Effect of dietary supplementation of *Azolla filiculoids*, *Azolla microphylla* on Fish Growth and Excretion of metabolites in *Oreochromis mossambicus* (Peters 1852)

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Abstract:

Now a day's eco friendly food alternatives are been used instead of chemical contents and hence utilization of natural plant based alternatives are in consideration, in present experimental trial *Azolla filiculoids, Azolla microphylla* powder rich diet were formulated and the study was carried out upon growth performance of *Oreochromis mossambicus* (Peters 1852), formulation of azolla rich four fish feeds containing concentration (0.30, 0.60, 0.80 and 1.0g 100 g-1 of fish feed) were formulated. A diet without azolla served as control. Significantly highest growth performance, carcass protein, apparent protein digestibility, nutrient retention , digestive enzyme activity were observed in the group fed diet containing azolla at a concentration of 0.80 g, 100 g-1 of diet. Excretion of metabolites remained low, while the values of VSI and HSI stayed elevated. Muscle glycogen and liver glycogen noted low, muscle protein were high in fish fed eD-3 containing Azolla concentration of 0.80g, 100g-1 of diet. Present investigation indicates that Azolla at appropriate concentration can be a useful.

Index terms: *Azolla filiculoids, Azolla microphylla*, Fish Health, *Oreochromis Mossambicus*, Growth Study.

Introduction:

Intensive culture of tilapia has resulted in problems such as deterioration of water quality and the outbreak of diseases. Currently, fish disease is managed through the use of antibiotics which have led to antimicrobial resistant pathogens, reduction in beneficial microbiota in the gastrointestinal (GI) ecosystem, including the accumulation of residual antibiotics in fish muscle making it unsuitable for human consumption. To overcome these problems a new feeding approach using natural health improving and

growth promoting feeds is explored. Vaccines are being developed and marketed and they generally cannot be used as a universal disease control measure in aquaculture. Juvenile fish are fully immunocompetent and do not always respond to vaccination. Vaccination by injection, sometimes are the only effective route of administration, is impractical when supplied to small fish or large number of fish. This situation is avoided by an alternative in the production system through the use of beneficial natural product The present research was conducted to evaluate the effect of dietary supplementation of Azolla *filiculoids*, *Azolla microphylla* on fish health , digestibility and excretion of metabolites in tilapia *Oreochromis mossambicus* (Peters 1852)

Here we consider hanging freshwater ferns genus Azolla as an alternative for the preparation of protein fish feed. While species diversity was higher in episodes in the geologic past,6 the genus Azolla exhibits seven species worldwide that flourish in tropical to temperate regions of the world.[1, 2, 3] Azolla ferns exhibit high relative growth rates when cultivated individually on an open water surface, i.e. not competing for light. relative growth rates of over 0.5 d−1, or biomass repetition times of less than 2 days have been reported much higher than generally encountered in land plants.(4, 5) A unique feature of Azolla ferns is that they host nitrogen (N2)-fixing cyanobacteria, *Nostoc azollae*, in the cavities of their leaves.[6, 7, 8] These phototrophic symbionts fix N2 during the day and likely release it as NH4 + in the leaf cavities to be taken up by the ferns. *Azolla* is of interest as a protein feed due to the high protein content of its biomass, which was reported to be between 200–400 g kg^{-1} of the dry weight (DW). [9.10]

I. Materials and Methods

II-1. Diet preparation:

Azolla filiculoids, *Azolla microphylla* were sun dried first and used in powdery form in fish diet. Here we made small pellets so as to become easy to consume fish fingerlings. The macronutrient analysis of *Azolla filiculoids*, *Azolla microphylla* were done by using standard method (AOAC, 2005) Five experimental diets with containing varying concentrations of *Azolla filiculoids*, *Azolla microphylla* (0.30, 0.60, 0.80 and 1.0%) were formulated using processed soybean as the protein source. The dietary ingredients and proximate composition of the formulated diets are given in Table 1

II-2. Experimental design:

Tilapia, *Oreochromis mossambicus* (Peters 1852) fry were collected from the fish farm Faridabad, Haryana, India. Experiment was conducted in biofloc system. With aeration facilities in the where the temperature was kept as 25±1°C after an initial 10-day acclimation period, fry (mean body weight: 1.22±0.04g) were randomly distributed among the portable plastic pool, with 25 fish per portable plastic pool. Each diet treatment was tested in replicate of four. All fish were fed 2 times daily, at 09:00 am and at 15:00 pm. The feeding rate was at 5% body weight day^{-1} for the whole rearing period

of 60 days, and the amount of feed was adjusted every 10^{th} day following a bulk weighing of each group of fish. The fish were exposed to their respective diet for 4h during each ration, afterward; the uneaten feed was siphoned out, stored and dried separately for calculating the feed conversion ratio (FCR). The faecal matter annulled by the fish in each portable plastic pool was also collected by siphoning, dried in a hot air oven (60^oC) and subsequently analyzed for digestibility estimations. The water in the aquaria was renewed daily with water that had been stored and adjusted to the laboratory temperature (25^oC). At the end of the experiment, fish from all the treatments were weighed and length was also recorded individually to the nearest gram and processed for succeeding analyses. 10 fish were obtained from each aquarium and kept on an ice tray. The viscera of the fish were extirpated for the calculation of the viscero-somatic index (VSI). Liver was removed for calculating HSI and also for the estimation of liver glycogen [11]

III-2 . Analytical Techniques

The feed ingredients, experimental diets, faecal matter samples and fish carcass (initial and final) were analyzed following [12] Cr_2O_3 levels in both the diets and the faecal samples were estimated spectrophotometrically following the method of Furukawa [13] pH and dissolved oxygen were maintained throughout experimental period . AT the end of the feeding schedule, water samples from each aquarium were collected at 2-h intervals over a 24-h period and used for estimating [14] the excretory patterns of total ammonia (NH_4-N) and reactive phosphate (o-PO_4) production; calculations were made method suggested [15] Ammonia/orthophosphate excretion rates (N-NH_4/o-PO_4 mg kg^{-1} fish per 2-h interval) were measured at 2-h intervals from the water and calculated

Daily ammonia/orthophosphate production (DP) rates (in milligrams per day) were estimated by summing up the concentrations obtained at 2-h intervals over a 24-h period. Live weight gain (in grams), growth percentage gain, specific growth rate [% body weight (BW) per day], feed conversion ratio (FCR), gross protein retention (GPR) and gross energy retention (GER) were calculated using standard methods (Steffens, 1989). Apparent protein digestibility (APD) of the diets was calculated according to Cho *et al.* (1982). Gross energy content of the diets and fish were calculated using the average caloric conversion factors of 0.3954, 0.1715 and 0.2364 kJ g^{-1} for lipid, carbohydrate and protein, respectively.

II-4.Statistics Analysis

ANOVA followed by Duncan's multiple range test [16] and student 't' test were applied to find out the significant differences between different treatments. Data were further subjected to orthogonal polynomials for trend analysis.

III- Results

III-1: Fish growth, digestibility and nutrient retention

Survival was not affected by the inclusion levels of azolla mix. Growth performance in terms of live weight gain (Fig.2), growth percent gain in BW and final length), SGR (3,) and nutrient retention (PER, GPR, GER and APD) (fig.4 to 7) increased when dietary Azolla level were increased from 0.30g to 0.80g 100 g^{-1} of diet; further increase in dietary azolla mix level (>0.80g 100g^{-1}) resulted in a significant ($P<0.05$) growth depression and nutrient depletion. Apparent protein digestibility (Fig. 8) was significantly ($P<0.05$) higher in fish which were fed diets containing Azolla mix. at 0.80g 100 g^{-1} than in fish fed Azolla mix free diet (Control) or diets containing low or high levels of Azolla mix. FCR values were also significantly ($P<0.05$) lower in fish fed diet containing Azolla mix at 0.80g 100g^{-1} than fish fed other dietary preparations including control diet (Table 2)

III-2: excretory levels of total ammonia (N-NH_4) and reactive phosphate (o-PO_4)

Total ammonia excretion and reactive phosphate production were significantly lower ($P<0.05$) in fish fed azolla mix at 0.80g 100 g^{-1} of diet than fish fed other dietary preparations or control diet (Table 3). Irrespective of the azolla mix level N-NH_4 excretion showed a peak at 6 h post-feeding, while o-PO_4 production showed an initial high level at 2 h post-feeding and a peak between 14 and 16h post-feeding (Table 3 and Figs. 9).

III-3: Fish carcass composition

The body composition of the fish was also affected by the azolla mix concentrations in diets. The accumulation of carcass protein, fat, phosphorus and gross energy were significantly ($P<0.05$) high in groups fed diets containing azolla mix at 0.5 to 0.80 g 100g^{-1} of diet. Carcass ash contents remained significantly ($P<0.05$) low at 0.80g of azolla mix level

IV: Discussion:

Filicophyta as the major source of feed during pond culture of macro-phytophagous fish species *Labeo rohita and Cirrihinus mrigala were shown better growth,*Feed acceptance trial was conducted for these seven species using *Azolla imbricata* and *Azolla pinnata* species. Had been recorded higher growth rates fed with azolla [[17]

Azolla filiculoides in pond and examined its potential as a fish feed. In a feeding experiment with *Tilapia nilotica*,. Diets C and D exerted the same effect on the growth of Tilapia after 3 weeks of feeding with a 17% decrease of growth compared to the control. Based on the results, it was observed that azolla can replace about 20% of tilapia feed, which indicates the beneficial effect of the use of aquatic plant. similar kind of study using dry azolla meal as a sole protein source for feeding tilapia *Oreochromis mossambicus (Peters 1852).* The inclusion levels of azolla meal were 0, 15, 20, 30, 40 and 45% on dry weight basis in diet. Comparing growth results, juveniles fed with 15% of azolla meal have exhibited best growth followed by azolla free diet. Considering cost of feed, the study recommended to use 45% azolla incorporated diet for tilapia in a fertilized pond. Another study carried out on Azolla pinnata shows that the feed macronutrients value along with protein, vitamins, and minerals. Which shows the better alternative for the commercial fish food *Barbonymus gonionotus* [18]

Significantly highest growth performance, carcass protein, apparent protein digestibility, nutrient retention , digestive enzyme activity were observed in the group fed diet containing azolla at a concentration of 0.80 g, 100 g-1 of diet. Excretion of metabolites remained low, while the values of VSI and HSI stayed elevated. Muscle glycogen and liver glycogen noted low, muscle protein were high in fish fed eD-3 containing Azolla concentration of 0.80g, 100g-1 of diet. Present investigation indicate that azolla at appropriate concentration can be a useful.

Table 1. Ingredient (%) and proximate analysis (% dry weight basis) of five experimental diets with different levels of Azolla Mix (g 100 g-1 of diet)

Ingredients	Experimental fish feed (Diets)				
	eD0	eD1	eD2	eD3	eD4
Groundnut Oil Cake	55.00	55.00	55.00	55.00	55.00
Rice Bran	3.20	2.90	2.60	2.40	2.20
Vitamine Premix	10.00	10.00	10.00	10.00	10.00
Maize Flour	3.20	3.20	3.20	3.20	3.20
Soybean Flour	26.60	26.60	26.60	26.60	26.60
Chromium oxide	1.00	1.00	1.00	1.00	1.00
Phosphorus	1.00	1.00	1.00	1.00	1.00
Azholla Mix *	-	0.30	0.60	0.80	1.00
Proximate analysis %					
Dry matter	80.12±0.02	90.32±0.03	91.16±0.02	91.21±0.04	91.23±0.04
Crude protein	38.80±0.22	39.90±0.24	40.01±0.19	40.13±0.2	40.18±0.20
Crude fat	7.16±0.29	7.40±0.30	7.50±0.28	7.51±0.31	7.58±0.26
Crude fibre	5.70±0.23	6.10±0.11	5.95±0.12	5.97±0.20	5.98±0.31

*Azolla was sun dried and processed in an autoclave to eliminate antinutritional factors

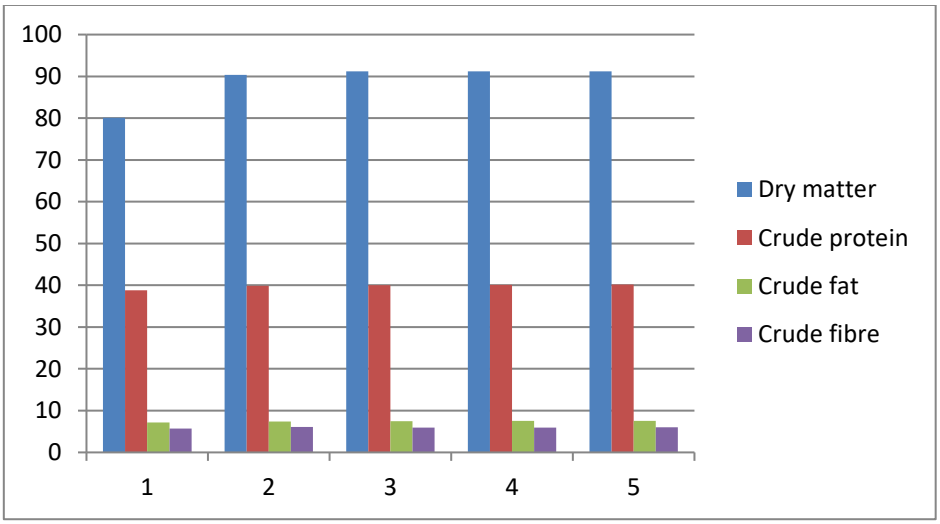

Figure 1: proximate analysis %

Table 2 :- Results of dissimilar levels of azolla supplement on growth performance, , nutrient retention , digestibility and excretion of metabolites in tilapia *Oreochromis mossambicus* (Peters 1852) fry under laboratory conditions .

Parameters	Experimental Diets				
	eD0 (control)	eD1	eD2	eD3	eD4
Initial weight (g) (IW)	1.30±0.03a	1.32±0.02a	1.33±0.02a	1.31±0.04a	1.34±0.02a
Initial length (cm) (IL)	4.10±0.05a	4.35±0.04a	4.24±0.06a	4.19±0.04a	4.30±0.07a
Final weight (g) (FW)	3.22±0.04e	3.23±0.04d	5.52±0.05b	6.97±0.31a	4.63±0.06c
Final length (cm) (FL)	5.86±0.07d	6.28±0.10cd	6.93±0.12b	7.72±0.12a	6.19±0.09c
Live weight gain (LWG)	1.92±0.04e	1.91±0.04d	4.19±0.06b	5.66±0.22a	3.29±0.02c
Specific growth rate (SGR)	1.76 ±0.02e	1.93±0.05d	2.69±0.04a	3.53±0.04b	1.89±0.02c
Feed conversion ratio (FCR)	2.33±0.05a	1.98±0.07a	1.99±0.05a	1.83±0.07b	2.04±0.08a
Gross Energy retention (GER)	18.99±0.90b	20.82±0.49b	20.97±0.50b	23.95±0.90a	20.51±1.02b
Gross Protein retention (GPR)	23.96±1.10b	24.90±0.68b	26.38±0.95b	30.92±1.40a	24.58±1.17b
Protein efficiency Ratio (PER)	1.28±0.04b	1.32±0.23b	1.36±0.02b	1.51±0.04a	1.37±0.05b
Apparent protein digestibility (APD%)	82.01±0.15d	82.98±0.35c	83.60±0.10b	84.17±0.26a	80.42±0.19c

All values are mean ± SE of mean. Means bearing different letters in the same row differ significantly (P<0.05)

Table 3: Excretory levels of total ammonia (N-NH_4) and reactive phosphate (o-PO_4)

Excretory levels	Experimental Feeds				
	eD0 (control)	eD1	eD2	eD3	eD4
Total ammonia excretion (mg kg^{-1} BW day^{-1})	1251.40±2.61a	980.45±6.56b	630.66±1.17 d	522.62±4.88 e	767.15±5.45c
Total phosphate production (mg kg^{-1} BW day^{-1})	232.82±0.17a	161.99±10.20c	140.71±5.32 d	139.78±2.21 d	195.77±3.94b

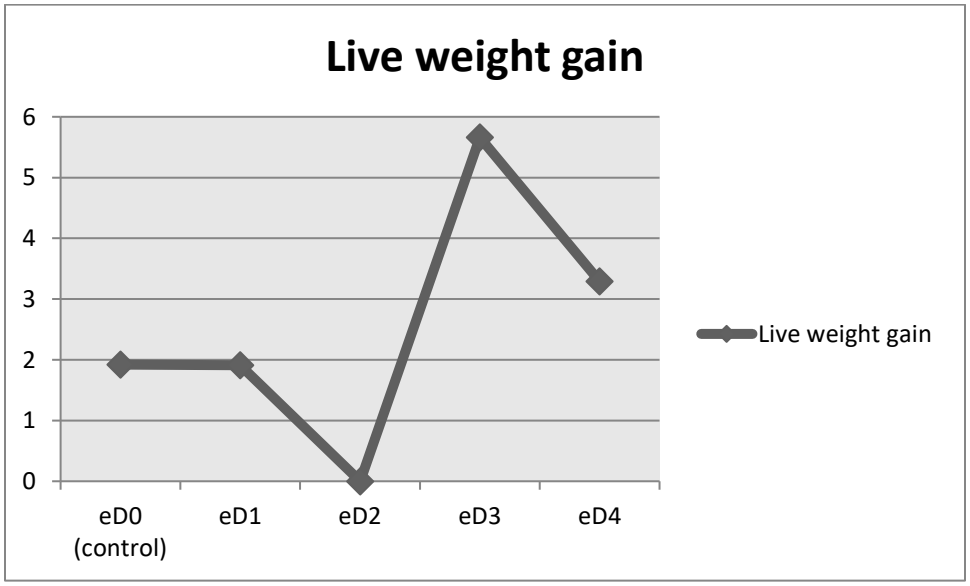

Figure 2: Live Weight Gain

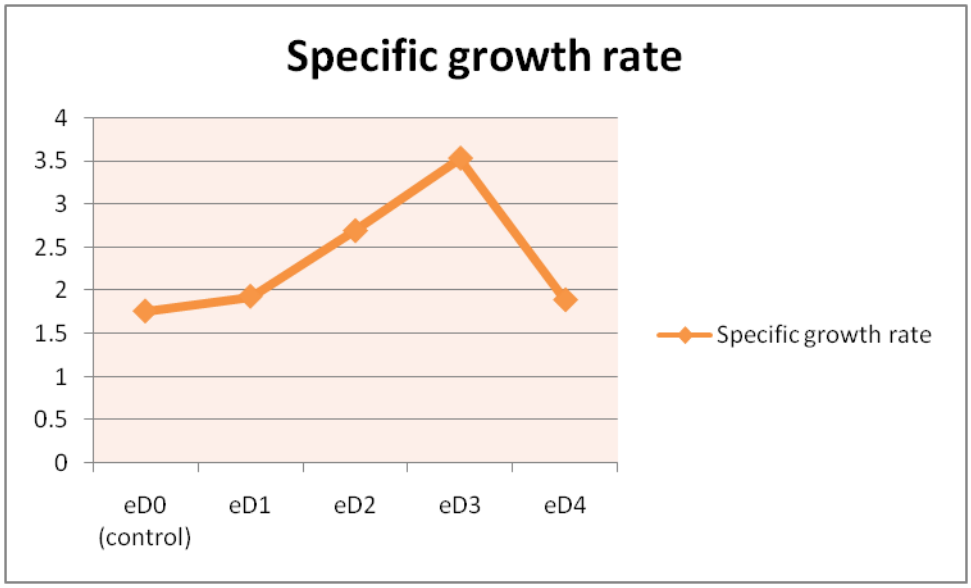

Figure 3: Specific Growth Rate

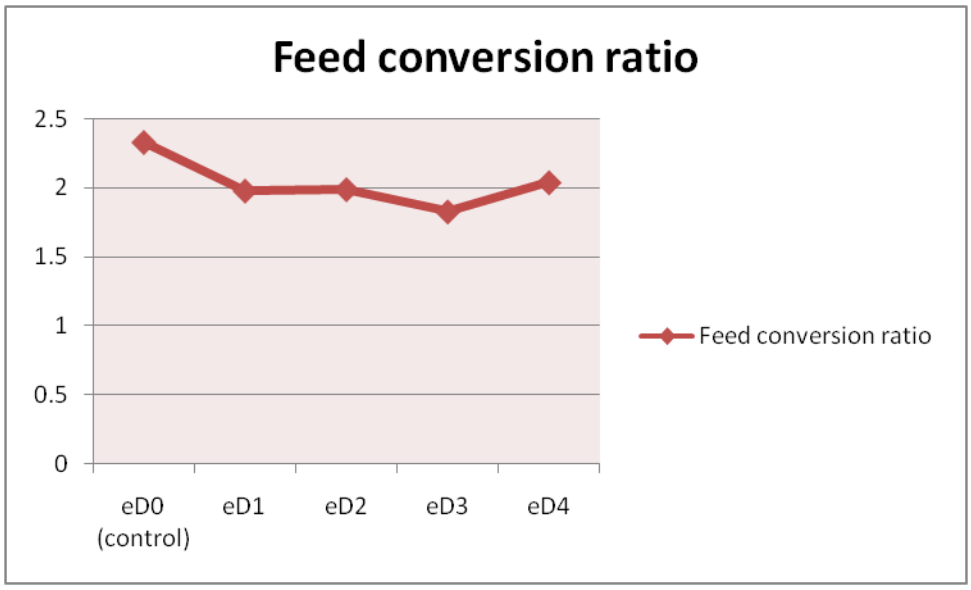

Figure 4: Feed Conversion Ratio

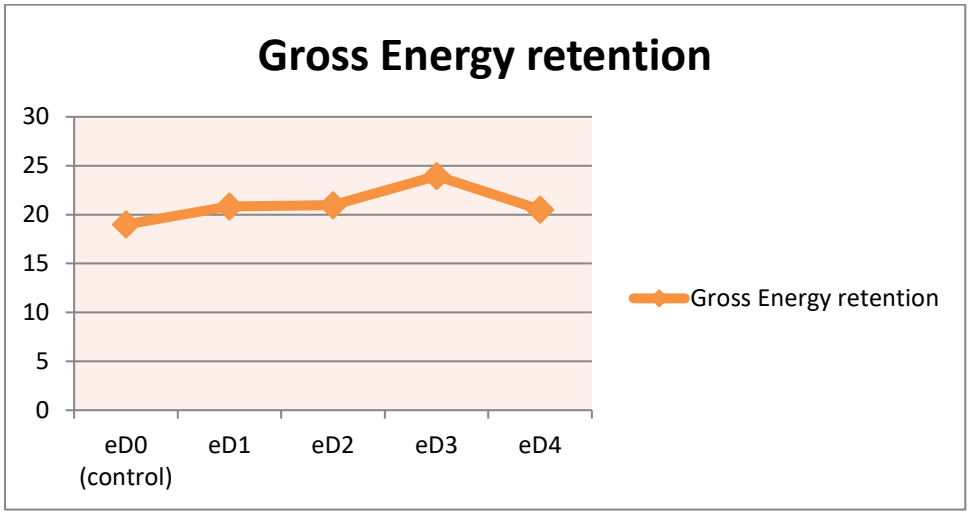

Figure 5: Gross Energy Retention

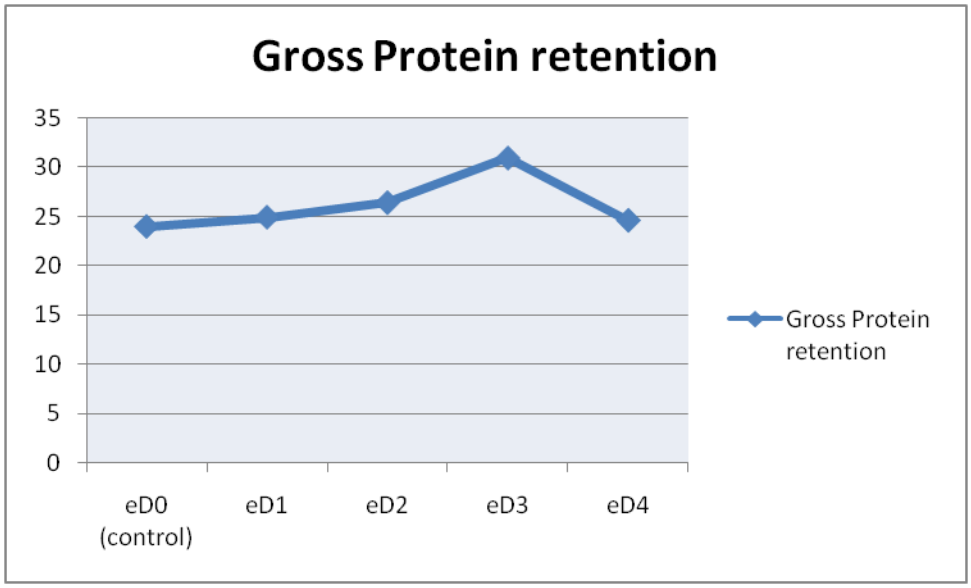

Figure 6: Gross Energy Retention

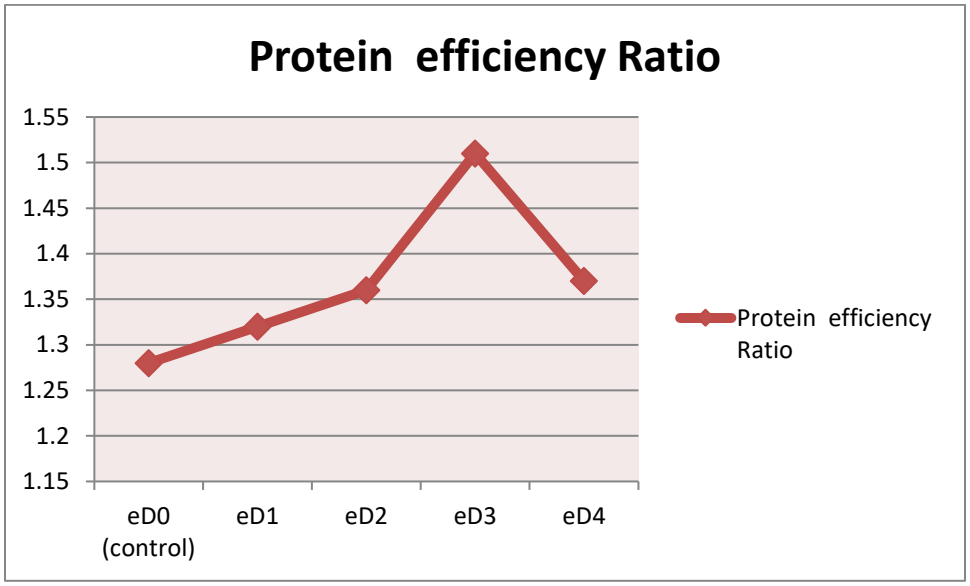

Figure 7: Protein Efficiency Ratio

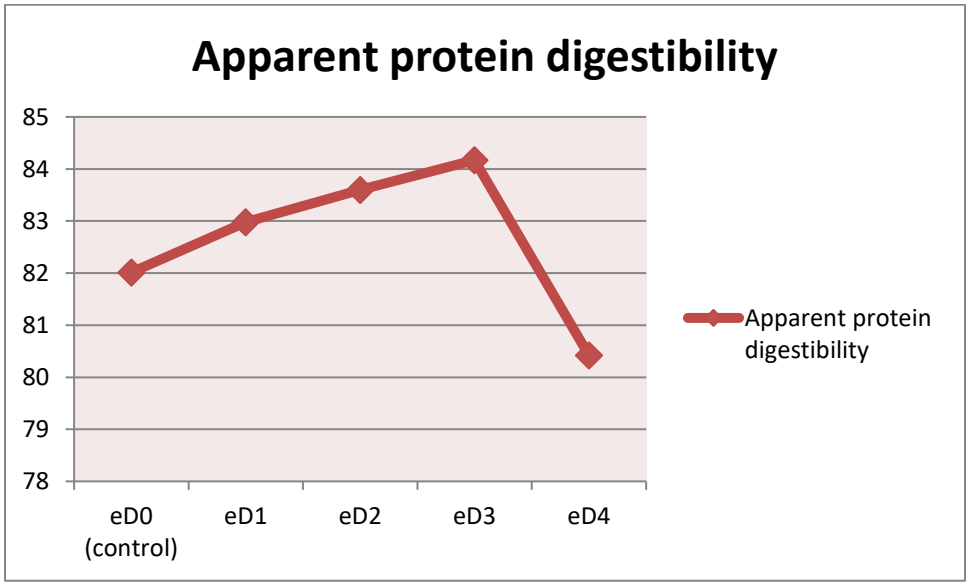

Figure 8: Apparent protein digestibility (APD %)

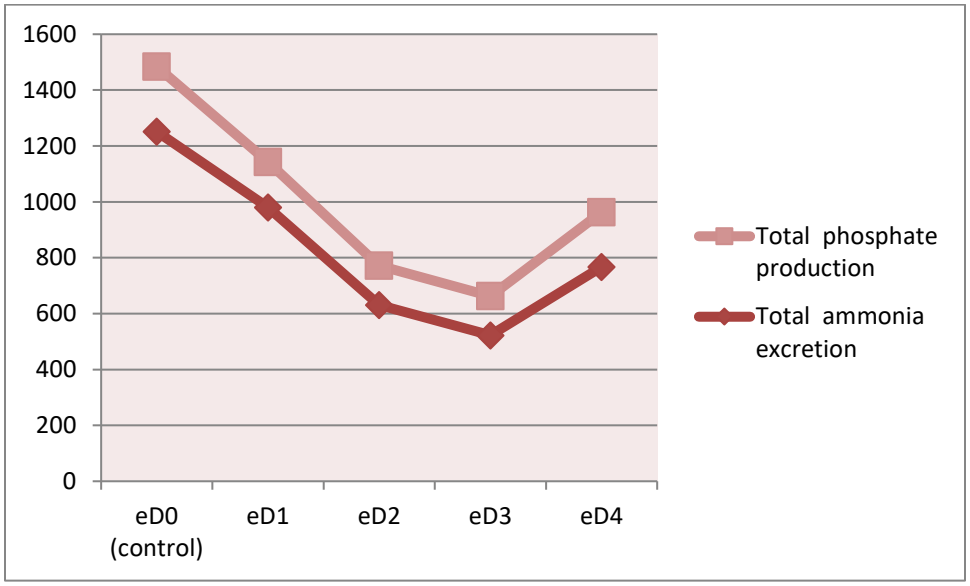

Figure 9: Excretory levels of total ammonia (N-NH4) and reactive phosphate (o-PO4)

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