EFFECT OF DIETARY SUPPLEMENTATION OF INORGANIC PHOSPHORUS ON FEED INTAKE, PROTEIN INTAKE, FEED CONVERSION AND PHOSPHORUS GAIN/LOSS OF THE HYBRID AFRICAN CATFISH *Heterobranchus bidorsalis* (ö) X *Clarias gariepinus* (Ò) FRY

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ABSTRACT

Sixteen experimental diets were formulated to include four groups of inorganic phosphorus (P) sources {monosodium phosphate (MSP), monopotassium phosphate (MPP), monocalcium phosphate (MCP), and dicalcium phosphate (DCP) at four levels {A(0.40%), B(0.60%), C(0.80% and D(1.20%)}. Two controls of a non-phosphorus supplemental diet (CD) and a purified diet (PD) were fed along with the other 16 experimental diets to the fry of Heterobranchus bidorsalis X Clarias gariepinus hybrid (mean weight, 1.5 ± 0.12 g) at 5% body weight per day for 70 days. The results showed that the feed intake (FI), the protein intake (PI), the food conversion ratio (FCR) and the phosphorus gain/loss (PGL) varies significantly among the 18 diets tested ($P < 0.001$). The effect of the inorganic P sources on FI, PI, FCR and PGL was also significantly different ($P < 0.001$). The MSP supplemented diets appeared to elicit better responses in the fish than any of the other P-supplemented (MCP, MPP and DCP) diets. A comparatively higher quantity of protein was consumed by the fish fed the MSP diets (15.28%) than other P-supplemented diets. A loss in the percent phosphorus content of fish flesh fed MSP diet was observed (-0.04%).Based on the above, MSP diets were the best for enhancing growth.

Keywords: Inorganic phosphorus**,** *Heterobranchus bidorsalis* X *Clarias gariepinus,* Catfish hybrid, Protein Intake, Food Conversion Ratio.

INTRODUCTION

Channel catfish (*Ictalurus punctatus*) utilize inorganic elements to maintain osmotic balance between fluids in their body and water. Essential minerals may be obtained from water by exchange across gill membrane or from body by absorption across the gut (Phillips, 1959). The salmonid requirements for calcium, magnesium, potassium, sodium and zinc can be met directly from the water (Podoliak, 1970). However, certain minerals such as chlorides, phosphates and sulphates are more efficiently obtained from feed sources (Phillips *et al*., 1963). Dissolved phosphorus is poorly absorbed by trout (Phillips *et al*., 1963), while dietary phosphorus is used to meet the majority of the salmonid requirement.

Phosphorus is one of the most important nutrients for growth and normal development of bones in fish (Shin and Ho, 1989). It represents the third most expensive nutrient following protein and energy (Potchanakorn and Potter, 1987). Phosphorus is a component of a wide variety of organic molecules and it is a major constituent of animal protoplasm. It is also the most limiting nutrient for plant and algal growth in freshwater pond ecosystems (Dobins and Boyd, 1976). Pond ecosystems receiving small applications of phosphorus would respond more to increased phosphorus cycling than those receiving high applications of phosphorus (Lichtkoppler and Boyd, 1977). Diets containing high levels of animal protein may not require supplemental inorganic phosphorus (Phillips *et al*., 1993). The supplementation of production diet containing 25% whole herring meal with inorganic phosphates produced no significant difference in weight gain, feed conversion and mortalities of rainbow trout ($P > 0.05$) (Reinitz *et al.*, 1978). Ketola (1975) demonstrated that mineral supplements are required when soybean meal is substituted for fishmeal in diets fed to Atlantic salmon. Atlantic salmon fed a diet containing 0.70% phosphorus from plant sources required a minimum inorganic phosphorus supplement of 0.60% of the diet for normal growth and survival. The level of available dietary phosphorus required to maintain normal growth in rainbow trout was estimated to be 0.70-0.80% of the diet (Ogino and Takeda, 1978).

Phosphorus in channel catfish pond effluents can be a source of pollution in receiving waters (Schwartz and Boyd, 1994). Therefore, methods for reducing the amount of phosphorus in pond water and effluents have been suggested (Boyd and Tucker, 1985). These include reducing stocking and feeding rates, managing ponds to minimize or eliminate effluents, discharging effluents through settling basins or constructed wetlands, conservative feeding practices and the use of low phosphorus feeds. Boyd (1995) stated that methods to lower the amount of phosphorus added to feeds without reducing fish production should be the primary consideration of fish nutritionists. He maintained that providing high quality feed in a manner that ensures essentially complete consumption of feed by fish could lower phosphorus inputs in ponds. Gross *et al.* (1998) observed that phosphorus recovered from harvested fish from a fishpond tended to increase by approximately 18% as the phosphorus levels in diets increased. They further reported that there was a clear increase in phosphorus adsorption by pond bottom soils as phosphorus in diets increased.

Eya and Lovell (1997) reported that an all-plant commercial type diet with no phosphorus supplement and containing 0.20% available phosphorus was sufficient for maximum weight gain by channel catfish grown to marketable size in ponds. This was lower than 0.27% recorded for the same fish by Robinson *et al.* (1996). Both studies, however, agreed that the phosphorus requirement for the growth of large channel catfish in ponds was lower than that for small channel catfish (1.60-6.00 g) grown in aquaria.

This study was therefore designed to supplement locally formulated diets fed to *H bidorsalis x C. gariepinus* hybrid with either of the four inorganic phosphorus (monosodium phosphate, monocalcium phosphate, monopotassium phosphate and dicalcium phosphate). The growth and survival of the hybrid catfish fry was evaluated. In this study, the phosphorus requirement (0.06 to 0.80%) of the channel catfish fry (Robinson *et al.,* 1996; Eya and Lovell,1997) was exceeded as a means of testing higher doses on the survival and growth of the hybrid African catfish fry.

MATERIALS AND METHODS

Sixteen isonitrogenous experimental diets (CP=38.00%)were formulated to include four groups of inorganic phosphorus sources {monosodium phosphate (MSP),monopotassium phosphate (MPP), monocalcium phosphate (MCP), and dicalcium (DCP) at four levels {A (0.40%), B(0.060%, C (0.80%) and D(1.20%)}. Two controls {a non- inorganic phosphorus supplemental diet and a purified diet (Table 1)} were also formulated and used along with the 16 diets to feed 4-weeks old hybrid of *H. bidorsalis* x *C. gariepinus* (mean weight, 1.5± 0.12g) at 5% body weight per day for 70 days.

One thousand and eighty (1080) advanced fry of the hybrid were randomly stocked into 54 aerated aquaria (55 x 30 x 30 cm). The aquaria received a continuous supply of clean water and their filtration systems helped in the collection of faeces and other residues. Batches of 20 fry were introduced into each aquarium and fed the compounded diets at 4 hourly intervals starting from 8.00 hours. The temperature range throughout the experimental period was between 24^{o}C and 28^{o}C while the water pH was 6.60-6.80±0.2. Cultured catfishes were weighed with the aid of a Mettler balance every 7 days. The diet was adjusted in accordance with the body weight of fish. Both the experimental fish and test diets were analyzed in the laboratory for their proximate compositions (AOAC, 1995) (Tables 2a, 2b, and 3).

The nitrogen contents of the specimens (fish and diets) were determined by the microkjeldahl technique of Fels and Veatch (1959) and converted to total protein equivalent by multiplying by 6.25. The crude fat was measured in a Soxhlet apparatus of lipid by petroleum ether (b.pt 40-60^{0}c) extraction. The dry weight was calculated gravimetrically after drying at 105^{0}C for 24 hours and ash by combusting in a muffle furnace at 550^{0}C for 12 hours. The nitrogen free extract (NFE) was derived thus: NFE= 100 -% ash - % moisture -% protein -% lipid. The phosphorus gain/loss (PGL) was estimated thus: PGL = final tissue phosphorus - initial tissue phosphorus. Analysis of variance and least significant difference were used to compare treatment means (Steel and Torrie, 1990).

Table 1: Gross composition of experimental diets fed the African catfish hybrid fry for 70 days

Ingredient	Inorganic P - supplementation A 0.40% P	B 0.60% P	C 0.80% P	D 1.20% P	Control diet 0% P	Purified diet	
Yellow maize	9.81	9.55	9.29	9.07	10.32	Casein	33.00
Soyabean meal	54.76	54.86	54.84	54.86	54.68	Dextrin	35.00
Fish meal	10.95	10.96	10.97	10.97	10.97	Corn starch	20.00
Palm oil	5.00	5.00	5.00	5.00	5.00	Cod –liver oil	3.00
Salt	0.25	0.25	0.25	0.25	0.25	Palm oil	3.00
Vitamin mix[a]	0.60	0.60	0.60	0.60	0.60	Carboxymethyl Cellulose	3.00
Calcium & phosphorus free mineral mix[b]	1.80	1.80	1.80	1.80	1.80	Vitamin mix[a]	1.20
Inorganic phosphorus Supplementation (MSP), MPP, MCP, DCP	0.40	0.60	0.80	1.20	0.00	Calcium & Phosphorus free Mineral mix[b]	1.80
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	

Key: *MSP = Monosodium phosphate, MPP = Monopotassium phosphate MCP = Monocalcium phosphate, DCP = Dicalcium phosphate.*

a. Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): Thiamin, 10; riboflavin, 20; pyridoxin, 10; folacin,5; pantothenic acid, 40; choline chloride, 3000; niacin, 150; vitamin $B_{12,}$ 0.06; retinyl acetate (500,000Iu/g), 6; menadione bisulphate, 0;inositol, 400,; biotin, 2; vitamin C, 200; ethoxyquin, 200; alphatocopherol, 50; cholecalcipherol (1,000,000 Iu/g).

b. Contained g/kg of premix, $FeS0_4$ $7H_20$,5; $MgS0_4$. $7H_20$, 132; K_2S0_4, 329.90; KI,0.15; NaCI, 45; Na_2S04,44.88; $AICI_3$,0.15; $CoCI_2.6H_20$,5; $CuS0_4.5H_20$,5; $NaSe0_3$,0.11; $MnS0_4.H_20$,07; and cellulose, 380.97.

Table 2a: Proximate composition of experimental diets

Nutrient (%)	Dietary Groups A (0.40% P)	B (0.60% P)	C (0.80% P)	D (1.20% P)	Control Diet (0% P)	Purified Diet
Crude Protein (CP)	38.88	37.85	37.58	36.96	37.65	37.84
Ether Extract (EE)	4.64	5.02	5.68	4.64	5.48	5.32
Ash (AS)	10.56	10.65	10.48	12.32	10.54	5.55
Dry matter (DM)	11.70	11.50	11.80	11.21	11.65	11.70
Nitrogen Free Extract (NFE)	36.28	34.98	34.46	34.87	34.68	39.59
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Key: Dietary Groups A (0.40% P) B (0.60%P) C(0.80%P) D(1.20%P)

A	B	C	D	
1	*2*	*3*	*4*	*-Monosodium phosphate (MSP)*
5	*6*	*7*	*8*	*-Monopotassium phosphate (MPP)*
9	*10*	*11*	*12*	*-Monocalcium phosphate (MCP)*
13	*14*	*15*	*16*	*-Dicalcium phosphate (DCP).*

Note: Diets with numbers in the same row were supplemented with the same inorganic phosphorus; Diets with numbers in the same column had the same inorganic phosphorus inclusion.

Table 2b: Phosphorus contents of experimental diets

Ingredient %	Dietary Group A 0.40% inorganic P-supplementation	Dietary Group B 0.60% inorganic P-supplementation	Dietary Group C 0.80% inorganic P-supplementation	Dietary group D 1.20% inorganic P–supplementation	Control Diet 0% inorganic P-Supplementation
Yellow maize (%)	0.12	0.12	0.12	0.12	0.12
Soyabean meal (%)	0.15	0.15	0.15	0.15	0.15
Fish meal (%)	1.90	1.90	1.90	1.90	1.90
Blood meal (%)	0.17	0.17	0.17	0.17	0.17
% Inorganic P-supplementation (i.e. MSP, MPP, MCP or DCP)	0.40	0.60	0.80	1.20	0.00
Total % Dietary P concentration (TPC)	**2.74**	**2.94**	**3.14**	**3.54**	**2.34**

Key: MSP = Monosodium phosphate, MCP = Monocalcium phosphate,
MPP = Monopotasium phosphate, DCP = Dicalcium phosphate, TPC = Total phosphorus concentration.

RESULT

Table 3 shows the proximate composition of the hybrid African catfish (*H. bidorsalis* X *C. gariepinus*) fry fed diets supplemented with four inorganic phosphorus sources. Fish fed diets supplemented with DCP had more crude protein deposited in their body tissue (15.28%) than those fed diets supplemented with MSP (15.28%), MCP (14.13%) and MPP (13.10%). The control diet (19.12%) had similar crude protein value as DCP (19.10%). The fish tissue ether extract (EE) recorded were MSP (7.05%), MPP (5.95%) MCP (4.39%) and DCP (3.50%). The EE of control diet (8.69%) and purified diet (8.66%) were higher than those obtained with the P-supplemented diets. Ash content (AS) also varied significantly among the test diets (P < 0.001).

The daily feed intake (FI) of fish ranged from 0.37g in the purified diet (PD) to 0.54g in the DCP diet. These values varied significantly among the 18 test diets (Table 4) (P < 0.001). The mean effects of the supplementary phosphorus on FI of fish were MCP (0.48g), DCP (0.47g), MSP (0.44g) and MPP (0.43g). The fish fed less of the purified diets (0.37g) than any of the P-supplemented diets and the control diet (0.43g). There were also significant effects of the dietary P sources on the daily consumption of feed by the fish (Table 4) (P < 0.001). There were positive correlations between FI and FCR (0.84, P <0.001) and the weekly protein intake (0.87, P < 0.001), while FI was negatively correlated with PGL (-0.20, P < 0.001). The protein intake (PI) of the fish ranged from 1.01% in the purified diet to 1.47% in the MSP diet. These values also varied significantly among the 18 test diets (Table 4) (P < 0.001). The effects of the supplementary phosphorus sources on PI were MSP (1.29%), MCP (1.28%), MPP (1.18%) and DCP (1.18%). Less protein was consumed by the fish fed the control (1.13%) and the purified (1.01%) diets than those fed the P –supplemented diets (Table 4). PI varied significantly with the dietary inorganic P sources (P < 0.001). PI was positively correlated with FI (0.87, P < 0.001) and FCR (0.84, P < 0.001) but was negatively correlated with PGL (-0.19, P < 0.001).

The food conversion ratio (FCR) ranged from 1.65 in the purified diet to 4.94 in the MPP diet. The values also varied significantly among the 18 test diets (Table 4) (P < 0.001). The effects of the supplemented phosphorus on FCR were MSP (3.51), MCP (3.72), DCP (4.04) and MPP (4.11). The FCR of fish fed the control (1.82) and the purified (1.65) diets were better than those fed the P-supplemented diets (Table 4). The FCR of fish also varied significantly among the P-supplemented diets and the controls (P < 0.001).

The phosphorus gain or loss (PGL) of the fish ranged from –0.05% in the MSP diet to 0.03% in the MCP diet. These values also varied significantly among the 18 test diets (Table 4) (P < 0.001). The effects of the supplementary phosphorus on PGL were MCP (0.02), MPP (0.01%), DCP (0.00%) and the purified (0.04%). Fishes fed the control (0.08%) and the purified (0.04%) diets gained more phosphorus than those fed any of the P-supplemented diets (Table 4). The PGL of fish varied significantly among the P-supplemented diets and the controls (P < 0.001). PGL showed significant negative correlations with FI (-0.20), PI (-0.19) and FCR (-0.25) (P < 0.001).

There were significant effects of duration (days) on FI, PI, FCR and PGL (Table 5) (P < 0.001). Whereas protein intake increased progressively from day 7 (0.20%) to day 70 (2.45%), there seemed to be moderate variability of the fish tissue phosphorus as the study progressed from day 7 (0.07%) to day 70 (0.01%) (Table 5).

DISCUSSION

Data on the hybrid African catfish fed supplemented phosphorus diets is scanty. Fish fed MCP-supplemented diets consumed more of this diet than those fed DCP, MSP and MPP. The significant differences in performance of the P-supplemented diets, the purified and the control diets on feed intake may be attributed to the levels of P-supplementation and sources of inorganic P in the diet (Table 4). The higher quantity of protein consumed by the fish fed with the MSP diet than any of the P-supplemented diets (Table 4) is consistent with the higher protein intake recorded by Andrews *et al*. (1973) for channel catfish, fed MSP diet when compared with those fed MCP and DCP diets. The range values of PI (1.30 –1.40%) for this study also compared favourably with 1.02 – 1.72% reported for *Oreochromis aureus* fingerling (Wu and Jan, 1977). The significant but negative correlation between PI and PGL (P < 0.001) may be an indication that increased protein intake by fish possibly had negative effect on the phosphorus gain/loss.

Table 3: Effect of inorganic phosphorus dietary supplementation on the proximate composition of the African catfish hybrid fry fed for 70 days

Proximate composition	Inorganic Phosphorus Source									
	A MSP	B MPP	C MCP	D DCP	CD	PD	Overall mean	S.E	L.S.D	Sign. Level
Crude Protein (CP)	15.28	13.96	14.13	19.10	19.12	19.00	14.75	0.19	0.42	* * *
Ether Extract (EE)	7.05	5.95	4.83	3.50	8.69	8.66	5.70	0.25	0.55	* * *
Ash (AS)	2.25	1.85	2.13	2.04	3.59	4.31	2.34	0.10	0.22	* * *
Moisture Content	7.12	68.46	65.36	66.12	66.34	65.53	67.16	1.14	0.56	* * *
Nitrogen Free Extract (NFE)	5.00	9.78	13.35	12.79	2.17	2.50	7.14	6.14	3.14	* * *
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**				

*MSP = Monosodium phosphate, MPP = Monopotassium phosphate, MCP = Monocalcium phosphate Dicalcium phosphate, S.E. = Standard error, L.s.d = Least significant difference; * * * = Significant at 0.1% (P<0.001*

Table 4: Growth performance of the African catfish hybrid fry fed different inorganic phosphorus supplemented diets

Diet	Feed intake (FI-g)	Protein intake (PI-%)	Food conversion ratio (FCR)	Phosphorus gain/loss (PGL-%)
	Supplementation with monosodium phosphorus			
Diet 1 (0.40%P)	0.39	1.31	4.33	-0.03
Diet 2 (0.60%P)	0.45	1.21	4.33	-0.04
Diet 3 (0.80%P)	0.46	1.17	3.52	-0.04
Diet 4 (1.20%P)	0.47	1.47	3.23	-0.05
Mean	0.44	1.29	2.95	-0.04
	Supplementation with monopotassium 3.51phosphate (MPP)			
Diet 5 (0.40%P)	0.45	1.18	4.94	0.00
Diet 6 (0.60%P)	0.38	1.03	4.02	0.01
Diet 7 (0.80%P)	0.41	1.09	3.84	0.00
Diet 8 (1.20%P)	0.46	1.43	3.65	0.02
Mean	0.43	1.18	4.11	0.01
	Supplementation with monocalcium phosphate (MCP)			
Diet 9 (0.40%P)	0.53	1.28	3.71	0.01
Diet 10 (0.60%P)	0.44	1.17	3.77	0.02
Diet 11 (0.80%P)	0.46	1.24	3.60	0.02
Diet 12 (1.20%P)	0.50	1.45	3.80	0.03
Mean	0.48	1.28	3.72	0.02
	Supplementation with dicacium phosphate (DCP)			
Diet 13 (0.40%P)	0.46	1.17	4.32	-0.01
Diet 14 (0.60%P)	0.45	1.03	3.74	-0.01
Diet 15 (0.80%P)	0.41	1.12	3.49	0.04
Diet 16 (1.20%P)	0.54	1.40	4.61	-0.01
Mean	0.47	1.18	4.04	0.003
Diet 17 (control diet)	0.43	1.13	1.82	0.08
Diet 18 (purified diet)	0.37	1.01	1.65	0.04
Overall mean	0.45	1.22	1.61	0.01
S.E. of mean	0.007	0.012	0.001	0.001
L.S.D.	0.021	0.033	0.004	0.001
Significant level	***	***	***	***

*Least significant difference; * * * = Significant at 0.1% (P < 0.001); S.E = Standard error.*

This was evident from the negative values of PI recorded for the fish fed with MSP diets and which had better protein intake than those fed other P-supplemented diets. Lovell (1978) reported that the availability of phosphorus in a diet ensures the maximal utilization of nitrogen, which is a major component of protein necessary for growth. This was not the case with the P-supplemented diets of this study.

The FCR of fish fed the control (1.82) and the purified (1.65) diets compared favourably with Donald and Robinson (1987) values of 1.60 – 1.90 for red drum *(Sciaenops ocellatus).*

Table 5: Effect of duration (days) on the growth performance of the African catfish hybrid fry fed different supplemental dietary phosphorus

Growth Parameter	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days	49 Days	56 Days	63 Days	70 Days	S.E±	L.S.D	Sign. Level
Feed intake (FI) (g)	0.09	0.17	0.24	0.31	0.39	0.48	0.58	0.63	0.70	0.86	0.006	0.016	* * *
Protein intake (PI (g %)	0.20	0.44	0.66	0.84	1.05	1.29	1.56	1.69	1.88	2.56	0.009	0.024	* * *
Food conversion ratio (FCR)	0.38	0.79	1.16	1.46	1.84	2.22	5.62	6.34	7.17	9.13	0.001	0.003	* * *
Phosphorus gain / loss (PGL) (%)	0.7	0.05	0.04	0.05	0.08	0.04	0.34	0.01	0.05	0.01	0.001	0.003	* * *

However, the data on red drum contrasted with the FCR values obtained when the fish in this study were fed with the P-supplemented diets (Table 4). Robinson *et al*. (1996) recorded FCR values of 1.43 – 1.45 for channel catfish (*I. punctatus*) fed isonitrogenous diets supplemented with MSP, while Eya and Lovell (1997) reported FCR values of 1.60 – 1.90 for the same species. However the results of Donald and Robinson (1987), Robinson *et al*. (1996) and Eya and Lovell (1997) compared favourably with the FCR of control diets (1.60 – 1.82) of this study. Feeding fingerlings of *Oreochromis niloticus* with MSP-supplemented diets for 12 weeks, Robinson *et al*. (1996) reported higher FCR values (2.30 – 2.50) relative to the present control (1.82) and purified (1.65) diets. This range values (2.30 – 2.50) was lower than the values (3.51 – 4.04) recorded with the P-supplemented diets of this study (Table 4).

Furthermore, FCR decreased as the P-supplementation levels increased from 0.04 – 1.20 % (Table 4). This result agreed with Sakamoto and Yone (1978) who reported that a decrease of dietary phosphorus resulted in a decrease in glycogen (energy) content of liver, crude ash, calcium and phosphorus content of vertebrae.

The phosphorus gain/loss (PGL) was estimated as the weekly increase or decrease in percent phosphorus content of fish. Despite the higher quantity of protein consumed by fish fed the MSP diet (Table 4), there was a loss in the fish percent phosphorus compared the gains recorded with the MCP and control diet. The availability of phosphorus in a diet ensures maximal utilization of nitrogen, which is a major component of protein necessary for growth (Lovell, 1978). The significant negative correlations between PGL and PI and FCR ($P < 0.001$) suggested that the above parameters (i.e. feed intake, protein intake and feed conversion) affected the percent phosphorus gain in fish negatively.

The effect of duration (days) on feed intake (Table 5) showed that the quantity of food consumed increased as the age of fish increased. This increased food consumption relative to increasing size and age may be due to the interaction of factors affecting internal motivation or drive for feeding such as season, temperature, time and nature of last feeding, food stimuli perceived by the senses, lateral line system, hunger curiosity and gluttony (Lagler, *et al*., 1977). As time progressed (7 – 70days), the fish species of this study must have developed a higher sense of perception of food stimuli, which enhanced their internal motivation to feeding. Furthermore, the protein intake increased with time from day 7 to day 70 (Table 5) in agreement with Lagler *et al.* (1988) who reported that increased food consumption relative to size and age is affected by time among other factors. Similarly, the food conversion ratio (FCR) values increased as the study progressed from day 7 to day 70 (Table 5). This implied that time affected the ability of the fish to convert a unit gram of diet consumed to a unit gram of flesh produced. It is possible that the development of the lateral line system and sense of perception of food stimuli of the fish with time increased their internal motivation to feeding (Lagler *et al*., 1977) and consequent rate of food conversion

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