (Table 3) indicating that these animals do have a dietary requirement for K. The small size of the fish used in this study precluded a tissue-by-tissue analysis of K content related to dietary K.

Na^+-K^+ ATPase is a universal membrane-bound enzyme that actively transports Na^+ out of and K^+ into animal cells. It is not only crucial for maintaining intracellular homeostasis, but also for providing a driving force for K^+ transport in a variety of osmoregulatory epithelia, including mammalian kidney tubules, bladder, and intestine, reptilian and avian nasal salt glands, the salt gland of the invertebrate brine shrimp, and elasmobranchial rectal glands and teleostean gills. Euryhaline teleosts inhabit environments ranging from fresh water to seawater of high salinity. Through effective mechanisms of osmoregulation, teleosts are able to retain an osmotic and ionic constancy in the internal milieus. Gills are the most important extra-renal organs responsible for osmoregulation in fish. The biochemical mechanisms for maintenance of constant levels of ions in body fluids depend on the activity of Na^+-K^+ ATPase, and the activities of gill Na^+-K^+ ATPase in euryhaline teleosts are affected by environmental salinities and ion concentrations (De Renzis & Bornancin, 1984; Hwang *et al.* 1989; Mayer-Gostan & Naon, 1992; McCormick, 1995). The altered gill Na^+-K^+ ATPase activity found in the present study suggests that this variable may permit a satisfactory evaluation of K status of the fish. The broken-line analysis of weight gain and gill Na^+-K^+ ATPase activity in tilapia (Fig. 1) suggests that gill Na^+-K^+ ATPase activity can be used to estimate the K requirements of fish.

The dietary K requirement of tilapia is similar to that of channel catfish (Wilson & El Naggar, 1992). A much higher dietary K requirement (i.e. 8 g K/kg diet) was reported for chinook salmon (Shearer, 1988). Our present study confirmed the K requirement for chinook salmon being questioned by Wilson & El Naggar (1992) as an overestimation. However, the possibility of species differences and/or differences between freshwater and seawater fish should also be noted. The study with channel catfish showed that weight gain of the fish did not respond to the dietary K supplementation. However, a requirement of 2·6 g K/kg diet was obtained when the whole-body K retention was calculated. In the present study, the dietary K requirement in tilapia was obtained on the weight-gain data. The whole-body K retention data was also used to quantify the requirement and the values from the two variables agree well (Figs. 1 and 2). The reason for growth responding differently in tilapia and channel catfish when fed graded supplementation levels of K is unknown. Note, however, that the initial weight of fish used in two studies differs (i.e. 0·77 g for tilapia *v.* about 15·9 g for channel catfish). Nevertheless, the dietary K requirement of the two species are similar. This similarity in requirement values between the tilapia and catfish is also the same as has been reported for other animals, i.e. 2·3 g K/kg diet for the rat (Bieri, 1977), 2·5 g K/kg diet for the chick (Leach *et al.* 1959) and 2·6 g K/kg diet for the young pig (Jensen *et al.* 1961) indicating that K may serve similar physiological functions in these animals.

In summary, weight gain of tilapia was improved as the level of K increased in the diet, reaching a peak after the requirement was met. A similar trend was observed in gill Na^+-K^+ ATPase activity in fish fed the different diets. The dietary K requirement in fish can also be obtained when the total body K balance is attained. Collectively, the minimal dietary K requirement for growing tilapia in water containing 2·4 mg K/litre water is about 2–3 g K/kg diet.

Acknowledgements

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