# DO OXALATES FROM PLANT-BASED AQUAFEEDS IMPEDE GROWTH OF COMMON CARP *CYPRINUS CARPIO*?

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## DA LI OKSALATI IZ HRANE ZA RIBE BILJNOG POREKLA OMETAJU RAST ŠARANA, *CYPRINUS CARPIO*?

*Apstrakt*

Jedan od najvažnijih izazova održivog razvoja u akvakulturi je održivo obezbeđenje hrane, koje zahteva upotrebu manje konvencionalnih sastojaka za hranu za ribe kao što su presovane pogače i uljarice. Neke od ovih sirovina imaju visok sadržaj oksalata, koji su poznati kao antinutricioni faktor za neke domaće životinje. Oksalat je proizvod metabolizma biljaka, ali se takođe pojavljuje u metabolizmu životinja. Zavisno od vrste biljaka oksalati se nakuplja: kao rastvorljiva so vezana za natrijum, kalijum ili amonijum; kao nerastvorljiva so vezana sa dvovalentnim jonima kalcijuma, magnezijuma ili gvožđa; i kao kombinacija oba navedena sliučaja. Negativni efekti oksalata kod sisara uključuju smanjenje raspoloživosti minerala iz hrane što izaziva njihov nedostatak, zatim formiranje kamenčića oksalata kalcijuma ili magnezijuma u bubregu i drugim tkivima, što rezultira artritisom ili simptomima gihta. Za nepreživare preporučen je maksimalni sadržaj od 0.5% u hrani. Hrana za ribe koja sadrži lišće, presovane pogače ili proizvode od pirinča može lako da dostigne ili pređe navedeni sadržaj oksalata, međutim, koliko je nama poznato, ova problematika nije do sada nikada bila ispitivana.

Da bi se ispitali efekti na ribe, šarani (*Cyprinus carpio*) su hranjeni hranom koja sadrži oksalate u količinama od 0%, 0.5%, 1.5% i 2.5% tokom 8 nedelja. Sva hrana je bila redovno konzumirana, a preživljavanje riba je bilo 100%. Prirast ribe koja je hranjena hranama koje su sadržale oksalate je bio bolji nego u kontroli (0%), sa značajnim razlikama između tretmana 1.5% i 2.5% ($p \leq 0.05$, Tab.1). Isto je važilo i za vrednosti SGR, MGR i PER (Tab.2). FCR je bio značajno niži za tretmane 1.5% i 2.5% u odnosu na kontrolu, dok u slučaju tretmana 0.5% to nije bio slučaj. PPV je bio viši za tretmane 1.5% i 2.5% u odnosu na tretmane sa nižim sadržajem oksalata. LPV je bio značajno viši u kontroli u odnosu na hrane sa 1.5% i 2.5% oksalata, dok za vrednosti EPV nije bilo razlike.

Utvrđene su statistički značajne razlike u telesnom hemijskom sastavu eksperimentalnih riba: kontrola i tretman 0.5% su imali više lipida i energije, kao i niži sadržaj vlage i pepela u odnosu na tretmane 1.5% i 2.5%. Nije bilo razlike u proteinskom sastavu (Tab.3). Retencija kalcijuma, magnezijuma i fosfora je bila statistički značajno niža u kontroli i hrani sa 0.5% oksalata u odnosu na hrane koje su sadržale 1.5% i 2.5% oksalata (Tab.4).

Oksalati kod šarana imaju pozitivne efekte na rast i pretpostavlja se da bi to moglo biti zbog njihovog antimikrobijalnog delovanja u crevima. Efekti na telesni sastav su bili izraženi: više oksalata rezultiralo je višim sadržajem minerala i nižim sadržajem lipida. Tokom trajanja ogleda nije bilo antinutricionih efekata, naročito nedostatka minerala, kao što je to opisano u literature; ipak, bilo bi neophodno dugoročno ispitivanje koje bi uključilo i histopatologiju da bi se doneo zaključak da li su oksalati blagotvorni u hrani za šarana, kao i da bi se osvetlili potencijalni mehanizmi poboljšanog rasta.

## INTRODUCTION

In the continuing drive to replace animal protein (mostly fishmeal) in fish feeds with plant protein, research has focused on identifying and minimizing the influence of anti-nutritional components present in most plant-based feedstuffs. Anti-nutritional components are defined as substances that interfere negatively with food utilization or adversely affect the health status of the feeding animals (Francis et al., 2001). Despite a range of treatment methods employed to minimize anti-nutritional factors in plant feedstuffs, such as dry heat treatment (“toasting”), moist heat treatment, aqueous or alcoholic extractions, some of these substances cannot be removed and end up in the feed (Francis et al., 2001).

A good example for these is oxalic acid and its salts (oxalates). Oxalic acid is a metabolic product formed through several pathways in plants and animals.Oxalatesof monovalent ions, such as sodium, potassium or ammonium are well soluble in water while those oxalates formed with divalent ions, such as calcium, magnesium and iron are almost insoluble (Libert&Franceschi, 1987; Savage et al., 2000).

Oxalate has long been considered an anti-nutritional factor in humans and consumption in the form of plants containing high amounts of oxalate, such as spinach, beet or rhubarb, has been recommended not to exceed an upper limit (Noonan & Savage, 1999). Documented adverse effects of dietary oxalate in mammals include the binding of oxalate with calcium in the intestinal lumen to form insoluble calcium oxalate making calcium unavailable for absorption and excreting it with the faeces (Noonan & Savage, 1999). This may lead to low blood calcium levels (Blaney et al., 1982) and in cases of long-term exposure, bone material may be excessively mobilized to compensate for the shortages of minerals (Rahman et al., 2013). Further, long-term exposure to a high-oxalate diet may lead to formation of Ca- or Mg-oxalate stones in the kidney, which can cause urine flow problems or kidney failure (Noonan & Savage, 1999). Rahman et al. (2013) recommended that for ruminants dietary oxalate consumption should be less than 2% in order to avoid oxalate poisoning and less than 0.5% for non-ruminants. Soluble oxalate content in some potential fodder crops used in aquaculture diets are within these critical ranges: saltbush *Atriplexhalimus* 1.0 – 3.0%, rice *Oryza sativa* 1.0 – 2.5%, alfalfa *Medicago sativa* 0.9-1.1%; *Jatropha curcas* 2.4%. Some authors have postulated potential anti-nutritional effects of oxalate in fish nutrition (Yousif et al., 1994; Francis et al., 2001), but the effects of dietary oxalate on aquaculture species remain to be investigated. In this trial, the effect of four different concentrations (0%, 0.5%, 1.5% and 2.5%) of soluble oxalate in a standard diet on the growth performance, body composition and mineral availability of common carp (*Cyprinus carpio*) were investigated.

## MATERIAL AND METHODS

Four isonitrogeneous and isoenergetic diets with fishmeal and wheatmeal as main ingredients were produced containing 0% (Control), 0.5%, 1.5% and 2.5% of di-sodium-oxalate. Protein content was formulated to be 32% and lipid content 12% (50% fish oil, 50% sunflower oil). Vitamins and minerals were supplied in the form of a premix. Essential amino acids were added to meet the requirements of carp (NRC, 2011).

*Cyprinus carpio* were hatched and reared to the initial experimental weight of 9.85 ± 0.23 g at the Thuenen Institute of Fisheries Ecology, Ahrensburg, Germany. A total of 100 experimental fish (five replicates for each treatment, each replicate containing 5 fish) were distributed into a total of twenty 40 L-aquaria connected to a recirculation system. Water temperature was 26°C, with 1.5 L / min/tank flow rate. The experimental duration was 8 weeks.

Fish were fed according to multiples of their basic metabolic maintenance ration (Richter et al., 2002). Fish were fed 5 times the maintenance ration on a daily basis throughout the experiments. The ration was given in three installments per day. Fish were weighed every two weeks individually and without anesthesia in a bowl containing water from the respective tank. Feed amount was adjusted accordingly after every weighing.

Before the experiment, 8 fish were sacrificed with an overdose of ethyleneglycolmonophenylether (Liasko et al., 2010) and stored in polyethylene bags at -20°C. At the end of the study, all experimental fish were sacrificed in the same manner. For further processing, fish were autoclaved. Subsequently, fish were homogenized using an ultra turrax blending device and transferred to a pre-weighed plastic container. The homogenized material was frozen and freeze-dried. After samples were completely dry, they were weighed and again homogenized in a standard electric coffee grinder to obtain a fine powder with which subsequent analyses were conducted.

For chemical analysis dried material from all 5 fish per replicate was pooled. Nitrogen analysis of feed and whole body fish was conducted with a TrueSpec N Macro (LecoInstrumente GmbH). Protein content was calculated as nitrogen x 6.25. Lipids were analyzed through accelerated solvent extraction (Dionex, ASE200). Ash content was measured after combusting the samples at 550°C for one hour in a muffle furnace (Nabertherm). Gross energy was measured with a bomb calorimeter (Parr 6100). Mineral analyses of feed, whole body and faeces were conducted with a Thermo Scientific Series 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) after digesting the material in concentrated salpetric acid for 20 minutes at 190°C in a CEM Mars Xpress microwave digester.

Growth of the fish was expressed as specific growth rate (SGR), feed conversion ratio (FCR), body mass gain (BMG), metabolic growth rate (MGR), protein efficiency ratio (PER), protein productive value (PPV), lipid productive value (LPV) and energy productive value (EPV).

Data was tested for normal distribution with the Shapiro-Wilk test. Percentages were arcsine transformed before analysis. One-way ANOVA ($p \leq 0.05$) was used to analyze treatments. Tukey's test was applied as post-hoc test. Statistics were conducted with Statistica 8 software.

## RESULTS

All diets were consumed at all times and fish survival was 100%. Body mass gain of fish was higher in all diets containing oxalate than in the control (0%) diet, with differences between treatments 1.5% and 2.5% being significant ($p \leq 0.05$, Table1). The same was true for SGR, MGR and PER (Table 2). FCR was significantly lower for treatments 1.5% and 2.5% than the control diet, while treatment 0.5% was not. PPV was higher for treatments 1.5% and 2.5% than for lower oxalate treatments. LPV was significantly higher in the control diet than in diets 1.5% and 2.5%, while there was no difference in EPV.

**Table 1:**Body mass gain of common carp fed different concentrations of soluble oxalate for 8 weeks.

| Treatment | Control | | | | 0.5% | | | | 1.5% | | | | 2.5% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IW (g) | 9.87 | ± | 0.20 | | 9.90 | ± | 0.29 | | 9.83 | ± | 0.27 | | 9.81 | ± | 0.23 | |
| FW (g) | 26.2 | ± | 1.09 | b | 28.4 | ± | 2.61 | a,b | 31.5 | ± | 2.23 | a | 32.1 | ± | 2.32 | a |
| BMG (g) | 16.3 | ± | 1.26 | b | 18.5 | ± | 2.48 | a,b | 21.6 | ± | 2.01 | a | 22.3 | ± | 2.28 | a |
| BMG (%) | 165.1 | ± | 15.9 | c | 186.9 | ± | 23.2 | b,c | 219.8 | ± | 16.0 | a,b | 225.9 | ± | 23.4 | a |

Values are mean +/- standard deviation, n = 5. Values with different superscripts are significantly different from each other ($p \leq 0.05$). IW: Initial weight; FW: Final weight; BMG: body mass gain.

There were significant differences in the body composition of experimental fish, the control treatment and treatment 0.5% being significantly higher than 1.5% and 2.5% in body lipid and energy and significantly lower in body moisture and ash contents.There was no difference in body protein content (Table 3)

**Table 2.** Nutrient utilization of common carp fed different concentrations of oxalate for 8 weeks.

| Treatment | Control | | | | 0.5% | | | | 1.5% | | | | 2.5% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SGR | 1.74 | ± | 0.11 | b | 1.88 | ± | 0.15 | a,b | 2.07 | ± | 0.09 | a | 2.11 | ± | 0.13 | a |
| FCR | 1.74 | ± | 0.10 | a | 1.61 | ± | 0.15 | a,b | 1.45 | ± | 0.07 | b | 1.47 | ± | 0.11 | b |
| MGR | 7.35 | ± | 0.43 | b | 7.96 | ± | 0.65 | a,b | 8.80 | ± | 0.42 | a | 8.95 | ± | 0.54 | a |
| PER | 1.72 | ± | 0.11 | a | 1.90 | ± | 0.18 | a,b | 2.14 | ± | 0.11 | b | 2.12 | ± | 0.16 | b |
| PPV | 22.9 | ± | 1.34 | b | 24.8 | ± | 2.51 | b | 28.2 | ± | 1.51 | a | 28.2 | ± | 2.64 | a |
| LPV | 43.5 | ± | 1.14 | a | 44.0 | ± | 6.26 | a | 36.8 | ± | 5.66 | a,b | 33.4 | ± | 2.71 | b |
| EPV | 20.6 | ± | 0.53 | | 21.4 | ± | 2.23 | | 21.0 | ± | 1.55 | | 20.8 | ± | 1.55 | |

Values are mean +/- standard deviation, n = 5. SGR: specific growth rate (% / day); FCR: feed conversion ratio; MGR: metabolic growth rate ($g * kg^{0.8}$ / day); PER: protein efficiency ratio; PPV: protein productive value (%); LPV: lipid productive value (%); EPV: energy productive value (%). Values with different superscripts are significantly different from each other ($p \leq 0.05$)

**Table 3.** Body composition of common carp fed different concentrations of oxalate for 8 weeks.

| **Treatment** | **Initial Fish** | **Control** | **0.5%** | **1.5%** | **2.5%** |
|---|---|---|---|---|---|
| Moisture (%) | 77.5 ± 0.67 | 75.1 ± 0.53 $^{b}$ | 75.6 ± 0.34 $^{b}$ | 76.8 ± 0.74 $^{a}$ | 77.0 ± 0.59 $^{a}$ |
| Protein (%) | 14.6 ± 0.33 | 13.8 ± 0.14 | 13.6 ± 0.16 | 13.6 ± 0.21 | 13.7 ± 0.31 |
| Lipid (%) | 3.82 ± 0.38 | 7.27 ± 0.36 $^{a}$ | 7.13 ± 0.48 $^{a}$ | 5.67 ± 0.67 $^{b}$ | 5.29 ± 0.27 $^{b}$ |
| Ash (%) | 3.35 ± 0.18 | 2.38 ± 0.08 $^{b}$ | 2.38 ± 0.07 $^{b}$ | 2.65 ± 0.09 $^{a}$ | 2.74 ± 0.09 $^{a}$ |
| GE (kJ/g) | 4.89 ± 0.27 | 6.13 ± 0.17 $^{a}$ | 5.94 ± 0.12 $^{a}$ | 5.44 ± 0.25 $^{b}$ | 5.31 ± 0.16 $^{b}$ |

Values based in wet weight. Values are mean +/- standard deviation, n = 5. Values with different superscripts are significantly different from each other ($p \leq 0.05$)

Calcium, magnesium and phosphorus retention was significantly lower in low oxalate treatments (0% and 0.5%) compared to higher oxalate treatments (1.5% and 2.5%, Table 4).

**Table 4**. Mineral retention of selected minerals of common carp fed different concentration of oxalate for 8 weeks.

| Treatment | 0% | **0.50%** | **1.50%** | **2.50%** |
|---|---|---|---|---|
| Mg | 6.15 ± 0.53 $^{a}$ | 6.51 ± 0.91 $^{a}$ | 9.22 ± 0.45 $^{b}$ | 9.88 ± 0.37 $^{b}$ |
| P | 12.7 ± 1.01 $^{a}$ | 14.1 ± 2.08 $^{a}$ | 21.4 ± 2.26 $^{b}$ | 22.6 ± 0.82 $^{b}$ |
| Ca | 9.83 ± 0.99 $^{a}$ | 11.0 ± 1.82 $^{a}$ | 18.2 ± 1.98 $^{b}$ | 20.2 ± 0.64 $^{b}$ |
| Zn | 30.8 ± 5.10 | 28.7 ± 6.74 | 31.5 ± 5.47 | 29.3 ± 2.92 |

Values in %. Values are mean +/- standard deviation, n = 5. Values with different superscripts are significantly different from each other ($p \leq 0.05$)

## DISCUSSION

To our knowledge the present study is the first to deal with the influence of oxalate as an anti-nutritional factor in carp. Di-sodium oxalate was chosen because of its high solubility at neutral pH values compared to calcium or magnesium oxalate. The maximum treatment level of 2.5% was chosen to represent a theoretical maximum oxalate content in a fish diet consisting largely of high-oxalate plant ingredients, such as detoxified *Jatropha curcas* kernel meal. The present study shows significant impacts of oxalate on growth development, body nutrient and mineral composition of the experimental fish. There was a tendency for treatments 0% and 0.5% to show similar results, while diets 1.5% and 2.5% were different from the lower oxalate treatments, but similar to each other.

*Growth*

There was a significantly positive effect of oxalate on body mass gain, specific growth rate and feed conversion ratio of experimental fish. This effect was unexpected as a decrease in performance, possibly through mineral deficiencies or direct oxalate poisoning of fish in the high-oxalate treatments, might have been expected.

Oxalate is the salt of oxalic acid, a strong, organic acid (Francesch i& Horner, 1980). Organic acids or their salts have received considerable attention as growth promoting agents (Lueckstädt et al., 2008).

*Body Composition*

There were significant differences in body composition of experimental fish for treatments 0% and 0.5% compared to 1.5% and 2.5%. Higher ash values in treatments 1.5% and 2.5% reflect improved mineral retention of these treatments. There was no impact of oxalate concentration on body protein content.

*Mineral composition of carcass and mineral retention*

Most existing literature regarding the effects of oxalate in the digestive tract of humans or livestock states that oxalate binds to divalent cations making these unavailable for absorption. This is shown by increased cation content in the faeces and concomitant lower digestibility and retention values for these cations (Noonan & Savage, 1999; Rahman et al., 2013). However, our results show the opposite trend with treatments 1.5% and 2.5% showing clear increases in calcium, magnesium and phosphorus retention compared to treatments 0% and 0.5%.

## CONCLUSION

The effects of different concentrations of dietary oxalate on common carp have not been previously investigated despite high contents of this anti-nutritional factor in popular feedstuffs. Oxalate in carp had a positive effect on growth and it is hypothesized that this may be due to antimicrobial effects exerted in the intestine. The effects on body nutrient composition were distinct, higher oxalate promoted higher mineral and lower lipid content. No anti-nutritional effects, predominantly mineral deficiencies, as described in the literature could be detected over the trial period; however, long-term studies including histopathology are required in order to conclude whether oxalate is beneficial in carp feeds and to elucidate potential mechanisms.

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