

Lessons learned from using wild-caught and captive-reared lesser scaup (*Aythya affinis*) in captive experiments.

C.R. Beach<sup>\*, †, ‡</sup>; C.N. Jacques<sup>\*, §</sup>; J.D. Lancaster<sup>#</sup>; D.C. Osborne<sup>||</sup>; A.P. Yetter<sup>†</sup>; R.A. Cole<sup>¶</sup>; H.M. Hagy<sup>&</sup>; A.M.V. Fournier<sup>†</sup>

<sup>\*</sup> Department of Biological Sciences, Western Illinois University, 1 University Circle, Macomb, Illinois, USA, 61455

<sup>†</sup> Forbes Biological Station–Bellrose Waterfowl Research Center, Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, Havana, Illinois, USA, 62644

<sup>‡</sup> Current address: Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy, DeKalb, Illinois, USA, 60115

<sup>§</sup> Current address: Illinois Department of Natural Resources, Lena, Illinois, USA, 61048

<sup>#</sup> Gulf Coast Joint Venture, Ducks Unlimited, Inc. 700 Cajundome Blvd., Lafayette, Louisiana, USA, 70506

<sup>||</sup> College of Forestry, Agriculture, and Natural Resources, University of Arkansas at Monticello, Monticello, Arkansas, USA, 71656

<sup>¶</sup> U.S. Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA, 53711

<sup>&</sup> National Wildlife Refuge System, United States Fish and Wildlife Service, Stanton, Tennessee, USA, 38069

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## ABSTRACT

Waterfowl are housed in captivity for research studies that are infeasible in the wild.

Accommodating the unique requirements of semi-aquatic species in captivity while meeting experimental design criteria for research questions can be challenging and may have unknown effects on animal health. Thus, testing and standardizing best husbandry and care practices for waterfowl is necessary to facilitate proper husbandry and humane care while ensuring reliable and repeatable research results. To inform husbandry practices for captive-reared and wild-caught lesser scaup (*Aythya affinis*; hereafter, scaup), we assessed body mass and fat composition across two different aspects of husbandry, source population (captive-reared or wild-caught), and housing densities (birds/m<sup>2</sup>). Our results suggest that housing scaup at low densities ( $\leq 0.6$  m<sup>2</sup>/bird,  $P=0.049$ ) relative to other species can minimize negative health effects. Captive-reared scaup were heavier ( $P=0.027$ ) with greater body fat ( $P<0.001$ ) and exhibited fewer signs of stress during handling than wild-caught scaup. In our experience, scaup which are captive-reared from eggs collected in the wild were better for long-term captivity studies as they maintained body mass between and recovered lost body mass following trials. Researchers would benefit from carefully evaluating the tradeoffs of using short- and long-term captive methods on their research question before designing projects, husbandry practices, and housing facilities for waterfowl.

**Keywords:** Captive Waterfowl, Diving Duck, Husbandry, Lesser Scaup

## LAY SUMMARY

Waterfowl are housed in captivity for research studies that are not possible in the wild. Housing and caring for waterfowl, given they are a semi-aquatic species can be challenging when also needing to meet the needs of the research. We examined body mass and fat composition of a diving duck, the lesser scaup (*Aythya affinis*) under different housing scenarios to inform future studies holding scaup in captivity. Our results suggest that housing at low densities ( $\leq 0.6$  m<sup>2</sup>/bird) can minimize negative health effects. Captive-reared scaup were heavier and had more body fat and exhibited fewer signs of stress during handling than wild-caught scaup. In our experience, scaup which are captive-reared from eggs collected in the wild were better for long-term captivity studies as they maintained body mass between and recovered lost body mass more quickly. Researchers would benefit from carefully evaluating the tradeoffs of using short- and long-term captive methods on their research question before designing projects, husbandry practices, and housing facilities for waterfowl.

**TEASER TEXT** Housing diving ducks in captivity at lower densities compared to dabbling ducks minimized negative health effects. Individuals captive raised from eggs collected in the wild were better for long-term captivity studies.

## INTRODUCTION

Wild animals are held in captivity in zoological collections, aquariums, and research facilities for conservation breeding programs, education, research, and recreation (Greenwell et al. 2023). Captive animal research is invaluable to the conservation of various wildlife taxa allowing for controlled data collection that is often infeasible in the wild (Rose et al. 2014). Captive animal research provides an understanding of the biological and physiological requirements of wild animals, such as immune function and nutritional and energy requirements, which are necessary to properly manage and restore wild populations (Moller et al. 1998; Hutchins et al. 2003). A key component of ethical captive animal research is proper husbandry specific to the taxa in question (Larivière et al. 2005; Tonkins et al. 2015). Poor or suboptimal husbandry practices can affect research results and inferences to wild counterparts as well as have consequences for test subjects (Mason 2010). There has been limited evaluation of the effects of husbandry practices on ducks (Anatidae) held in captivity for research purposes, which are needed to provide consistent and replicable data across research projects and institutions (Gross et al. 2020, Wu et al. 2020).

The Three Rs principle for the use of animals in experiments establishes Replacement Alternatives, Reduction alternatives, and Refinement alternatives as important considerations when doing experimental work on animals (Russell and Burch 1959). When designing a study to address a question about a population of wild birds, it can be necessary to use individuals from that population, but there can be tradeoffs because animals brought into captivity from the wild may have altered behavior or physiology, and this needs to be considered in study design, because working with individuals in captive-reared populations also have potential biases, such as increased tameness, which may affect study results (Feenders et al. 2011, Cabezas et al. 2013,

Gross et al. 2020). Therefore, researchers would need to carefully consider the tradeoffs between holding wild-caught birds in captivity versus raising birds from eggs collected in the wild in captivity with limited information on the physiological and behavioral consequences of either, relative to husbandry practices. It is also important to consider Reduction alternatives, and to ensure that the study is carefully designed to minimize the number of individuals that need to be part of a study (Russell and Burch 1959). The third principle, Refinement alternatives, speaks to the need for the work we have done in this paper, where when it is necessary to work directly with individual birds, we need to work to refine and modify our techniques and practices to minimize distress and enhance welfare. For some species, there is very limited literature is available to inform that refinement during study design, which means that learning may occur during the study itself.

Lesser scaup (*Aythya affinis*; hereafter, scaup) are the most abundant diving duck in North America and have been used in captive research since the 1960s to evaluate their biology, physiology, and nutritional needs (Anteau et al. 2020, Table 1). Husbandry of captive-reared scaup has varied widely across previous studies, with birds housed at densities ranging from 0.1 m<sup>2</sup>/bird to 51.7 m<sup>2</sup>/bird ( $\bar{x} = 8.2 \pm 5.5$  [SE] m<sup>2</sup>/bird, Table 1). To date, we are aware of only one study that housed wild-caught scaup, and those individuals were held in captivity for <24 hr (Smith et al. 2021), suggesting their husbandry and housing practices may not provide strong guidance for long-term care of wild-caught scaup in captivity. In preparation for experimental trematode (Digenea) infections in captive scaup to evaluate the effects of sub lethal infections on body condition and immune function, we evaluated aspects of published husbandry protocols to determine best practices for this semi-aquatic species (Table 1). Herein, we compare quantitative and qualitative metrics on spacing, handling acclimation, food, and source of population to assist

future researchers in selecting husbandry protocols for lesser scaup and similar species in long-term captivity.

## **STUDY AREA**

We conducted this work at Forbes Biological Station (FBS; Illinois Natural History Survey, University of Illinois at Urbana-Champaign) near Havana, Illinois, USA, between March 2019 and October 2020. Forbes Biological Station has maintained secure indoor and outdoor housing facilities and housed numerous waterfowl species for a wide variety of research since the 1940s (Jordan and Bellrose 1951; Gross et al. 2020).

## **METHODS**

We housed wild-caught captive-reared scaup in captivity under three different scenarios: wild-caught scaup housed at relatively high densities (0.3 m<sup>2</sup>/bird), wild-caught scaup housed at relatively low densities (0.9 m<sup>2</sup>/bird), and captive-reared, wild scaup housed at low densities (0.9 m<sup>2</sup>/bird). All scaup were used in trials evaluating effects of experimental trematode (Class: Digenea) infections and served as either control (uninfected) or experimental (infected) specimens (Beach 2021). To evaluate husbandry methods, only individuals in the control group from each captive experiment were used in the statistical analysis. University of Illinois Urbana-Champaign and Western Illinois University Institutional Animal Care and Use Committees provided ethical review of our work before it began. All methods were approved by the Institutional Animal Care and Use Committee at University of Illinois at Urbana-Champaign and Western Illinois University (Protocol #18128 and #009-20, respectively), U.S. Fish and Wildlife Service (Permit #MB145466-3, #MB145466-4, and #MB145466-6), Illinois Department of Natural Resources (Permit #W19.6079 and #W20.6079A), and North Dakota Game and Fish Department (Permit #GNF04969331).

## High Density Wild-Caught Lesser Scaup

During spring 2019, we captured 37 female scaup near Havana, Illinois (lat 40.30° N, long 90.06° W) using swim-in traps (Haramis et al. 1982). To trap the birds, we would find areas along the Illinois River where lesser scaup were spending time during migration and would bait that area with field corn. Once, once the birds were readily using the bait, we would encircle the bait with a trap. The trap is cylindrical in shape, approximately 7 feet high and 4 feet in diameter. At the bottom on one side there is an opening where the birds can dive into the trap to get access to the corn. Due, due to the shape of the opening, it is difficult for the birds to then dive out of the trap. The traps are secured in place with long pieces of metal conduit. The traps are never used in more than 4 feet of water, giving the birds abundant area to rest on the water's surface when they are not feeding. We caught both male and female scaup, but for the study on the sub lethal impacts of parasites on Lesser Scaup we were initially only interested in females because if they were negatively affected by sub lethal infections it may affect their ability to successfully lay eggs, thus contributing to population declines. Two scaup were randomly selected and placed in each indoor galvanized wire cage at a density of 0.3 m<sup>2</sup>/bird (Cornell University Duck Research Laboratory [CUDRL] 2016). We provided scaup with Purina Game Bird Maintenance Chow (Purina Animal Nutrition, LLC, Arden Hills, Minnesota, Table 2), dried mealworms (*Tenebrio molitor*), and dried shelled corn *ad libitum* by means of a 475-mL feeding cup attached to the inner wall of each pen (Table 2). Fresh water was provided in the same manner as food, and a 3.8-L rubber pan of water was placed inside each cage that allowed scaup to drink, clean, and preen. We subjected scaup to a normal daylight regime that matched the time of year via full spectrum bulbs and handled scaup daily to measure body mass changes and acclimate them to captivity. We anticipated the scaup would acclimate to captivity within 6 days of capture based



on our previous experience bringing wild dabbling ducks into captivity for research. However, scaup did not acclimate to captivity within 6 days, as indicated by decreased body mass and apathy towards food items, and we extended the initial acclimation period for an additional 6 days. At day 12, when scaup continued to decrease in body mass, we moved them to outdoor pens at a density of 0.9 m<sup>2</sup>/bird to ensure recuperation and recovery of body mass (Table 2; Cornwell and Hartung 1963; Sanderson et al. 1998). In the outdoor pens, scaup had access to continuously replenished fresh water from stock tanks (12 m<sup>2</sup> surface area) that were flush to the floor of the pen. We provided scaup with feed with increased fat and protein (Sportsman's Choice Floating Pond and Catfish Food; Cargill, Inc., Minneapolis, Minnesota; Table 2) compared to the Purina Game Bird Maintenance Chow. We allowed scaup to acclimate to outdoor captive conditions, with minimal handling, for approximately 3 months before they participated in a research trial. Recovery was determined by observation of stable and continued feeding over time; a return to normal behavior such as preening, stretching, walking, swimming, and loafing; and observation of a stable body mass that was at least 89% of their capture weight on the first day of the 10-day trial.

### **Captive-Reared Lesser Scaup**

We collected wild scaup eggs from nests near Devils Lake, North Dakota (48°07'N, 98°52'W) in June 2019. We incubated 24 scaup eggs at FBS following the procedure of Ward and Batt (1973), keeping ducklings of similar age in groups of ≤15 in brooder pens (76 cm × 76 cm × 45 cm) and rearing them to 22–23 days of age (Ward and Batt 1973; Lightbody and Ankney 1984). We handled ducklings daily to acclimate them to humans. When scaup were 23-days post hatch, we placed them in an outdoor pen at a density of 0.9 m<sup>2</sup>/bird (Table 2). Ducklings were exclusively fed non-medicated Purina Flock Raiser crumbles *ad libitum* until 30

days of age to ensure consistent growth (Table 2; Lightbody and Ankney 1984). From 30 to 60 days of age, Sportsman's Choice Floating Pond and Catfish Food was added into their diet in dry feeders (Table 2). From 60 days of age, captive-reared scaup were exclusively fed Sportsman's Choice Floating Pond and Catfish Food *ad libitum* to ensure nutrients and growth were similar to captive wild-caught scaup. Once in outdoor pens, captive-reared scaup were handled at least once a week to continue their acclimation to humans. At approximately 3-months post hatch, we weighed scaup weekly to monitor mass changes and acclimate them to the handling requirements for the captive experiment. At approximately 5 months of age, when the scaup were fully fledged, we conducted the 10-day captive experiment.

### **Low Density Wild-Caught Lesser Scaup**

In spring 2020, we captured 16 male and 19 female scaup using baited swim-in traps (Haramis et al. 1982) and housed them outdoors as a group at a density of 0.6 m<sup>2</sup>/bird (Table 2). Scaup were fed Sportsman's Choice Floating Pond and Catfish Food *ad libitum* in dry feeders (Table 2). Scaup were held in captivity without handling for approximately 5 months; whereafter, we weighed them biweekly for an additional 2 months to acclimate them to handling before the 10-day captive experiment.

### **Health Evaluation, Tissue Collection, and Processing**

Upon capture, or once fully flighted for captive-reared scaup, we weighed ( $\pm 1$  g) them (Trauger 1974; Carney 1992). We determined body mass ( $\pm 1$  g) again on day 0 of the experiment. At the end of each experiment, we euthanized and necropsied all birds. We exposed the abdominal cavity by peeling away the skin and cutting the ribcage, enabling the ribcage to be pushed aside (England et al. 2018). The liver, intestinal tract (esophagus to cloaca), bill, feet, and

feathers were removed from each carcass. To determine percent fat, moisture, protein, and ash of each carcass, we conducted proximate analysis following Klimas et al. (2020).

### Statistical Analysis

To examine the effects of source (wild-caught versus captive-reared), sex, enclosure density on body mass (g) and body fat (%) of lesser scaup, we used linear models. We only included data from control birds in this analysis, to avoid any effects from trematode infection. This resulted in 13 females housed under the high-density wild-caught scenario, 4 males and 4 females under the captive-reared scenario, and 6 males and 6 females under the low density wild-caught scenario. In the first model set, we compared body mass and body fat among source (wild-caught versus captive-reared) populations and sexes using males and females across trials. In the second model set, we examined the effect of enclosure density (low or high) on scaup body weight and body fat. For this model we only used data from female scaup because no males were held under the high-density conditions. All statistical analysis were done in R version 4.2.2 (R Core Team 2022), and we used  $\alpha = 0.05$  to determine significant differences across categorical variables.

### RESULTS

Males were 90 g ( $\pm 22$ ;  $p < 0.001$ ) heavier than females across both wild-caught and captive reared birds. Wild-caught scaup were 54 g ( $\pm 24$ ;  $P = 0.027$ ) lighter than captive-reared scaup. Our model of body mass had an adjusted coefficient of determination ( $R^2$ ) of 0.45. Wild-caught scaup had 26% ( $\pm 4$ ;  $p < 0.001$ ) less body fat than captive-raised individuals, and we did not find an effect of sex on body fat ( $P = 0.315$ ). Our model of body fat had an adjusted  $R^2$  of 0.58.

Scaup housed in lower densities were 55 g ( $\pm 26$ ;  $p=0.049$ ) heavier at the start of experimental trials and had 16% ( $\pm 6$ ,  $p=0.01$ ) more body fat at the end of experimental trials compared to scaup housed in higher densities. Our model of body mass, had an adjusted  $R^2$  of 0.13, and our model of body fat had an adjusted  $R^2$  of 0.21.

## DISCUSSION

Captive-reared scaup at the beginning of trials were heavier with more body fat than wild-caught scaup regardless of housing density, and our captive-reared scaup had fat levels greater than free-ranging, spring-migrating scaup (Anteau and Afton 2004). Captive-reared animals may acclimate and adjust to the rigors of captive experiments more readily than wild-caught animals, but they may not be as representative of wild populations because of their enhanced body condition and calm demeanor in confinement (Kohl and Dearing 2014). Wild-caught animals may provide more representative data of wild populations because their body condition, behavior, and gastrointestinal microbe and parasite communities are more similar to that of free-ranging animals; however, there are increased costs (i.e., time, resources, and permitting, and patience) associated with acclimating wild-caught animals to captivity (Anteau and Afton 2004; Kohl and Dearing 2014). For example, we originally planned for a short ( $<6$  day) acclimation period based on the performance of previously captive wild-caught scaup and other diving ducks (Smith et al. 2021, Bouton et al. 2023), but we instead found they need several months to acclimate. However, if data from both groups of wild-caught scaup in our study are combined, it can be inferred that with adequate space (i.e.,  $\geq 0.6$  m<sup>2</sup>), food, and handling, wild-caught scaup can acclimate to captivity within a 4-month period (i.e., 2 months without handling followed by 2 months of biweekly handling and mass monitoring).

We found female scaup have better body condition in captivity at lower densities ( $\geq 0.6$  m<sup>2</sup>/bird) than what is broadly recommended for ducks in captivity (0.3 m<sup>2</sup>/bird, CUDRL 2016, 0.3251 m<sup>2</sup>/bird, Tucker et al. 2020). Because we did not house male scaup at each density we cannot confirm if they would have a similar body condition response. Although we did not evaluate the effects of different food types on captive lesser scaup, we did adjust the type of food provided from a low protein (12.5%) poultry maintenance chow to a high protein (32%) pellet, and we observed the birds' weight and body condition improved with the increase in protein content.

For scaup and other diving ducks, husbandry practices can present a challenge and necessitate a variety of experimental design complexities. Based on our experience, three general options are available for captive studies with scaup and similar species that appear to be more difficult to acclimate to captive conditions: (1) short-term captivity (<72 hr hold) where acclimation to handling and feeding in captivity is unnecessary or can be overcome using force feeding designs (Larson 2021; Smith et al. 2021; Bouton et al. 2023), (2) long-term captivity where wild-caught birds are acclimated to handling and captive feeding over multiple months (Gross et al. 2020), or (3) long-term captivity with captive-reared, wild birds (e.g., eggs collected from the wild and hatched in captivity) acclimated to handling and captive feeding over multiple months (this study). The first, short-term captivity scenario has been used recently by researchers to avoid potential long acclimation periods (Larson 2021; Smith et al. 2021; Bouton et al. 2023); but this method is not suitable for some research questions where experimental treatment is longer than ~48 hr.

After using all three methods, we found that wild, captive-reared scaup are appropriate for experimental situations in which testing time is prolonged or when individual birds must be

subjected to multiple trials, as in this study. Although each method has potential biases, and these biases (e.g., stress effects, digestive system changes, behavioral changes) are largely unexplored at this time relative to most research questions, efforts such as this to more clearly document and test husbandry practices may improve reliability of study results and conditions for study subjects.

This paper is the summation of observations made while adaptively developing a husbandry and feeding protocol that provided birds with conditions to support an adequate body condition for experimental work with trematodes (Beach 20212021). Thus, food sources and sex ratios were not standardized across the three groups because researchers were working through an adaptive process to develop husbandry methods for scaup due to inadequate information in the published literature. Although captive experiments with wild-caught and/or captive-raised, wild waterfowl can help address certain research questions, researchers would ideally test husbandry and experimental methods over a long period before initiating captive experiments. The published literature of husbandry practices on captive waterfowl is scant, and those available studies are heavily skewed towards dabbling ducks, especially mallards (*Anas platyrhynchos*). We also noted that when we traced the sources of husbandry practices in those studies, most were from the poultry industry, and limited experimentation has been published on effects of captive conditions on behavioral, physical, and physiological responses of waterfowl. We have discovered and begun to quantify the differences in best husbandry practices for diving ducks compared to previous studies with dabbling ducks and geese.

When investigating a question that is not behavioral in nature, such as in our studies, we found positive effects on body condition of birds that underwent a period of acclimation to handling; thus, and suggest such an acclimation period would be important to consider and accommodate in research timelines. Based on our results and experiences working with scaup in captivity, we found that housing scaup with at least 0.6 m<sup>2</sup>/bird, with constant access to water and high protein (e.g., 20-30%) food, would be beneficial. Planning. We also recommend planning for an acclimation period of at least 4 months before beginning research data collection on wild-caught diving ducks, including 2 months without handling after capture and then two months of biweekly handling and weight checks to acclimate birds, would be a best practice. In combination with recommendations from other recent work with waterfowl to improve husbandry practices in captivity (Lancaster et al. 2019, Gross et al. 2020, Larson 2021), we highlight possible tradeoffs in study designs with husbandry practices and potential effects on data from captive waterfowl. We have learned from this study (Beach 20212021) and nearly a decade of previous work with captive waterfowl the critical nature of husbandry choices on waterfowl conditions, and that diving ducks present additional and nuanced challenges, which warrant being explored intentionally and carefully prior to the initiation of new research projects to ensure humane treatment of test subjects and minimal biases of resulting data.

## DATA AVAILABILITY STATEMENT

Data are available from Auriel Fournier ([auriel@illinois.edu](mailto:auriel@illinois.edu)) upon request.

## DISCLOSURES

We have no conflicts of interest to declare.

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Table 1. Density, diet, and housing conditions of captive lesser scaup (*Aythya affinis*) from published literature.

Study	Source	Density (m <sup>2</sup> /bird)	Acclimation Period (week)	Length of Experiment (week)	Food; % Protein, % Fat	Housing
Brennan et al. 2017	Captive egg	6.2	-	104	Commercial duck pellet (for breeders); -, -	Outdoor facility
Brady et al. 2013	Captive egg	51.7	3	23	Mazuri Sea Duck Diet; 21.5%, 5%	Outdoor facility
Cornwell and Hartung 1963	-	0.4	-	-	-	Outdoor elevated wire pens
DeVink et al. 2008	Captive egg	9	-	10	Mazuri Sea Duck Diet; 21.5%, 5%	Outdoor facility
Kaseloo and Lovvorn 2005	1st or 2nd generation captive egg	-	3	> 42 hrs.	Commercial poultry laying pellet and mixed grain (corn, wheat, milo); 14.3%, 2.2%	Outdoor water tank

Laberge and McLaughlin 1991	Wild eggs	-	0	6	Laying feed; 18%, -	-
Lightbody and Ankney 1984	Wild eggs	5.4	0	10	Feed Rite custom mix; -, -	Outdoor Water tank
Longcore and Cornwell 1964	-	0.4	-	10	Submersed aquatic vegetation, invertebrates, corn, and wheat; -, -	Outdoor elevated wire pens
Richman and Lovvorn 2004	Wild eggs	0.3	-	8	2 parts turkey starter pellets and 1 part wheat; 25%, 2.5%	Indoor aviary
Smith et al. 2021	Wild adults	0.3	0	24 hrs	1 part layer crumble, 0.5 parts scratch grain, and 0.25 parts mealworms; -, -	Outdoor wire cages
Shave and Howard 1976	Wild eggs	-	0	52	Custom pellet; 18%, -	Outdoor facility



Sugden and Harris 1972	Wild eggs	0.1	0	12	Commercial duck starter; 19.1%, 4.3	Indoor wire cages
Stephenson et al. 1989	Captive eggs	0.7	-	-	-	Outdoor tank

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Table 2. Density, acclimation period, food, and housing provided to wild-caught and captive-reared lesser scaup (*Aythya affinis*) prior to and during the 2019 and 2020 captive experiments.

Captive Status	Density m <sup>2</sup> / bird	Acclimation Period	Food; % Protein, % Fat, % Fiber, Selenium (mg/kg)	Housing
High density wild-caught	0.3	8–12 days	Purina Game Bird Maintenance Chow, mealworms, and corn; 12.5–50%, 2.5– 25%, 7–10%, -	Indoor galvanized wire cages, 76 cm × 76 cm × 45 cm
	0.9	3 months – no handling	Sportsman's Choice Floating Pond & Catfish Food – dry; 32%, 4%, 7.5%, 0.25 mg/kg	Outdoor elevated wire pens with pools of water (2.7 m × 1.9 m × 1.1 m) and 30% hard surfaces, Pen 1; 4.9 m × 4.3 m and Pen 2; 2.1 m × 4.9 m
Captive- reared	0.04	1 month – daily handling	Purina Flock Raiser crumbles; 20%, 3.5%, 5%, -	Indoor galvanized wire cages
	0.9	2 months – no handling, but daily human presence	Purina Flock Raiser crumbles supplemented with Sportsman's Choice Floating Pond & Catfish Food – dry; 20– 32% 3.5–4%, 5–7.5%, 0.25 mg/kg	

			Outdoor elevated wire pens with pools of water (2.7 m × 1.9 m × 1.1 m) and 30% hard surfaces, Pen 1; 4.9 m × 4.3
	2 months – weekly handling	Sportsman's Choice Floating Pond & Catfish Food – dry; 32%, 4%, 7.5%, 0.25 mg/kg	
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Low density wild-caught	0.6	5 months – no handling	Outdoor elevated wire pens with pools of water (2.7 m × 1.9 m × 1.1 m) and 30% hard surfaces, Pen 1; 4.9 m × 4.3
	2 months bi-weekly handling	Sportsman's Choice Floating Pond & Catfish Food – dry; 32%, 4%, 7.5%, 0.25 mg/kg	
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