Kafkas Univ Vet Fak Derg
16 (Suppl-B): S271-S275, 2010
DOI:10.9775/kvfd.2010.2079

RESEARCH ARTICLE

The Effects of Diets Supplemented with $CaCl_2$ and Rock Salt on Growth, Feed Efficiency and Some Serum Mineral Levels in Rainbow Trout, *Oncorhynchus mykiss* [1]

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[1] *The study was granted by Ministry of Agriculture and Rural Affairs (MARA) of Turkiye (TAGEM-GYAD-11/02/076)*

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Makale Kodu (Article Code): KVFD-2010-2079

Summary

In this study, the effects of $CaCl_2$ (2%) supplemented diet, rock salt (RS, 2%) supplemented diet and control diet (no supplement) on the weight gain, specific growth rate, daily feed intake, feed conversion rate, protein efficiency rate and serum mineral concentration (Na, K, Cl) were investigated in rainbow trout. RS was used for increasing the CAD and Na/K ratio of the control diet and $CaCl_2$ was used for decreasing the CAD level of the control diet. A total of 300 fish, 2 replications for each treatment consisting of 50 fish with a mean initial body weight of 88.2 g were used in the experiment. The experiment lasted fifteen weeks. The diet supplemented with 2% RS improved significantly the weight gain, feed conversion rate and protein efficiency rate in rainbow trout compared to 2% $CaCl_2$ supplemented group and control ($P<0.05$). Dietary RS supplementation also improved the specific growth rate and daily feed intake. However, this improvement was not statistically significant ($P>0.05$). Dietary 2% $CaCl_2$ supplementation did not significantly affect the weight gain, specific growth rate, daily feed intake, feed conversion rate and protein efficiency rate in rainbow trout compared to control diet ($P>0.05$). Na, K and Cl level of the serum and mortality rate were not statistically different among the groups ($P>0.05$). The dietary 2% RS supplementation produced a better growth and feed efficiency for rainbow trout compared to dietary 2% $CaCl_2$ supplementation and control diet. Added RS to diet was appropriate for making cationic the diet and increasing the CAD level and Na/K ratio of diet.

Keywords: *Rainbow trout, $CaCl_2$, Rock salt, Cation-anion difference (CAD), Growth, Feed efficiency, Serum mineral level*

$CaCl_2$ ve Kaya Tuzu Ilave Edilmiş Rasyonların Gökkuşağı Alabalığının *(Oncorhynchus mykiss)* Büyüme ve Yemden Yararlanma ve Kimi Serum Mineral Düzeylerine Etkisi

Özet

Bu çalışmada, $CaCl_2$ (%2) ve kaya tuzu (%2) ilave edilmiş diyetlerin kontrol diyetine (ilave katkı yok) kıyasla Gökkuşağı alabalıklarında *(Oncorhynchus mykiss)* canlı ağırlık kazancı, spesifik büyüme oranı, günlük yem alımı, yem dönüşüm oranı, protein etkinlik oranı ve serum mineral konsantrasyonu (Na, K, Cl) üzerine etkileri araştırıldı. Kaya tuzu ilavesi kontrol diyetinin Katyon-anyon farkını ve Na/K oranını arttırmak için, $CaCl_2$ ilavesi ise kontrol diyetinin katyon-anyon farkını düşürmek için kullanıldı. Her birinde 50 balığın kullanıldığı 2 tekerrürlü denemelerde başlangıç ortalama ağırlıkları 88.2 g olan, 300 gökkuşağı alabalığı kullanıldı. Deneme on beş hafta sürdürüldü. %2 Kaya tuzu ilave edilmiş diyet, %2 $CaCl_2$ ilave edilen diyete ve kontrol diyetine göre gökkuşağı alabalığında canlı ağırlık kazancını, yem dönüşüm oranını ve protein etkinlik oranını önemli şekilde iyileştirmiştir ($P<0,05$). Yeme kaya tuzu ilavesi spesifik büyüme oranını ve günlük yem alımını da ilerletmiştir. Ancak bu ilerleme istatistiksel olarak önemsiz bulunmuştur ($P>0,05$). %2 $CaCl_2$ ilave edilen diyet ile kontrol diyeti arasında canlı ağırlık kazancı, spesifik büyüme oranı, günlük yem alımı, yem dönüşüm oranı, protein etkinlik oranı açısından fark bulunmamıştır Serum Na, K ve Cl seviyeleri ve ölüm oranları açısından gruplar arasında farklılık bulunmamıştır ($P>0,05$). Diyete %2 Kaya tuzu ilavesi, kontrol grubuna ve %2 $CaCl_2$ ilave edilen gruba kıyasla gökkuşağı alabalığında daha iyi büyüme ve yem değerlendirme sağlamıştır. Gökkuşağı alabalığı diyetine kaya tuzu ilavesi, diyeti katyonik hale getirmek ve diyetin katyon-anyon farkını ve Na/K oranını artırmak için uygundur.

Anahtar sözcükler: *Gökkuşağı alabalığı, $CaCl_2$, Kaya tuzu, Katyon-anyon farkı (KAF), Büyüme, Yem değerlendirme, Serum mineral seviyesi*

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INTRODUCTION

Dietary cation anion difference, CAD, is defined as Na+K-Cl and expressed as mEq kg^{-1}. Dietary CAD level has an influence on blood pH, feed intake and growth in dairy cows [1], dairy calves [2], growing steers, lambs [3], chicken [4], horses [5] and juvenile African catfish, Clarias gariepinus [6]. In juvenile African catfish, Dersjant-Li et al. showed that feed intake and growth increased linearly with dietary cation anion difference from -100 to 700 mEq kg^{-1}, and the level of 700 mEq kg^{-1} was optimal [6]. In their later study, Dersjant-Li et al.[7] investigated the effect of dietary Na/K ratios on feed intake, growth and nutrient utilisation of juvenile African catfish at an optimal dietary CAD level of 554-657 mEq kg^{-1} and showed that the dietary Na/K ratio of 1.5 to 2.5 produced the best growth in African catfish.

Some researchers reported that effects of CAD were no clear on growth in rainbow trout, *Oncorhynchus mykiss* [8-11]. We have only limited data on the effects of dietary CAD level and dietary Na/K ratio on growth and feed efficiency in rainbow trout. However Arabacı, Nursoy and Diler [12] concluded that growth increased linearly, feed conversion rate decreased linearly with increasing dietary Na/K ratio-CAD level and daily feed intake was not significantly influenced in rainbow trout. They observed that the best growth was taken with the dietary Na/K ratio and dietary CAD level between 2.15-560 and 3.53-981 (mol mol^{-1}-mEq kg^{-1}) for rainbow trout. For regulating the dietary Na/K ratio and dietary CAD level, they supplemented the rainbow trout diet with $NaHCO_3$ and Na_2CO_3.

In this study, rainbow trout diet were supplemented with cationic (Rock salt, RS) and anionic salt ($CaCl_2$). 2% RS was used for increasing the CAD and Na/K ratio of the control diet and 2% $CaCl_2$ was used for decreasing the CAD level of the control diet.

The objective of the study was therefore to determine the effects of diets, supplemented with $CaCl_2$ (2%) and rock salt (2%), on growth, feed efficiency and serum mineral concentration in rainbow trout.

MATERIAL and METHODS

Fish and Facilities

The study was conducted at Institute of Mediterranean Fisheries Research, Production and Training, Kepez Unit, Antalya, Türkiye. A total of 300 rainbow trout, 2 replications for each treatment consisting of 50 fish with a mean initial body weight of 88.2±2.4 g (mean±SE) were used in the experiment. Before the commencement of the study, fish were acclimated to rearing conditions for 2 weeks. The study, dietary 2% $CaCl_2$ and RS supplementation were tested, lasted fifteen weeks. Fish were reared at a temperature of 11.2-15°C, under natural light regime. The O_2 concentration was maintained above 6 ppm. Six concrete ponds were used in the study. Each tank, having 40x40x200 cm of dimensions and with 280 liters capacity, received a constant flow of water (1.0 $l.sn^{-1}$). Hardness and conductivity of water used in the experiment was 370 mg/lt $CaCO_3$ and 650 µmho.cm^{-1}, respectively.

Diets and Feeding

The basal diet (Control) supplemented with rock salt and $CaCl_2$. Supplementation level was selected as 2% for seeing clearly the effects of the salts in the diet. The basal diet was given in *Table 1*.

Diets were pelleted at the size of 3 mm. During fifteen weeks of feeding period, meals were supplied to fish manually three times daily (at 09:00, 13:00 and 17:00 h) according to feeding rate. Feeding rate was predetermined according to live weight and water temperature (1-20 days, 2% at 15°C; 21-41 days, 1.8% at 13°C; 42-62 days, 1.7% at 12°C; 63-83 days, 1.5% at 11.5°C; 84-105 days, 1.5% at 11.2°C). However, feed was supplied in small quantities, assuring that fish ate to satiation *(ad libitum)* and no feed was wasted. When predetermined feed amount was not sufficient for satiation, more feed was given to fish. The amount of given feed per day was recorded. Live weight was determined 6 times with 21-day intervals from the initiation of experiment to the end of experiment.

Blood (1-2 ml) was sampled from 12 fish in each group, by inserting a syringe into the caudal vessels. The blood samples were centrifuged (1.500 g for 20 min) to obtain serum. Serum samples were stored -20°C until analysis of mineral contents. In each serum sample, Na^+, K^+ and Cl^- levels were measured in Easy Lyte Plus Na/K/Cl electrolyte analyser by ISE method.

Dietary pH was measured as described by Tarakçı [16]. Dry matter (105°C, overnight), ash (550°C, overnight), crude protein (nitrogen×6.25), ether extract, crude fiber of diets were analysed by methods of AOAC [15]. Dietary Na^++K^+-Cl^- or CAD level (mEq kg^{-1}) were determined by Horst et al. and dietary Na/K ratio (mol/mol) was measured from the Na^+, K^+ and Cl^- in diets which were analysed according to Kaçar [1,17]. Calculations used in this study were given in *Table 2* [18,19].

Statistical Analysis

Results are presented as mean values ± standard

Table 1. *Ingredients and chemical composition of the diets used in the experiment. RS; Rock salt*

Tablo 1. *Deneme diyetlerinin içerikleri ve kimyasal analizler. RS; Kaya tuzu*

Ingredients %	**Treatment Diets**		
	2% $CaCl_2$ Suppl.	**Control**	**2% RS Suppl.**
Fish meal	45	45	45
Soybean meal	15	15	15
Full fat soybean meal	10	10	10
Blood meal	3	3	3
Wheat middlings	12	14	12
Fish oil	10	10	10
$CaCl_2$	2	-	-
Rock salt	-	-	2
Vitamin premix [1]	2	2	2
Mineral premix [2]	1	1	1
Vitamin C [3]	0.3	0.3	0.3
Choline [4]	0.15	0.15	0.15
Methionine	0.15	0.15	0.15
Pellet binder	0.4	0.4	0.4
Antioxidant	0.3	0.5	0.3
TOTAL	100.0	100.0	100.0
Analysed Composition			
Dry matter, %	89.25	90.20	89.36
Crude protein, %	46.80	48.01	48.52
Ether extracts, %	13.31	14.81	14.11
Crude fiber, %	2.24	2.01	2.05
Crude ash, %	10.61	10.08	10.66
Na, %	0.23	0.26	0.84
K, %	1.01	1.01	0.93
Cl, %	0.98	0.96	0.94
DE*, kcal kg^{-1}	3262	3262	3322
Feed pH	5.62	5.96	6.09
Dietary calculated CAD ** level, mEq kg^{-1}	83	101	338
Dietary Na/K, mol/mol	0.35	0.4	1.39

*[1] **Vitamin mixture:** Included of per kg; 18.000 IU Vit A, 2.000 IU Vit D_3, 200 IU Vit E, 12 mg Vit K, 150 mg Vit C, 30 mg Vit B_2, 20 mg Vit B_1, 0.05 mg Vit B_{12}, 20 mg pyridoxine, 10 mg panthotenic acid, 220 mg niacin, 210 mg inositol, 5 mg folic acid, 0.5 mg biotine, 2.000 mg choline. Hoffman La-Roche Inc, Istanbul, Turkiye*
*[2] **Mineral mixture:** Included of per kg: 70 mg Zn, 60 mg Mn, 60 mg Mg, 4 mg Fe, 2 mg Cu, 1.5 mg I, 0.5 mg Co, 0.05 mg Se. Hoffman La-Roche Inc, Istanbul, Turkiye*
*[3] **Vitamin C,** Hoffman La-Roche Inc, Istanbul, Turkiye*
*[4] **Choline,** Ufuk Kimya Ilaç San. ve Tic. Ltd. Sti Istanbul, Turkiye*
** **DE:** Digestible Energy which was calculated with NRC (1993) and New (1987) of tables using for diet ingredients [13,14]*
*** Calculated from Na, K, Cl content of diet*

error. Results were analyzed by one-way analyses of variance (ANOVA) and the treatment means were compared by Duncan's multiple range tests. Significance was tested at the P<0.05 level. Homogeneity of variance was performed by Levene test and variance was found to be homogenous (SPSS 17.0, released version) [20].

Table 2. *Growth performance, feed efficiency and serum mineral levels in experimental groups (Mean±Standard error). RS; rock salt*

Tablo 2. *Deneme gruplarında büyüme performansı, yem değerlendirme ve serum mineral konsantrasyonu (Ortalama ±Standart hata). RS; Kaya tuzu*

Growth±SE	**Treatment**		
	2% $CaCl_2$ Suppl.	**Control**	**2% RS Suppl.**
WG [1]	139.5±1.5 [b]	138.5±3.5 [b]	158±5 [a]
SGR [2]	0.92±0.05 [a]	0.88±0.03 [a]	1.01±0.03 [a]
Feed efficiency	-	-	-
DFI [3]	1.42±0.03 [a]	1.39±0.01 [ab]	1.31±0.01 [b]
FCR [4]	1.63±0.08 [a]	1.67±0.04 [a]	1.38±0.02 [b]
PER [5]	1.28±0.06 [b]	1.28±0.03 [b]	1.49±0.02 [a]
Minerals in serum *	-	-	-
Na	152.81±8.31 [a]	144.26±9.7 [a]	150.79±2.79 [a]
K	13.34±1.98 [a]	15.69±3.57 [a]	14.18±0.30 [a]
Cl	125.08±2.56 [a]	121.92±3.26 [a]	133.59±2.61 [a]

Different superscript in a raw indicates significant differences
** The initial values for serum mineral levels were 140.04±0.93, 13.24±0.63 and 118.76±0.62 mmol l^{-1} for Na, K and Cl, respectively (n=12 for each group).*
[1] The weight gain (WG, g) = last biomass-first biomass.
[2] The specific growth rate (SGR, % day^{-1}) = 100 x ([ln w2-ln w1]/feeding days)
[3] The Daily feed intake (DFI, %) = 100 x (Feed consumed, g/feeding days/fish number)/((initial biomass, g + final biomass, g)/2).
[4] The feed conversion rate (FCR) = Feed consumed/(Increase in biomass + dead fish biomass).
[5] Protein Efficiency Rate (PER) = Increase in biomass, g/Protein consumed, g

RESULTS

The diet supplemented with 2% RS improved significantly the weight gain, feed conversion rate and protein efficiency rate in rainbow trout compared to 2% $CaCl_2$ supplemented group and control (P<0.05, *Table 2*). Dietary RS supplementation also improved the specific growth rate and daily feed intake. However, this improvement was not statistically significant (P>0.05). Dietary 2% $CaCl_2$ supplementation did not significantly affect the weight gain, specific growth rate, daily feed intake, feed conversion rate and protein efficiency rate in rainbow trout compared to control diet (P>0.05, *Table 2*).

Na, K and Cl level of the serum were not statistically different among the groups *(Table 2)*. Mortality was 2% in all groups and insignificantly influenced by supplementation of $CaCl_2$ and RS.

DISCUSSION

The results of the present study indicated that the diets supplemented with 2% RS improved the weight

gain, feed conversion rate and protein efficiency rate comparing to control and 2% $CaCl_2$ groups in rainbow trout. Rock salt used in the experiment increased either dietary Na/K ratio or dietary CAD level more than three times (dietary Na/K increased from 0.4 to 1.39 meq kg^{-1}, dietary Na/K ratio increased from 101 to 338 mol/mol, *Table 1*). On the other hand, $CaCl_2$ supplementation decreased the dietary CAD level only 20% *(Table 1)*. Purity of additive and/or interaction among the ingredients of diet may be cause of this. $CaCl_2$ supplementation to diet is generally appropriate for making diet anionic and decreasing the dietary CAD level.

The studies about the effects of dietary CAD level and dietary Na/K ratio on growth performance in fish species are few in number. The most studied fish species on this topic are African catfish [6,7] and rainbow trout [8-11].

Different dietary CAD and Na/K regulations caused to different response in African catfish in respect of growth performance and feed efficiency. When only dietary CAD was increased within the range -100 to 700 mEq kg^{-1}, feed intake and growth increased linearly with an increase in dietary CAD and feed conversion rate was not significantly influenced [6]. When only dietary Na/K ratio was increased (from 0.2 to 2.5 mol mol^{-1}) at optimal dietary CAD level (554-657 mEq kg^{-1}), growth increased quadratically, feed conversion rate decreased quadratically and feed intake was not significantly influenced [7]. In previous studies, it was reported that effects of CAD were no clear on growth in rainbow trout, *Oncorhynchus mykiss* [8-11]. Recently, Arabacıet al. [12] reported that growth increased linearly, feed conversion rate decreased linearly with increasing dietary CAD level and Na/K ratio, and daily feed intake was not significantly influenced in rainbow trout.

In the present study, RS supplementation, increasing dietary CAD level and dietary Na/K ratio, improved the weight gain and feed efficiency compared to $CaCl_2$ supplemented group and control group ($P<0.05$). However, specific growth rate increased, but this was not statistically significant ($P>0.05$). The cause of the partial improvement in growth may be an insufficient level of dietary CAD level and dietary Na/K ratio. Because Arabacı et al. [12] observed that the best growth was taken with the dietary Na/K ratio and dietary CAD level between 2.15-560 and 3.53-981 (mol mol^{-1}-mEq kg^{-1}) for rainbow trout. On the other hand, in this study growth and feed efficiency didn't improve in 2% $CaCl_2$ supplemented group having low dietary CAD level (83 meq kg^{-1}) and low dietary Na/K ratio (0.35 mol mol^{-1}) like control (CAD level; 101 mEq kg^{-1}, Na/K ratio; 0.4 mol-mol^{-1}).

Improving in daily feed intake in RS supplemented group was not significantly different from the control group, but was significantly better than $CaCl_2$ supplemented group ($P<0.05$). Feed conversion rate decreased by 15-17% in RS supplemented group compared to $CaCl_2$ supplemented group and control group respectively. Additionally weight gain significantly increased by 12% in RS group compared to $CaCl_2$ supplemented group and control group ($P<0.05$). These significant differences in weight gain and feed conversion rate in treatment groups probably associated with differences in energy costs. Because of the significant decreasing at the protein efficiency rate in 2% $CaCl_2$ supplemented group and control *(Table 2),* it can be expected that more energy is needed to maintain mineral balance and homeostasis for the fish feeding with diets having low dietary CAD-Na/K levels. Dersjant-Li et al. also stated that, feed efficiency improved with increasing dietary Na/K in African catfish, so more fat was deposited and increased linearly the body nitrogen, fat and energy levels [7]. Likewise D'Cruz and Wood, reported that 263 mmol/kg salt (NaCl) supplementation to diet of juveniles of rainbow trout improved the growth and FCR in acidic water conditions [21].

The hardness of water used in this study was very high (370 mg lt^{-1} $CaCO_3$). Water hardness is very important for fish culture. It is a measure of the quantity of divalent ions such as calcium, magnesium and/or iron in water. Calcium and magnesium are essential in the biological processes of fish. The presence of free (ionic) calcium in culture water helps reduce the loss of other salts (e.g., sodium and potassium) from fish body fluids. Environmental calcium is also required to re-absorb these lost salts [22]. In this study, supplementation of fish diet with 2% RS caused to improve in the weight gain and feed efficiency of rainbow trout in very hard water providing the advantages to fish in respect of iono-regulation and biological processes. We estimated that high hardness of water did not mask the effects of RS supplementation on the weight gain and feed efficiency in rainbow trout. There are need to further studies, for determining the effects of different levels of dietary RS supplementation on growth performance of rainbow trout in water having different hardness values.

Na, K and Cl levels in the serum samples were not significantly influenced with dietary $CaCl_2$ and RS supplementation. These results are consistent with the research of Arabacı et al.[12]. In previous studies, different regulations in fish diet affected differently the plasma Na, K and Cl levels of fish [6-8,11].

Mortality rate was similar at a range of 2% among groups. Arabacı et al.[12] were found that there was no clear effect of dietary CAD-Na/K level on mortality.

However Dersjant-Li et al.[7] observed that mortality and disease rate was high in the fish receiving low dietary Na/K ratio of 0.2. For determining the effect of very low dietary Na/K ratio and/or CAD level on mortality rate in rainbow trout, there is need to further research.

The results of the present study imply that RS supplementation to diet of rainbow trout could be used to increase both dietary CAD level and dietary Na/K ratio and this caused to positive effects on the weight gain and feed efficiency in rainbow trout.

It was concluded that the weight gain and protein efficiency rate increased significantly and the feed conversion rate decreased significantly. Specific growth rate and daily feed intake improved slightly but not a significant level with RS supplementation. Na, K, Cl level in the serum was not significantly influenced with $CaCl_2$ and RS supplementation. However, there are need to further studies for determining the optimum levels of RS supplementation to rainbow trout diets and for clearing the interactions between dietary salt supplementations and dietary CAD level-Na/K ratios.

According to our present study, 2% RS supplementation produced a better growth and feed efficiency for rainbow trout compared to 2% $CaCl_2$ supplementation and control diet. Added RS to diet was appropriate for making cationic the diet and increasing the CAD level and Na/K ratio of diet.

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