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Terminal-Phase Replacement of Artemia with Enriched Moina in Indonesian Mahseer (Tor soro) Larviculture: Effects on Growth, Survival, Intestinal Morphometry, and Feed Economics

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Abstract

Reliance on commercial *Artemia* cysts raises hatchery costs and makes hatcheries susceptible to supply fluctuations. The potential of using enriched *Moina* as an alternative to *Artemia* for the last stage of Indonesian mahseer (*Tor soro*) larviculture without compromising the production performance was evaluated. Larvae were reared for 28 days and assigned to five terminal-phase replacement windows: 0, 7, 14, 21 or 28 days of enriched *Moina* feeding, or 0, 25, 50, 75 or 100% of the rearing period (five replicate containers per treatment). Growth, survival, supplied-feed utilization, feed economics, live-feed composition and intestinal morphometry were assessed. Dunnett tests were applied to compare each replacement treatment with the 100% *Artemia* control. Enriched *Moina* had a higher crude protein but lower lipid, ash and total measured amino acid concentration than *Artemia* on a dry matter basis. However, even at 25% substitution, the final weight and feed efficiency were reduced but the survival and three measured intestinal morphometric indices were not significantly different from the control. At ≥ 50% substitution, area and diameter of intestinal folds were reduced and at 75–100% substitution, all measured intestinal indices were reduced. Complete replacement decreased survival from 98.0 to 88.4% and increased the economic conversion ratio by about 6 times. Treatment 25% reduced total feed cost not. Replacing *Artemia* with enriched *Moina* only during the last 7 days can therefore reduce *Artemia* use while maintaining survival and measured intestinal morphology but is not nutritionally or economically equivalent to continuous *Artemia* feeding.

1. Introduction

Indonesian mahseer (dewa, *Tor soro*) is a high value freshwater cyprinid species of interest for aquaculture diversification, conservation hatchery and local seed production. Larval rearing is still a critical stage since early larvae are very sensitive to feed quality, prey characteristics and rearing conditions. Larval performance is sensitive to temperature and natural-feed selection in *T. soro* (Subagja et al., 2021; Ismarica et al., 2024), which suggests that the selection of feeding strategies compatible with the changing requirements of growing larvae is critical for consistent seed production.

Live prey is particularly important in initial feeding because larvae have an immature digestive system and need prey of appropriate size, motility, digestibility and nutrient availability (Samat et al., 2020; Joshua et al., 2022; Ciji et al., 2026). *Artemia* nauplii fulfilll many of these requirements: they are easy to hatch, are reliably accepted and are operationally predictable. However, reliance on commercial *Artemia* cysts increases production costs and exposes hatcheries to price and supply fluctuations (Lipscomb et al., 2020; Joshua et al., 2022; Eiras et al., 2023).

Moina spp. is a freshwater cladoceran that can be cultured locally and enriched before feeding. Their suitability as *Artemia* alternatives is however species and stage-specific and depends on prey size, ingestion, digestibility, culture conditions, enrichment method and nutrient composition (Rasdi et al., 2020; Samat et al., 2020; Joshua et al., 2022). *Moina* has been tested as a partial or total replacement of *Artemia* in shrimp postlarvae, ornamental fish and catfish (Martín et al., 2006; Eiras et al., 2023; Yusof

Hanan et al., 2024). Across these studies, partial replacement is more likely to retain survival or acceptable larval performance than is complete replacement. Economic evaluations are still relatively rare, but available studies suggest lower feed prices do not necessarily improve production economics when feed conversion or biomass gain is reduced (Lipscomb et al., 2020; de Oliveira Ramiro et al., 2023). In *Tor*, feed type and live-feed enrichment affect larval growth, biochemical composition and immune response of *T. tambroides* (Asaduzzaman et al., 2016; Joshua et al., 2024), and weaning strategy influences larval performance and digestive enzyme dynamics in *T. putitora* (Ciji et al., 2026). Together, these results demonstrate that the value of a live-feed is dictated by both the proximate composition and prey characteristics, as well as the ability of the developing larval gut to utilize the feed provided.

Enrichment can improve the functional value of *Moina*, but the response depends on the enrichment medium, exposure duration, and target species (Rasdi et al., 2020; Joshua et al., 2022). The present enrichment emulsion was made up of egg yolk, fish oil, vitamin C and yeast. Yeast-derived compounds such as β-glucans, mannans and nucleotides have been linked to digestive function and epithelial integrity in fish larvae (Amir et al., 2018; Cigarroa-Ruiz et al., 2023). Enrichment, however, does not necessarily overcome all nutrient limitations and should be assessed based on feed composition and larval performance.

Most *Artemia–Moina* replacement studies have used graded mixtures within the daily ration or complete replacement throughout larval rearing. Such designs do not directly address the practical hatchery question of how late *Artemia* can be withdrawn without unacceptable production losses. This question is analogous to weaning-timing studies in which live prey is withdrawn at different developmental stages (Saputra et al., 2024; Eiras et al., 2024; Ciji et al., 2026). Because digestive and absorptive capacity develops progressively (Ciji et al., 2026), tolerance to a nutritionally and physically different prey may change during rearing. A terminal-phase design, in which *Artemia* is withdrawn for progressively longer periods counted back from the end of rearing, directly tests this management question. However, the duration of *Moina* exposure and the larval age at which replacement begins are inherently linked in this design and therefore cannot be separated mechanistically. This approach has received little attention in mahseer larviculture, particularly when intestinal morphometry and production economics are evaluated together.

We therefore evaluated terminal-phase replacement of *Artemia* with enriched *Moina* in *T. soro* larvae during a 28-day trial, with *Moina* replacing *Artemia* for the final 0, 7, 14, 21, or 28 days. We characterised live-feed proximate and amino acid composition and quantified larval growth, survival, supplied-feed utilisation, feed cost, economic conversion, and intestinal morphometry. We hypothesised that a short terminal replacement window would have less effect on survival and intestinal morphometry than longer windows beginning earlier in rearing, whereas growth and feed efficiency would decline progressively as the replacement window lengthened because the enriched *Moina* would not fully reproduce the nutritional and physical properties of *Artemia*.

2. Materials and methods

2.1. Experimental site and larvae

The experiment was conducted at Mina Kancra Ciburial Hatchery, Sumedang Regency, West Java, Indonesia. Dewa larvae from a single selected spawning batch were used at 3–5 days after hatching. After acclimatisation in aerated green water, 1,250 larvae were randomly allocated to 25 plastic rearing containers at 50 larvae per container. Each container held 10 L of working water and served as one experimental unit. Larvae were reared for 28 days under continuous aeration and a 12 h light:12 h dark photoperiod. No routine water exchange was performed, and only green water was added to compensate for evaporative losses. Dead larvae were removed and recorded each day.

The trial measured water temperature, pH, and dissolved oxygen. All containers were subject to the same husbandry procedures, green-water supplementation, aeration and photoperiod to minimize environmental variation among treatments. Water temperature, pH and dissolved oxygen varied across monitoring occasions with treatment means ranging from 25.04 to 28.56°C, 7.41 to 7.89 and 3.02 to 4.36 mg L^{-1}, respectively.

All procedures involving live animals were conducted in accordance with applicable ethical and professional standards for animal research and followed Regulation No. 6/PERMEN-KP/2020 of the Ministry of Marine Affairs and Fisheries, Indonesia. This regulation provides the applicable framework for responsible aquaculture practices in Indonesia.

2.2. Live-feed production and *Moina* enrichment

Artemia cysts (Golden West Artemia, Golden West Artemia Inc., Ogden, UT, USA, LOT #241823) were hatched in saline water at 30–31 ppt, and nauplii were collected before feeding. The enrichment emulsion was prepared to a final volume of 1 L from 15 g egg yolk, 5 g fish oil, 1.2 g of a taurine and vitamin C mixed feed additive (Henan Muxiang Biotechnology Co., Ltd., Zhengzhou, China), and 4.5 g bakery yeast (*Saccharomyces cerevisiae*; NEVADA Instant Dry Yeast, distributed by Biomex, Paris, France), made up to volume with fresh water and homogenised at 5,000 rpm for 8 min. Harvested *Moina* biomass was enriched for 3 h under continuous aeration at 28–30°C and pH 6–7 in fresh-water medium containing 0.63 g *Moina* biomass L-1 and 42 mL enrichment emulsion L-1. The enriched *Moina* was then harvested with a plankton net, rinsed twice with clean water, and provided fresh to the larvae.

2.3. Experimental design and feeding treatments

A completely randomised design was used with five feeding treatments and five replicate containers per treatment. Treatments were defined by the terminal-phase window during which enriched *Moina* replaced *Artemia* over the 28-day rearing period: (A) *Artemia* alone, days 1–28 (0% replacement); (B) *Artemia*, days 1–21, followed by enriched *Moina*, days 22–28 (25% replacement); (C) *Artemia*, days 1–14, followed by enriched *Moina*, days 15–28 (50% replacement); (D) *Artemia*, days 1–7, followed by enriched *Moina*, days 8–28 (75% replacement); and (E) enriched *Moina* alone, days 1–28 (100% replacement). Replacement level therefore represented the proportion of the 28-day rearing period during which *Artemia* was replaced by enriched *Moina*, not the proportion of the two live feeds within a mixed daily

ration. Live feed was supplied three times daily (morning, midday, and afternoon) at 20% of estimated larval biomass per day according to the treatment schedule. The cumulative live-feed mass supplied to each container was recorded separately for feed-utilisation and economic calculations.

2.4. Proximate composition and amino acid profile of live feeds

Yeast, *Artemia* nauplii, unenriched *Moina*, and enriched *Moina* were analysed at IPB University, Bogor, Indonesia, according to AOAC International procedures. Moisture was determined by drying samples at 105°C to constant weight (AOAC 1990, method 934.01); crude protein was determined by the Kjeldahl method (AOAC 1990, method 984.13) using a nitrogen-to-protein conversion factor of 6.25; crude lipid was determined by diethyl ether extraction using the Soxhlet method (AOAC 1990, method 920.39); crude fibre was determined according to AOAC 1990, method 978.10; and ash was determined by combustion in a muffle furnace at 550°C for 6 h (AOAC 1990, method 942.05). Proximate measurements were performed in duplicate. Nutrient values were converted to a dry-matter basis as follows: nutrient on a dry-matter basis = nutrient as analysed / (100 - moisture) × 100. Amino acid composition was determined following acid and performic-acid hydrolysis based on AOAC Official Method 994.12, followed by HPLC separation and quantification.

2.5. Growth, survival, and feed utilisation

Ten larvae per container were measured on days 0, 7, 14, 21, and 28 for total length and individual body weight, and container means were used for analysis. Survival was calculated as 100×final number of larvae/initial number of larvae. The specific growth rate (SGR) was calculated as 100 × [ln (final body weight) – ln (initial body weight)] / days of rearing. Biomass gain was determined as the difference between final and initial biomass. Feed conversion ratio (FCR) was calculated as total live-feed mass supplied / biomass gain and feed efficiency was calculated as 100 × biomass gain / total live-feed mass supplied. All feed-utilisation calculations were made on the same mass basis as the recorded data set. FCR and feed efficiency are apparent feed-use indices based on feed supplied rather than feed ingested, because consumption was not measured directly.

2.6. Histology and morphometry of the intestine

At day 28, one larva from each of three independently chosen replicate containers per treatment was randomly chosen for intestinal histology. Tissue pieces were fixed for 24 h at room temperature in 10% neutral buffered fixative, dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. Sections, 5 µm thick, were cut with a rotary microtome and stained with hematoxylin and eosin (H&E). Histological preparations were observed under a light microscope with ×40 and ×100 magnifications. Photomicrographs were taken to enable morphometric measurement and descriptive evaluation of intestinal architecture.

For each larva, three non-consecutive transverse sections were examined, and in each section five intact mucosal folds were measured. Morphometric endpoints included mucosal fold height, mucosal fold

area, and intestinal diameter Mucosal fold height measured from the base to the tip of the fold. Fold area traced along the epithelial boundary. Intestinal diameter measured along the broadest transverse axis. The replicate value for each larva was the mean of all valid measurements. No formal lesion-severity scoring or goblet-cell quantification was performed; therefore, histological interpretation was limited to the measured morphometric endpoints.

2.7. Economic evaluation

Total feed cost was calculated from the amount and unit price of *Artemia* and *Moina* supplied to each replicate. The economic conversion ratio (ECR) was calculated as total feed cost divided by biomass gain and expressed as IDR g-1 biomass gain. This metric relates feed expenditure to biomass produced rather than to final standing biomass.

2.8. Statistical analysis

The replicate container was the experimental unit for growth, survival, feed-utilisation, and economic variables (n = 5 per treatment). Three independent replicate samples per treatment were used for intestinal morphometry. Data are presented as mean ± standard deviation. Residual normality and homogeneity of variance were assessed using the Shapiro-Wilk and median-centred Levene tests, respectively. Survival proportions were arcsine square-root transformed for inferential analysis, while untransformed percentages are reported. Treatment effects were tested by one-way analysis of variance, followed by prespecified two-sided Dunnett comparisons of each replacement treatment with the 100% *Artemia* control. Second-order polynomial regressions were fitted to the independent replicate observations to visualise responses across the replacement gradient. These polynomial fits were used descriptively and not to infer an optimum replacement level because *Moina*-feeding duration and larval age at replacement onset were linked by design. The body-weight trajectory across sampling days was displayed descriptively and was not analysed as a repeated-measures model. Proximate composition and amino acid data were used for feed characterisation. Statistical significance was accepted at $p < 0.05$. Analyses were conducted using SPSS version 25 and independently checked from the replicate-level data.

3. Results

3.1. Nutrient composition of live feeds

On a dry-matter basis, enriched *Moina* contained 37.52% crude protein, compared with 36.29% in unenriched *Moina* and 34.77% in *Artemia*. However, enriched *Moina* contained less crude lipid than *Artemia* (3.55 vs 4.23%) and had the highest ash concentration (9.97% dry matter; Table 1). Thus, the enrichment protocol increased measured crude protein but did not reproduce the overall proximate composition of *Artemia*.

Table 1
Proximate composition of yeast and live feeds used in the experiment.

Material	Dry matter (% as received)	Crude protein (% DM)	Crude lipid (% DM)	Crude fibre (% DM)	Ash (% DM)
Yeast	95.55 ± 0.02	35.71 ± 0.00	3.21 ± 0.02	1.89 ± 0.04	5.83 ± 0.38
Artemia	95.73 ± 0.23	34.77 ± 0.45	4.23 ± 0.06	1.96 ± 0.15	7.58 ± 0.34
Moina	95.24 ± 0.27	36.29 ± 0.04	3.99 ± 0.06	1.76 ± 0.03	7.06 ± 0.26
Enriched *Moina*	93.76 ± 0.06	37.52 ± 0.07	3.55 ± 0.01	1.98 ± 0.22	9.97 ± 0.37

Note. Values are mean ± SD of duplicate analytical determinations. DM = dry matter. Duplicate laboratory determinations characterise the analysed material and were not treated as biological replicates.

The total measured amino acid concentration was 8.744 g 100 g-1 dry matter in *Artemia*, 9.565 in unenriched *Moina*, and 8.544 in enriched *Moina* (Table 2). The measured essential-to-total amino acid ratio was similar among the live feeds (43.49–44.02%). Enriched *Moina* contained lower measured concentrations of threonine, valine, phenylalanine, lysine, and cysteine than unenriched *Moina*. Its total measured amino acid concentration was also slightly lower than that of *Artemia* despite its higher crude protein concentration.

Table 2
Amino acid profile of the live feeds on a dry-matter basis.

Amino acid	***Artemia***	***Moina***	**Enriched *Moina***
Aspartate	1.201	1.186	1.141
Glutamate	1.557	1.638	1.472
Serine	0.637	0.672	0.736
Glycine	0.366	0.472	0.395
Histidine	0.104	0.073	0.064
Arginine	0.648	0.756	0.715
Threonine	0.313	0.409	0.299
Alanine	0.637	0.672	0.533
Proline	0.366	0.441	0.309
Tyrosine	0.136	0.199	0.181
Valine	0.710	0.735	0.640
Methionine	0.052	0.084	0.075
Cysteine	0.042	0.073	0.021
Isoleucine	0.627	0.693	0.619
Leucine	0.533	0.577	0.587
Phenylalanine	0.198	0.220	0.160
Lysine	0.616	0.661	0.597
Total measured amino acids	8.744	9.565	8.544
Total measured essential amino acids	3.803	4.210	3.754
Essential/total amino acids (%)	43.49	44.02	43.95

Note. Values are expressed as g 100 g-1 dry matter, except for the essential/total ratio. Essential amino acids comprise the measured histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. Tryptophan was not available. Each feed type had one analytical amino acid profile; comparisons are therefore descriptive.

3.2. Growth and survival

Body-weight trajectories diverged progressively as the terminal-phase replacement window lengthened (Fig. 1). The 25% replacement group (*Artemia* on days 1–21, then enriched *Moina* on days 22–28) followed the control trajectory through day 21 and diverged after the switch to *Moina*. Similar divergence

occurred after *Moina* was introduced in the 50, 75, and 100% replacement groups, beginning on days 15, 8, and 1, respectively.

Final length, final weight, and specific growth rate differed among treatments ($F_{4,20} = 116.42$, 47.51, and 56.55, respectively; all $p < 0.001$). Dunnett comparisons showed that the 25% replacement treatment already had lower final length, final weight, and specific growth rate than the 100% *Artemia* control. Survival also differed among treatments ($F_{4,20} = 3.72$, $p = 0.020$). Survival did not differ significantly from the control at 25–75% replacement, whereas complete replacement reduced survival to 88.4% (Table 3). Quadratic fits described most of the variance in final length ($R^2 = 0.955$), specific growth rate ($R^2 = 0.914$), and final weight ($R^2 = 0.904$). The fit for survival was weaker ($R^2 = 0.380$), reflecting relatively stable survival from 0 to 75% replacement and a decline at complete replacement (Fig. 2).

Table 3
Growth and survival of *Tor soro* larvae under terminal-phase replacement of *Artemia* with enriched *Moina* during the 28-day feeding trial.

Terminal phase replacement of *Artemia* (%)	**Initial length (mm)**	**Final length (mm)**	**Initial weight (g)**	**Final weight (g)**	**Weight gain (g)**	**Survival (%)**	**SGR (% day-1)**
0	9.67 ± 0.98	24.00 ± 1.56	0.0126 ± 0.0019	0.132 ± 0.030	0.119 ± 0.030	98.0 ± 2.0	8.26 ± 0.95
25	9.67 ± 0.98	21.36 ± 0.09***	0.0126 ± 0.0019	0.087 ± 0.004***	0.074 ± 0.004***	98.8 ± 1.8	6.95 ± 0.68*
50	9.67 ± 0.98	18.52 ± 0.61***	0.0126 ± 0.0019	0.052 ± 0.007***	0.039 ± 0.007***	96.4 ± 3.3	4.85 ± 0.39***
75	9.67 ± 0.98	15.92 ± 0.61***	0.0126 ± 0.0019	0.034 ± 0.007***	0.021 ± 0.007***	97.6 ± 2.2	3.41 ± 1.04***
100	9.67 ± 0.98	14.64 ± 0.09***	0.0126 ± 0.0019	0.023 ± 0.002***	0.010 ± 0.002***	88.4 ± 9.3*	2.04 ± 0.48***

Note. Values are mean ± SD, n = 5 replicate containers. Asterisks indicate Dunnett-adjusted differences from the 100% *Artemia* control: *p < 0.05, **p < 0.01, ***p < 0.001. Survival was tested after arcsine square-root transformation. SGR = specific growth rate. Initial length (9.67 ± 0.98 mm) and initial weight (0.0126 ± 0.0019 g; n = 15 larvae) were measured once from the common source population immediately before random allocation to replicate containers and are therefore identical across treatments. Weight gain = final weight − initial weight.

3.3. Feed utilisation and economic performance

Feed conversion ratio increased with *Moina* replacement ($F_{4,20} = 9.95$, $p < 0.001$), whereas feed efficiency declined ($F_{4,20} = 32.49$, $p < 0.001$). After Dunnett adjustment, FCR at 25% replacement did not differ significantly from the control, but feed efficiency was lower. Complete replacement produced the

highest FCR and the lowest feed efficiency (Table 4). Quadratic fits accounted for substantial variation in feed efficiency (R^2 = 0.848) and total feed cost (R^2 = 0.762), and less variation in FCR (R^2 = 0.645) and ECR (R^2 = 0.600), consistent with wider dispersion of FCR and ECR at high replacement levels (Fig. 3).

Table 4
Feed utilisation and economic performance of *Tor soro* larvae under terminal-phase replacement of *Artemia* with enriched *Moina* during the 28-day feeding trial.

Terminal phase replacement of *Artemia* (%)	**FCR**	**Feed efficiency (%)**	**Total feed cost (IDR)**	**ECR (IDR g-1 gain)**
0	1.41 ± 0.32	74.16 ± 17.42	14,276.56 ± 3,458.06	2,503.13 ± 576.05
25	2.52 ± 0.33	40.34 ± 5.57***	14,982.76 ± 2,064.15	4,107.86 ± 532.77
50	3.91 ± 1.01	27.31 ± 8.66***	10,680.69 ± 2,735.28	5,825.00 ± 1,509.11
75	5.80 ± 2.37	19.47 ± 7.10***	6,928.39 ± 715.06***	7,794.29 ± 3,191.03
100	12.71 ± 6.59***	9.67 ± 4.60***	4,649.65 ± 344.78***	15,254.63 ± 7,903.91***

Note. Values are mean ± SD, n = 5 replicate containers. Asterisks indicate Dunnett-adjusted differences from the 100% *Artemia* control: *p < 0.05, **p < 0.01, ***p < 0.001. FCR = total live-feed mass / biomass gain. Feed efficiency = 100 × biomass gain / total live-feed mass. ECR = total feed cost / biomass gain.

Total feed cost differed among treatments ($F_{4,20}$ = 20.85, p < 0.001). The 25% replacement treatment did not reduce total feed cost relative to the control, whereas significant cost reductions occurred at 75 and 100% replacement. Economic conversion ratio also differed among treatments ($F_{4,20}$ = 8.16, p < 0.001). ECR did not differ significantly from the control at 25–75% replacement, but complete replacement significantly increased cost per unit biomass gain. Thus, lower feed expenditure at high replacement levels was accompanied by poorer biological and economic conversion (Fig. 3).

3.4. Intestinal morphology

Treatment affected mucosal fold height, mucosal fold area, and intestinal diameter ($F_{4,10}$ = 10.27, 9.83, and 48.55, respectively; p ≤ 0.002). At 25% replacement, none of the three morphometric variables differed significantly from the 100% *Artemia* control. At 50% replacement, fold area and intestinal diameter were lower than the control, whereas fold height did not differ significantly after Dunnett adjustment. At 75 and 100% replacement, all three measured intestinal indices were lower than the control (Table 5; Fig. 4). Fold area was numerically higher at 100% than at 75% replacement, unlike fold height and intestinal diameter, which declined monotonically across the replacement gradient. Given the small number of independent histology replicates (n = 3 per treatment), this non-monotonic fold-area

pattern should be interpreted cautiously. Quadratic fits explained most of the variation in intestinal diameter (R^2 = 0.862) and a moderate proportion of the variation in mucosal fold height (R^2 = 0.703) and mucosal fold area (R^2 = 0.621).

Table 5
Intestinal morphometry of *Tor soro* larvae under terminal-phase replacement of *Artemia* with enriched *Moina* during the 28-day feeding trial.

Terminal phase replacement of *Artemia* (%)	**Mucosal fold height (µm)**	**Mucosal fold area (µm²)**	**Intestinal diameter (µm)**
0	140.48 ± 16.50	233.45 ± 42.69	715.11 ± 11.96
25	145.30 ± 9.00	243.91 ± 14.44	714.59 ± 6.47
50	115.83 ± 4.33	174.83 ± 3.22*	619.80 ± 17.48***
75	102.55 ± 10.49**	153.39 ± 5.54**	557.78 ± 29.20***
100	98.13 ± 14.02**	164.11 ± 24.17*	551.74 ± 25.53***

Note. Values are mean ± SD, n = 3 independent replicate samples. Asterisks indicate Dunnett-adjusted differences from the 100% *Artemia* control: *p < 0.05, **p < 0.01, ***p < 0.001.

Representative haematoxylin-and-eosin sections are shown in Fig. 5 to illustrate the structures quantified in the morphometric analysis. Because no formal lesion-scoring system was applied, histological interpretation was restricted to the measured fold dimensions and intestinal diameter.

4. Discussion

The results partially supported the study hypothesis and identified a clear trade-off between reducing *Artemia* use and maintaining larval performance. Replacement during only the final 7 days (25% of the rearing period) produced no detectable difference from the control in survival or the three intestinal morphometric endpoints, yet final size, specific growth rate, and feed efficiency were already lower. Therefore, the absence of detectable intestinal change at 25% replacement should not be interpreted as nutritional equivalence. From 50% replacement onward, reductions in growth were accompanied by lower fold area and/or intestinal diameter, and at 75–100% replacement all three morphometric indices were reduced. The production penalty associated with extending the *Moina*-feeding window thus appeared before a clear survival penalty and before all measured intestinal dimensions changed.

The growth response was strong and non-linear. All replacement treatments produced lower final length, final weight, and specific growth rate than the 100% *Artemia* control. The 25% group followed the control growth trajectory through day 21 and diverged after enriched *Moina* replaced *Artemia* during the final week, while earlier switches were followed by earlier divergence in the 50, 75, and 100% treatments. A similar advantage of *Artemia*-based feeding over *Moina*-based feeding has been reported in *T. tambroides* (Joshua et al., 2024), and partial replacement has generally been more reliable than complete replacement in other larval fishes (Eiras et al., 2023). However, because replacement duration

and larval age at the onset of replacement were linked in the present design, the observed response cannot distinguish an effect of longer *Moina* exposure from greater sensitivity at earlier developmental stages.

The feed-composition data provide one plausible explanation for the growth response, but they do not establish causation. Although enriched *Moina* had a higher crude protein concentration than *Artemia*, it contained less crude lipid, more ash, and a slightly lower total measured amino acid concentration. Several measured amino acids, including threonine, valine, phenylalanine, lysine, and cysteine, were also lower in enriched than in unenriched *Moina*. These differences show that crude protein alone was an inadequate descriptor of live-feed quality. Nevertheless, digestibility, gross energy, fatty-acid composition, and species-specific amino acid requirements were not measured; therefore, the present data cannot identify a specific nutrient deficiency (Samat et al., 2020; Joshua et al., 2022).

Differences in prey intake provide an alternative, non-exclusive explanation. The feed-utilisation indices were based on live-feed mass supplied rather than feed actually ingested, and *Moina* differs from *Artemia* in prey size, swimming behaviour, and potentially palatability, all of which can influence capture and ingestion by fish larvae (Samat et al., 2020; Joshua et al., 2022). Consequently, poorer growth under *Moina* replacement could reflect lower ingestion, lower nutrient availability per unit ingested, or both. The present design cannot separate these mechanisms. Direct measurements of prey size, gut fullness, or ingestion rate would be required to resolve their relative contributions.

Intestinal morphometry adds a structural perspective to these performance data. Mucosal folds contribute to effective absorptive surface, and diet-related changes in fold dimensions and intestinal diameter can accompany differences in gut development during larval rearing (Eiras et al., 2024). At 25% replacement, none of the measured intestinal indices differed significantly from the control despite reduced growth and feed efficiency. At 50% replacement, fold area and intestinal diameter were lower, and at 75–100% replacement all three indices were reduced. The higher fold area at 100% than at 75% replacement was non-monotonic and should not be interpreted as recovery under complete *Moina* feeding because only three independent larvae per treatment were analysed. Overall, the concurrent reductions in growth, feed efficiency, and intestinal dimensions at higher replacement levels indicate an association, but they do not establish whether altered intestinal development caused poorer growth or arose from the same underlying limitation in feed intake or nutrient supply.

The enrichment treatment may have contributed functional components in addition to nutrients, but the present study was not designed to test that mechanism. The emulsion contained egg yolk, fish oil, a taurine and vitamin C additive, and yeast, and yeast-derived β-glucans, mannans, and nucleotides have been associated with epithelial integrity, digestive function, and stress responses in fish larvae (Amir et al., 2018; Cigarroa-Ruiz et al., 2023). Feeding strategies involving *Moina* can also influence larval intestinal histomorphometry in other species (Eiras et al., 2024). However, immune markers, digestive enzymes, microbiota, and lesion scores were not quantified here. Accordingly, the data support only the

conclusion that the measured intestinal dimensions were not detectably reduced at 25% terminal-phase replacement; they do not demonstrate a probiotic or immunostimulatory effect of the enrichment.

Feed economics must be interpreted together with biomass production because lower feed expenditure does not necessarily improve production efficiency. Feeding strategies are most informative when growth, feed conversion, and economic return are considered jointly rather than feed cost alone (Chadli et al., 2025). The 25% replacement treatment did not reduce total feed cost relative to the control, and its ECR was numerically higher (4,107.86 vs 2,503.13 IDR g-1 biomass gain), although the Dunnett-adjusted difference was not significant. Only at 75 and 100% replacement was total feed cost significantly reduced, but less biomass and poorer feed conversion were produced in these treatments. Complete replacement had the lowest feed expenditure and highest cost per unit biomass gain. This pattern is in line with previous economic evaluations, which propose ingredient cost to be interpreted in combination with biological conversion and production output (Lipscomb et al., 2020; de Oliveira Ramiro et al., 2023).

From a hatchery-management perspective, 25% terminal-phase replacement is a compromise and not an adequate substitute for continuous *Artemia* feeding. Of the replacement treatments tested, it was the only level at which none of the three intestinal morphometric endpoints differed significantly from the control, nor did survival differ significantly; however, growth and feed efficiency were lower and total feed cost was not reduced. Consequently, its utility will depend on management priorities. Hatcheries with an unreliable or expensive supply of *Artemia* may be prepared to trade off some growth in favor of less reliance on commercial cysts. Hatcheries wishing to maximize the rate of biomass gain and feed-use efficiency would be better off with continuous *Artemia* feeding under the conditions tested.

There are several limitations to the generality and mechanistic interpretation of these findings. The larvae were from a single spawning batch and thus responses should be validated in other genetic and maternal cohorts before a general terminal-phase replacement threshold can be recommended. Only one analytical amino acid profile was available for each feed type, and fatty acids, digestibility, gross energy, digestive-enzyme activity, whole-body composition, and nutrient retention were not measured. Histology was based on three independent larvae per treatment, thereby limiting the precision for smaller structural differences. Live-feed mass was recorded as supplied, not ingested, and only three morphometric endpoints were quantified. Future work should directly quantify prey size and ingestion, determine digestible amino acid and fatty acid profiles, evaluate functional markers of digestion and intestine, test alternative enrichment protocols, and validate the response across multiple batches of spawners.

In general, continuous feeding of *Artemia* provided the best biological and economic results in this 28-day larviculture experiment. Enriched Moina reduced the dependence on *Artemia*, but this advantage was accompanied by progressively poorer growth and feed-use efficiency as the replacement window increased. Therefore, the 25% terminal-phase strategy should be considered as an operational alternative to reduce the reliance on *Artemia* while maintaining survival and the measured intestinal dimensions, and not as a nutritionally or economically equivalent replacement. This difference is

important for hatchery decisions because reduced dependence on imported or externally supplied live feed has practical value only if the associated loss in production performance is acceptable.

5. Conclusions

The larvae of *Tor soro* fed continuously with *Artemia* for 28 days recorded the best growth and feed efficiency under the conditions of this trial. Replacing *Artemia* with enriched *Moina* only during the last 7 days (25% terminal phase replacement) did not significantly reduce survival or the three intestinal morphometric indices measured but significantly reduced growth and feed efficiency and did not reduce total feed cost. Longer replacement windows resulted in a progressive decrease in growth and intestinal morphometry, while complete replacement also significantly decreased survival and increased the economic conversion ratio. *Moina* thus enriched can be used to reduce the dependence on *Artemia* in situations where supply security is a priority but the 25% terminal phase strategy should be considered as a compromise rather than a nutritional or economic equivalent replacement. Validation across multiple spawning batches, and with direct measures of ingestion and nutrient digestibility, is warranted prior to hatchery-scale adoption.

Declarations

Ethical statement

The research was performed according to the ethical procedures for the treatment, care and use of experimental animals, and the basic principles of experimentation under Ministry of Marine Affairs and Fisheries Regulation No. 6/PERMEN-KP/2020.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Author Contribution

Mas Tri Djoko Sunarno: Conceptualization, budget funding, proposal, methodology, supervision, writing—original draft, and writing—review and editing.Ediwarman Ediwarman: Methodology, investigation, resources, data curation, and writing—review and editing.Novita Panigoro: Methodology, investigation, formal analysis, validation, and writing—review and editing.Ena Sutisna: Investigation, resources, data curation, and writing—review and editing.Lisa Ruliaty: Methodology, investigation, data curation, and writing—review and editing.Mas Bayu Syamsunarno: Formal analysis, validation, visualization, writing—original draft, and writing—review and editing.Budi Wardono: formal analysis, and writing—review and editing.Habson Batubara: Investigation, validation, and writing—review and editing.Mat Syukur: Formal analysis, validation, and writing—review and editing.Ali Suman: supervision, and writing—review and editing.All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

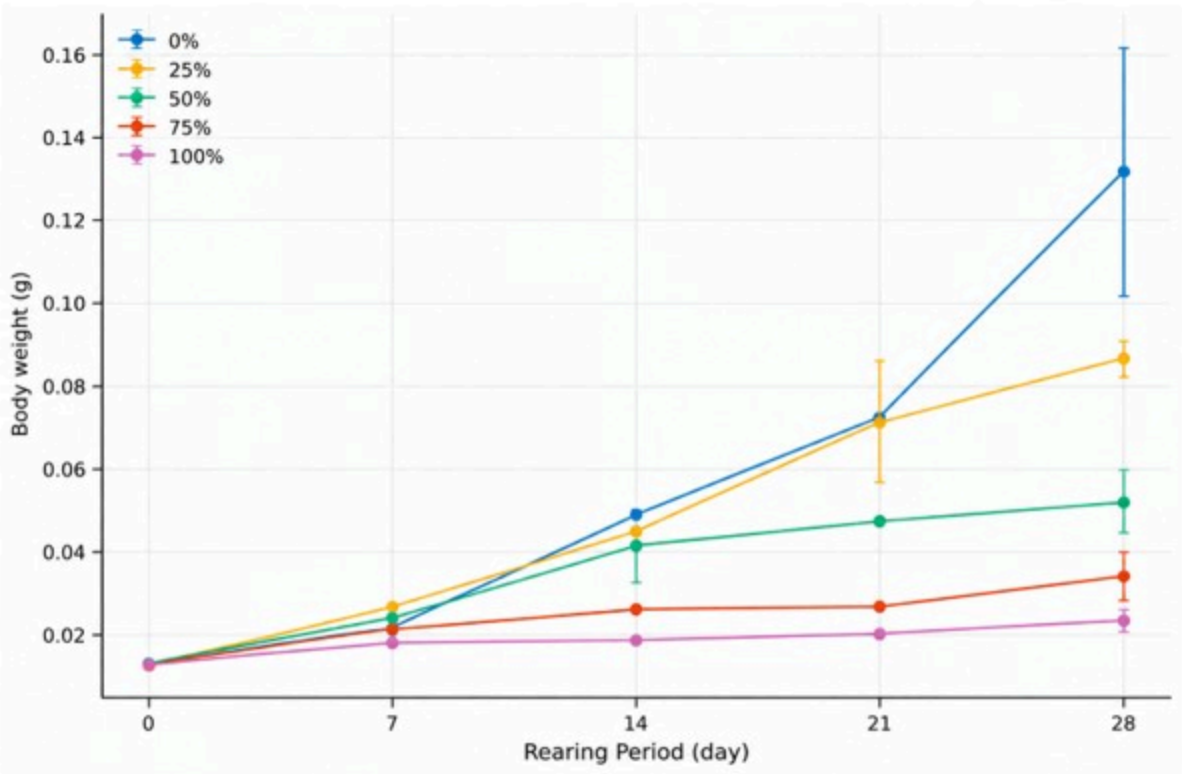


Figure 1

Body weight of *Tor soro* larvae under terminal-phase replacement of *Artemia* with enriched *Moina*during the 28-day feeding trial. Values are mean ± SD of five replicate containers. Lines show the observed sampling trajectories and do not represent a repeated-measures inferential model.

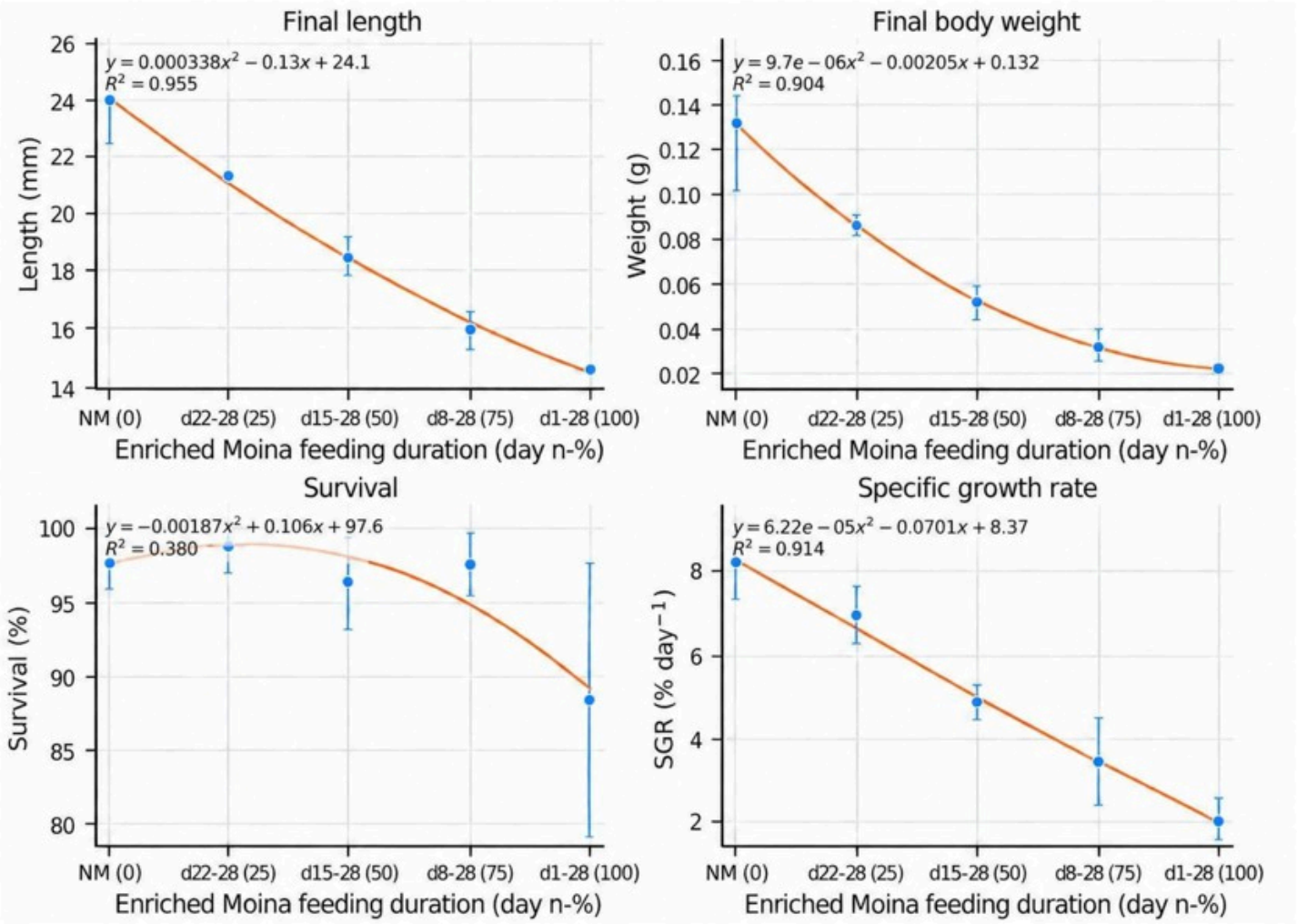


Figure 2

Quadratic responses of final length, final body weight, survival, and specific growth rate to terminal-phase replacement of *Artemia* with enriched *Moina* during the 28-day feeding trial. NM denotes no enriched *Moina* (0% replacement). Points are treatment means ± SD, n = 5. Curves are descriptive second-order polynomial fits.

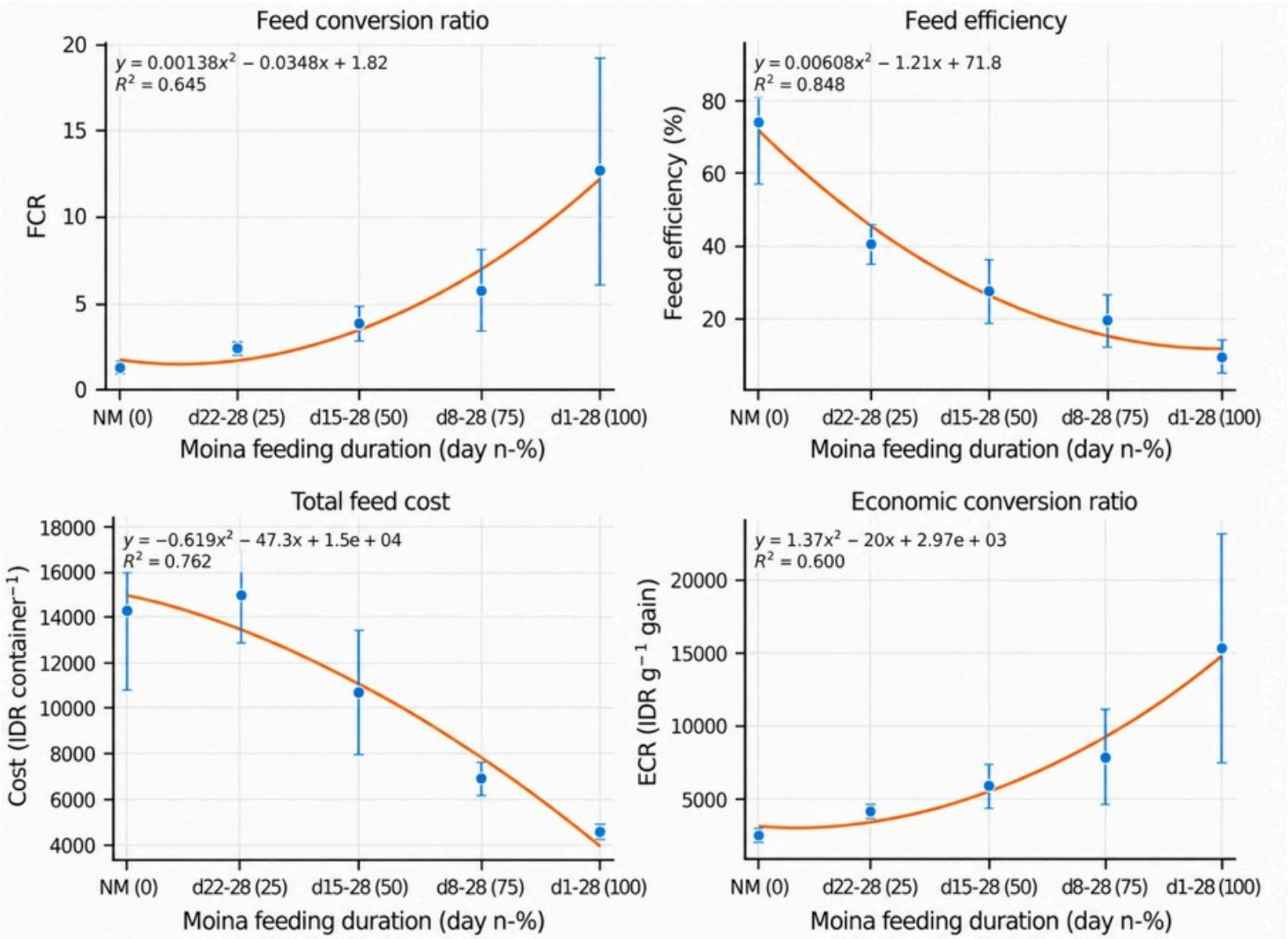


Figure 3

Quadratic responses of feed conversion ratio, feed efficiency, total feed cost, and economic conversion ratio under terminal-phase replacement of *Artemia*with enriched *Moina* during the 28-day feeding trial. NM denotes no enriched *Moina*(0% replacement). Points are treatment means ± SD, n = 5. Curves are descriptive second-order polynomial fits.

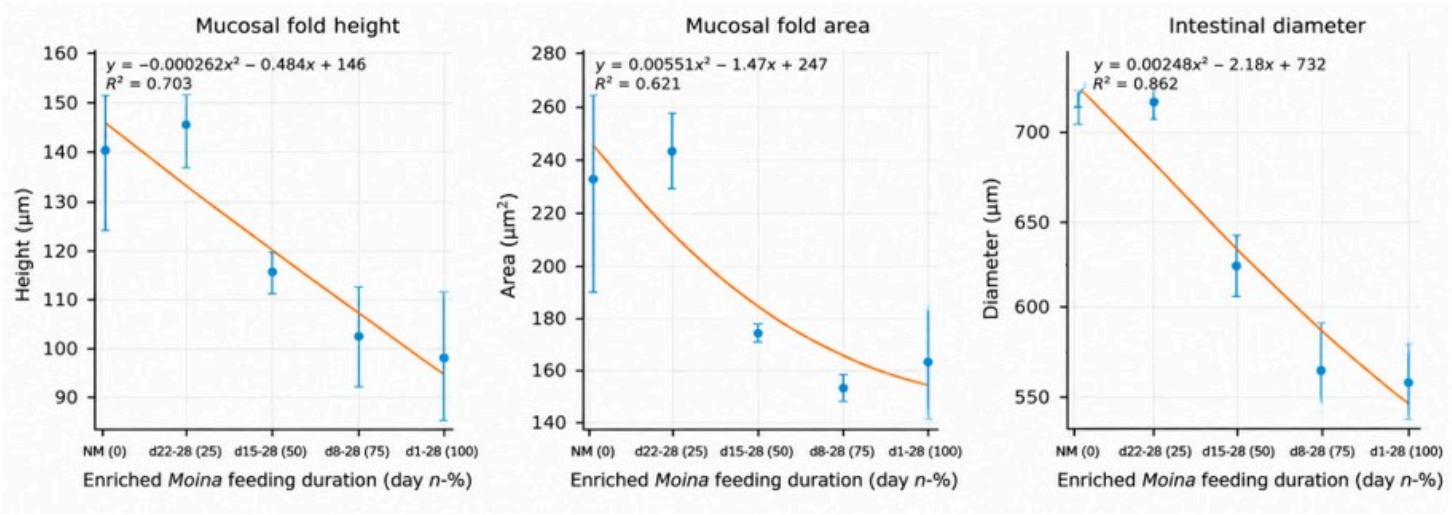


Figure 4

Quadratic responses of intestinal mucosal fold height, mucosal fold area, and intestinal diameter under terminal-phase replacement of *Artemia* with enriched *Moina*during the 28-day feeding trial. NM denotes no enriched *Moina* (0% replacement). Points are treatment means ± SD, n = 3. Curves are descriptive second-order polynomial fits.

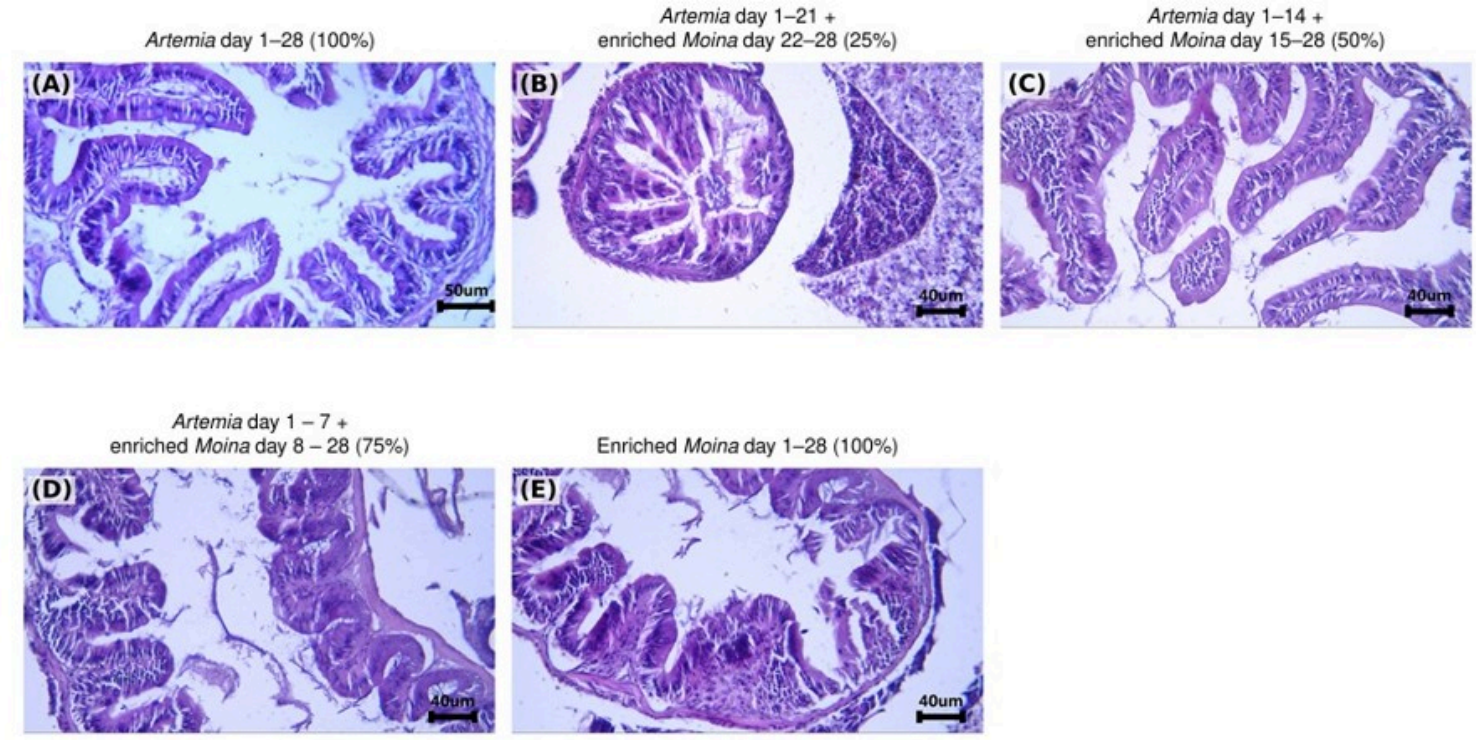


Figure 5

Transverse sections of representative intestines of Tor soro larvae during the terminal-phase replacement of Artemia with enriched Moina in the 28-day feeding trial: (A) 0% replacement (100%

Artemia control). (B) 25% replacement. (C) 50% replacement. (D) 75% replacement. (E) 100% replacement. Haematoxylin and eosin staining. Scale bars are given in each micrograph.