



Growth performance and survival of guppies (*Poecilia reticulata*) associated with dried *Wolffia* spp. supplementation in mixed-diet formulations

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Abstract

Background and Objective: Feed costs represent a major constraint in aquaculture, making alternative protein sources such as *Wolffia* spp. of increasing interest. Although *Wolffia* spp. contains 20–48% protein, its practical inclusion for guppies remains unclear. This preliminary study examined growth and survival in guppies fed 0–60% dried *Wolffia* spp. (base feed weight basis) under a non-isoproteinous, mixed-diet formulation.

Methodology: A 6-week feeding trial was conducted with 135 female guppies (0.48 g initial mean weight) fed diets containing 0, 15, 30, 45, or 60% dried *Wolffia* spp. powder mixed with commercial feed. Growth, feed efficiency, condition factor, and survival were evaluated and analyzed using one-way ANOVA or Kruskal-Wallis test ($P < 0.05$).

Main Results: Dried *Wolffia* spp. contained 22.16% protein, 3.78% fat, 49.20% carbohydrate, and 319.46 kcal/100 g (AOAC analysis). Significant differences in growth performance were observed beginning at week 5 ($P < 0.05$). The highest growth trends were observed in the 45% inclusion group, with an average daily gain of 0.00429 g/day, specific growth rate of $0.74 \pm 0.06\%$ /day. Survival remained high across all treatments (88–100%). Median apparent feed conversion ratio remained comparable across treatments ($P = 0.217$), while apparent protein efficiency ratio

($P = 0.006$) and condition factor ($P = 0.022$) differed significantly among treatments. The 60% group demonstrated growth patterns comparable to the 45% group ($P = 0.508$) with a similar survival rate.

Conclusions: This study indicates that 45% dried *Wolffia* spp. inclusion (base feed weight basis; 31.03% of total diet) was associated with improved growth trends in guppies. However, as the design could not isolate the effect of *Wolffia* spp. per se from changes in overall diet composition, these exploratory results likely reflect increased dietary protein. Further isonitrogenous trials are required to support practical dietary recommendations.

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INTRODUCTION

Feed is fundamental to the survival, growth, and reproduction of aquatic animals, and in aquaculture, it represents a major determinant of production efficiency. Feed costs typically account for 50–70% of total operational expenses (Rana *et al.*, 2009), with protein being the most critical and costly dietary component due to its substantial influence on growth and feed conversion (Sankian *et al.*, 2017). Fishmeal, containing 60–72% crude protein, has long served as the primary protein source in aquafeeds (Cho and Kim, 2011) and is used at levels up to 50–70% of fish feed formulations (Macusi *et al.*, 2023). However, rising demand associated with the expansion of intensive aquaculture has led to overexploitation of wild stocks and increased fishmeal prices (Tantikitti, 2014). Consequently, considerable research has focused on identifying alternative or supplementary ingredients that can reduce dependence on fishmeal while maintaining growth performance, sustainability, and fish health.

Several agricultural and animal-derived by-products, such as insect meal, algae, yeast, and poultry by-products, have been evaluated as potential

alternatives (Aragão *et al.*, 2022). Nonetheless, their performance has often been inconsistent due to differences in digestibility, nutrient utilization, and amino acid balance (de Francesco *et al.*, 2004; Serra *et al.*, 2024). Thus, efforts to identify reliable plant-based protein sources with adequate protein content and essential amino acid profiles comparable to fishmeal remain ongoing (Serra *et al.*, 2024).

Wolffia spp., a minute, free-floating aquatic plant lacking leaves and roots, has recently gained attention as a promising candidate for aquafeed supplementation. Depending on species, cultivation conditions, and processing methods, *Wolffia* spp. contains approximately 20–30% protein (Appenroth *et al.*, 2018) and up to 48.2% under optimized production systems (Ruekaewma *et al.*, 2015; Prosrdee *et al.*, 2023), and it provides all essential amino acids at levels consistent with World Health Organization (WHO) recommendations (On-Nom *et al.*, 2023). Its rapid growth and ease of cultivation further enhance its potential suitability for aquaculture use. While duckweed species have been identified as sustainable feed resources, digestibility constraints have also been reported (Takács *et al.*, 2025). Despite its

favorable nutritional attributes, studies evaluating *Wolffia* spp. in ornamental fish species, particularly guppies, remain lacking.

Guppies (*Poecilia reticulata*) are widely used as model organisms in nutritional research due to their hardiness, rapid growth, ease of culture, and clear responses to dietary manipulation. Given the absence of information on *Wolffia* spp. supplementation in this species, a key question arises: whether increasing *Wolffia* spp. inclusion levels would be associated with observable differences in growth performance and survival in guppies. To address this knowledge gap and considering the practical advantages of direct supplementation methods commonly used in small-scale aquaculture, a mixed-diet approach was adopted in the present study.

Accordingly, this study explored whether increasing dietary *Wolffia* inclusion would be associated with dose-dependent trends in growth performance and feed-use parameters in guppies. To address this objective, growth performance, feed efficiency, condition factor, and survival were evaluated across five dietary inclusion levels of dried *Wolffia* spp. powder (0, 15, 30, 45, and 60%) based on base feed weight.

MATERIALS AND METHODS

Ethical Statement

The experiment was conducted at the Animal Teaching Hospital, Chiang Rai Rajabhat University, from March to May 2024. Animal use and care were supervised by an attending veterinarian and approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Mahanakorn University of Technology (ACUC-MUT-2024/008).

Preparation of *Wolffia* spp.

Fresh *Wolffia* spp. was purchased from a local market in Chiang Rai province and initially collected using a stainless-steel sieve. The material was rinsed thoroughly under running tap water to

remove visible debris and then spread in a thin layer on trays for drying. Sun-drying was performed in a nylon mesh box for 2–4 days under ambient outdoor conditions (approximately 25–35°C) until the material was crisp to the touch. Drying temperature and final moisture content were not recorded, representing a methodological limitation. The dried *Wolffia* spp. was pulverized into a fine powder using a household grinder and stored in a sealed container at room temperature until use. A 300 g sample was sent to the Environmental and Product Testing Center (EPTEC), Chiang Rai Rajabhat University, for proximate analysis following AOAC (2019) methods: protein (991.20), lipid (948.15), ash (920.153), moisture (950.46 B), and carbohydrate (by difference). Energy content was calculated using standard conversion factors.

Diet Formulation and Preparation

Five experimental diets were freshly prepared each week using a consistent formulation throughout the 6-week trial. Dried *Wolffia* spp. powder was premixed with 2% cornstarch (binder), lightly moistened with sterile water, and manually coated onto a commercial herbivorous fish feed (CP 9931; 18% crude protein). *Wolffia* inclusion levels were set at 0 (control), 15, 30, 45, and 60% of the base feed weight, corresponding to actual proportions of 0.00, 13.04, 23.08, 31.03, and 37.50% of the total mixed diet, respectively (Table 1). After coating, the diets were spread on trays, air-dried overnight in the shade, and stored in sealed bags at room temperature until feeding.

The nutrient composition of the mixed diets was estimated from the manufacturer's labeled values for the commercial feed and the analyzed composition of the *Wolffia* spp. powder (22.16% CP, as fed; Table 1). As proximate analysis was performed on the raw *Wolffia* spp. powder only, the final formulated diets were not analytically verified, and nutrient levels were assumed based on calculated inclusion rates.

Table 1 Formulation and calculated protein content of experimental diets containing different inclusion levels of dried *Wolffia* spp. powder mixed with commercial fish feed (18% crude protein)

Treatment	<i>Wolffia</i> spp. (g)	Final diet (g)	<i>Wolffia</i> (%; actual, of total diet)	Feed (%; of total diet)	Protein (%; of total diet)
Control	0.00	5.00	0.00	100.00	18.00
15% <i>Wolffia</i> spp.	0.75	5.75	13.04	86.96	18.54
30% <i>Wolffia</i> spp.	1.50	6.50	23.08	76.92	18.96
45% <i>Wolffia</i> spp.	2.25	7.25	31.03	68.97	19.29
60% <i>Wolffia</i> spp.	3.00	8.00	37.50	62.50	19.56

Note: Target inclusion levels are based on base feed weight. Actual % shows final proportions in mixed diets. Protein values were calculated from feed protein (18%) and *Wolffia* protein (22.16%) using: (Feed % × 18.0) + (*Wolffia* spp. % × 22.16). No proximate analysis of mixed diets was performed; values are estimates only.

Fish Preparation and Acclimatization

A total of 135 healthy female guppies (*P. reticulata*), approximately 3 months old with an average purchase weight of 0.25 g, were obtained from a local aquarium shop in Chiang Rai province. Fish were randomly distributed into 15 plastic tanks (30 × 11 cm; 4.5 L capacity), each containing 4 L aerated water. During 7 days of acclimatization, tanks were maintained in an air-conditioned room at 25°C and fish were fed the commercial standard diet at 3% body weight twice daily. After this period, the overall mean body weight increased to approximately 0.48 g (week 0).

Sample size calculation was performed using G*Power 3.1 for a one-way ANOVA with five treatment groups, each replicated in three tanks (15 experimental units). Based on Cohen's (Cohen, 1988) guidelines, a medium effect size ($f = 0.25$) was selected because preliminary data were unavailable. The analysis assumed a statistical power of 0.80 and an alpha level of 0.05.

Experimental Design and Feeding Regime

The feeding trial followed a completely randomized design (CRD) with five dietary treatments corresponding to *Wolffia* spp. inclusion levels of 0

(control), 15, 30, 45, and 60%. Each treatment had three tank replicates, giving a total of 15 tanks and 135 fish. The daily feed ration was set at 3% of body weight, calculated from the week 0 mean weight of each group and adjusted weekly, and offered in two equal feedings per day.

Water quality was monitored throughout the experiment. Water temperature ranged from 25–29°C, dissolved oxygen (DO) from 5–10 mg/L, and pH from 6.2–7.8. Total and free chlorine, nitrate, and nitrite remained within acceptable ranges (0, 0–3, and 0–0.5 mg/L, respectively). While total ammonia nitrogen was not directly measured, which is recognized as a limitation, water quality was strictly maintained through regular 30% partial water changes performed every 3 days. The maintenance of safe ammonia levels was justified by the consistently low nitrite and nitrate concentrations, the absence of stress-related behaviors, and high fish survival across all treatments. Water turbidity ranged from 25 to 50 Nephelometric Turbidity Units (NTU) in aged water.

Data Collection and Determination of Growth Performance

Body weight (g) and total length (cm; snout to caudal fin tip) were measured weekly. The number

of live fish per tank was recorded to calculate survival. Fish health and welfare were monitored daily for signs of distress, including abnormal swimming patterns, reduced feeding activity, lethargy, surface gasping, or unusual coloration changes.

The total dry feed offered to each tank was weighed and recorded at each feeding throughout the 42-day experimental period. Uneaten feed could not be reliably collected due to feed disintegration; therefore, true feed intake could not be determined. Accordingly, apparent feed utilization indices were calculated based on total feed offered rather than actual intake, following Savanguane *et al.* (2022), as follows:

Apparent feed conversion ratio (apparent FCR) = Total dry feed offered (g) / Total wet weight gain per tank (g)

Apparent protein efficiency ratio (apparent PER) = Total wet weight gain per tank (g) / Apparent protein intake (g)

Growth performance indices were calculated according to standard methods (Ahmed and Ahmad, 2020; Khieokhajonkhet and Surapon, 2020; Fadel *et al.*, 2021; Kim *et al.*, 2021), as follows:

Average daily gain (ADG, g/day) = (Final body weight – Initial body weight) / Duration (day)

Specific growth rate (SGR, %/day) = $100 \times (\ln \text{Final body weight} - \ln \text{Initial body weight}) / \text{Duration (day)}$

Survival rate (%) = (Number of surviving fish / Number stocked) $\times 100$

Condition factor (CF; Fulton's K) = $100 \times W / L^3$ where W is the final body weight (g) and L is the final body length (cm)

Statistical Analysis

Data were analyzed using IBM SPSS Statistics (version 25.0). Normality and homogeneity of variances were verified via Levene's test. Growth parameters (initial and final weight, weight gain, ADG, SGR), apparent PER, and CF were analyzed by one-way ANOVA followed by Fisher's LSD test. However, apparent FCR and survival rate violated the homogeneity assumption or contained zero variance; thus, they were analyzed using the non-parametric Kruskal-Wallis test. Statistical significance was set at $P < 0.05$, and results are presented as treatment means $\pm$ standard deviations.

RESULTS AND DISCUSSION

The nutritional composition of dried *Wolffia* spp. indicated its potential as a supplementary protein source, with moderate energy density and a balanced macronutrient profile (Table 2). The CP content (22.16%) falls within the 20–30% range reported for *Wolffia* spp. (Appenroth *et al.*, 2018), but is lower than values reported under optimized conditions, such as outdoor pond cultivation (41.81%) or high-protein

Table 2 Proximate composition (g/100 g dry matter) of dried *Wolffia* spp. powder

Sample	Parameters	Content	Unit	Method of analysis
<i>Wolffia</i> spp.	Protein	22.16	g/100 g	AOAC (2019) 991.20
	Fat	3.78	g/100 g	AOAC (2019) 948.15
	Ash	11.79	g/100 g	AOAC (2019) 920.153
	Moisture	13.07	g/100 g	AOAC (2019) 950.46 (B)
	Carbohydrate	49.20	g/100 g	Method of analysis for nutrition labeling (1993),
	Energy	319.46	kcal/100 g	Chapter 6 Proximate and mineral analysis

Note: Analyses were conducted according to AOAC (2019) standard procedures. Values are expressed on a dry-matter basis, except for moisture content.

strains (up to 50.89%) (Hu *et al.*, 2022; Prosrdee *et al.*, 2023). Such variation, influenced by species, cultivation, and processing, is exemplified by high-temperature drying (70°C for 4–6 h), which reduces protein content from 41.91% to 34.08% (Prosrdee *et al.*, 2023). This variability provides important context for interpreting the growth responses observed in this study (Tables 3–5).

Higher *Wolffia* inclusion levels, defined on a base feed weight basis, were accompanied by modest increases in dietary protein and coincided with progressively higher ADG and SGR. The highest values were observed at 45% inclusion (31.03% of the total diet), with comparable performance at 60% (37.50% of the total diet) ($P = 0.508$) (Table 5; Figures 1A–B). These growth patterns may be related to the slightly elevated dietary protein levels in the *Wolffia*-supplemented diets. However, because dietary protein and energy levels varied across treatments, the contribution of *Wolffia* spp. per se cannot be fully disentangled. Consequently, the observed improvements should be interpreted as indicative trends associated with overall dietary composition.

Body length followed a similar pattern; the 60% group showed the highest mean length at week 6 (Table 4), which was significantly greater than that of the control and 15% groups ($P < 0.001$). The proportional increases in weight and length suggest that both measures followed similar directional trends. However, without body composition data, no conclusions regarding tissue-level responses can be drawn.

Feed utilization patterns were consistent with the growth responses. Apparent PER was significantly higher in the 45% and 60% groups ($P = 0.006$; Table 5; Figure 1D), which may indicate relatively higher weight gain per unit of calculated protein intake. Median apparent FCR values remained relatively high and did not differ significantly among treatments (Kruskal-Wallis test; $P = 0.217$), although numerical

improvements were observed in groups fed with *Wolffia* spp. diets (Table 5; Figure 1C). Lower apparent FCR and higher apparent PER were observed in *Wolffia*-supplemented groups, which may reflect combined dietary effects of the present formulation. CF also differed significantly among treatments ($P = 0.022$; Table 5; Figure 1E), with higher inclusion levels showing slightly higher CF values, which appears consistent with the observed growth trends (Froese, 2006; Zhang *et al.*, 2023).

The unusually high apparent FCR values, particularly in the control group, require careful interpretation. The extreme variance observed in this group, driven by replicates exhibiting negligible or negative weight gain, necessitated reporting apparent FCR values as median (range) and the application of the non-parametric Kruskal-Wallis test to ensure statistical validity. These elevated median values primarily reflect a mathematical artifact associated with near-zero growth rather than true inefficiency in feed utilization. Although daily feed rations were adjusted weekly based on biomass (3% body weight), weight gain in some replicates remained minimal under nutritional stress, leading to disproportionate inflation of apparent FCR values and variance. This effect was further exacerbated by methodological constraints, including the inability to recover uneaten feed due to rapid disintegration in water, which resulted in apparent FCR calculations based on total feed offered. In addition, calculated dietary crude protein levels (18.0–19.6% CP, Table 1) were substantially below reported requirements for guppies (30–40% CP; Fadel *et al.*, 2021; Xing *et al.*, 2024), likely contributing to growth failure and elevated apparent FCR. Accordingly, apparent FCR values are presented for descriptive purposes and should not be interpreted as absolute measures of feed efficiency or directly compared with values reported from nutritionally optimized feeding systems.

Survival remained high (88–100%) and was not significantly affected by *Wolffia* spp. inclusion (Kruskal-Wallis test; $P = 0.183$; Table 5; Figure 1F). The slight, non-significant decline in median survival at higher inclusion levels may reflect unknown dietary factors related to the overall diet composition, though the cause cannot be determined from the present data. Potential contributors, such as dietary fiber or anti-nutritional factors, were not assessed and remain speculative (Francis *et al.*, 2001; Said *et al.*, 2022). Nevertheless, the observed survival trends should be interpreted with caution, as the limited replication ($n = 3$) and short trial duration may have constrained the statistical power to detect biologically meaningful differences.

Several limitations of the feeding trial should be considered. First, as previously discussed, the non-standardized dietary levels (CP and energy) create a confounded design that prevents isolating *Wolffia*-specific effects. The experimental design, therefore, does not allow determination of whether the observed growth and feed utilization responses were attributable to *Wolffia* spp. per se or to the resulting changes in overall diet composition, including increased dietary protein and energy levels. Second, the lack of proximate analysis on the final mixed diets introduces nutritional uncertainty. Since the actual nutrient intake was not analytically confirmed, this limits the confidence with which growth and feed-utilization responses can be linked to specific nutrient levels. Calculated values may have deviated from the true composition due to factors such as

uneven mixing, moisture variability, or the physical leaching of the *Wolffia* spp. coating upon immersion in water. Consequently, these results should be viewed as indicative of a practical supplementation scenario rather than a precise dose-response study of specific nutrients. Third, the limited replication ($n = 3$) and high variance observed in certain parameters (e.g., apparent FCR and survival rate), which necessitated the use of median values and rank-based non-parametric analyses, may have constrained the statistical power to detect subtle or longer-term physiological effects. Finally, the absence of biochemical and immunological measurements means interpretations must remain limited to growth-based responses.

Given these methodological constraints, the present study should be considered exploratory rather than definitive. The findings indicate preliminary supplementation trends that warrant further investigation under controlled conditions but cannot support robust dietary recommendations or claims of *Wolffia*-specific benefits at this stage. Despite these limitations, the results reflect a realistic supplementation scenario in which plant-based materials are added directly to commercial feeds at the point of use, without prior diet reformulation. To distinguish *Wolffia*-specific effects from those associated with changes in overall nutrient content, future studies should employ nutritionally standardized diets and include comprehensive assessments of digestibility, amino acid availability, intestinal morphology, microbiome composition, and long-term health indicators.

Table 3 Mean body weight (g) ± standard deviation of guppies fed different dietary formulations over a 6-week trial

Trial group	Mean body weight (g)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	0.48 ± 0.07	0.47 ± 0.07 ^b	0.48 ± 0.09	0.48 ± 0.06	0.49 ± 0.09	0.50 ± 0.10 ^b	0.50 ± 0.14 ^b
15% <i>Wolffia</i> spp.	0.50 ± 0.08	0.48 ± 0.10 ^b	0.48 ± 0.09	0.48 ± 0.10	0.50 ± 0.11	0.54 ± 0.12 ^b	0.59 ± 0.12 ^b
30% <i>Wolffia</i> spp.	0.47 ± 0.07	0.47 ± 0.07 ^b	0.47 ± 0.07	0.48 ± 0.09	0.48 ± 0.08	0.54 ± 0.08 ^b	0.57 ± 0.08 ^b
45% <i>Wolffia</i> spp.	0.46 ± 0.08	0.51 ± 0.08 ^a	0.47 ± 0.10	0.52 ± 0.10	0.53 ± 0.13	0.60 ± 0.12 ^a	0.64 ± 0.11 ^a
60% <i>Wolffia</i> spp.	0.50 ± 0.09	0.56 ± 0.07 ^a	0.52 ± 0.08	0.53 ± 0.10	0.55 ± 0.11	0.66 ± 0.09 ^a	0.66 ± 0.09 ^a
P-value	0.443	0.002	0.242	0.110	0.148	0.000	0.000

Note: Means within the same column followed by different superscript letters (a, b) are significantly different at $P < 0.05$ (one-way ANOVA followed by Fisher's LSD test).

Table 4 Mean body total length (cm) ± standard deviation of guppies fed with different dietary formulations

Trial group	Mean body total length (cm)						
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	3.30 ± 0.13	3.41 ± 0.11	3.43 ± 0.12	3.50 ± 0.12	3.50 ± 0.12 ^b	3.56 ± 0.15 ^b	3.60 ± 0.10 ^b
15% <i>Wolffia</i> spp.	3.29 ± 0.16	3.41 ± 0.13	3.43 ± 0.17	3.46 ± 0.17	3.47 ± 0.20 ^b	3.60 ± 0.10 ^a	3.64 ± 0.10 ^b
30% <i>Wolffia</i> spp.	3.31 ± 0.15	3.37 ± 0.16	3.42 ± 0.18	3.43 ± 0.17	3.48 ± 0.20 ^b	3.62 ± 0.11 ^a	3.72 ± 0.11 ^b
45% <i>Wolffia</i> spp.	3.36 ± 0.16	3.37 ± 0.18	3.43 ± 0.14	3.51 ± 0.17	3.56 ± 0.23 ^a	3.65 ± 0.13 ^a	3.73 ± 0.13 ^a
60% <i>Wolffia</i> spp.	3.36 ± 0.19	3.47 ± 0.16	3.53 ± 0.15	3.53 ± 0.18	3.61 ± 0.17 ^a	3.73 ± 0.14 ^a	3.78 ± 0.11 ^a
P-value	0.323	0.122	0.094	0.225	0.048	0.000	0.000

Note: Means within the same column followed by different superscript letters (a, b) are significantly different at $P < 0.05$ (one-way ANOVA followed by Fisher's LSD test).

Table 5 Growth performance parameters of guppies fed different dietary formulations

Parameter	Control	Trial group				F-test	P-value
		15% <i>Wolffia</i> spp.	30% <i>Wolffia</i> spp.	45% <i>Wolffia</i> spp.	60% <i>Wolffia</i> spp.		
Initial weight (g)	0.48 ± 0.07	0.50 ± 0.08	0.47 ± 0.07	0.46 ± 0.08	0.50 ± 0.09	0.940	0.443
Final weight (g)	0.50 ± 0.14 ^c	0.59 ± 0.12 ^b	0.57 ± 0.08 ^b	0.64 ± 0.11 ^a	0.66 ± 0.09 ^a	8.125	0.000
Weight gain (g)	0.02 ± 0.07 ^c	0.09 ± 0.02 ^b	0.10 ± 0.05 ^b	0.18 ± 0.06 ^a	0.16 ± 0.03 ^a	8.816	0.000
ADG (g/day)	0.00048 ± 0.001 ^c	0.00214 ± 0.0025 ^b	0.00238 ± 0.0012 ^b	0.00429 ± 0.0007 ^a	0.00381 ± 0.0006 ^{ab}	8.427	0.003
SGR (%/day)	0.11 ± 0.22 ^c	0.42 ± 0.08 ^b	0.44 ± 0.20 ^b	0.74 ± 0.06 ^a	0.64 ± 0.11 ^{ab}	7.528	0.005
Apparent FCR	9.92 (-109.01–174.47)	7.85 (7.59–11.13)	7.07 (5.39–19.81)	4.77 (4.22–5.32)	6.38 (4.47–6.64)	-	0.217
Apparent PER	0.18 ± 0.33 ^c	0.68 ± 0.18 ^b	0.71 ± 0.36 ^b	1.19 ± 0.17 ^a	0.97 ± 0.15 ^{ab}	7.065	0.006
Condition factor	1.07 ± 0.05 ^b	1.23 ± 0.02 ^a	1.11 ± 0.08 ^b	1.24 ± 0.09 ^a	1.22 ± 0.04 ^a	4.686	0.022
Survival rate (%)	100 (88–100)	100 (88–100)	100 (88–100)	88 (88–88)	88 (88–88)	-	0.183

Note: Values for initial weight, final weight, weight gain, ADG, SGR, apparent PER, and condition factor are expressed as mean ± standard deviation; means within the same row followed by different superscript letters (a, b, c) are significantly different at $P < 0.05$ (one-way ANOVA followed by Fisher's LSD test). Values for apparent FCR and survival rate are presented as median (range) with P-values determined using the Kruskal-Wallis test due to non-homogeneity of variances; F-values are not reported for parameters analyzed using non-parametric tests. ADG = average daily gain, SGR = specific growth rate, FCR = feed conversion ratio, PER = protein efficiency ratio.

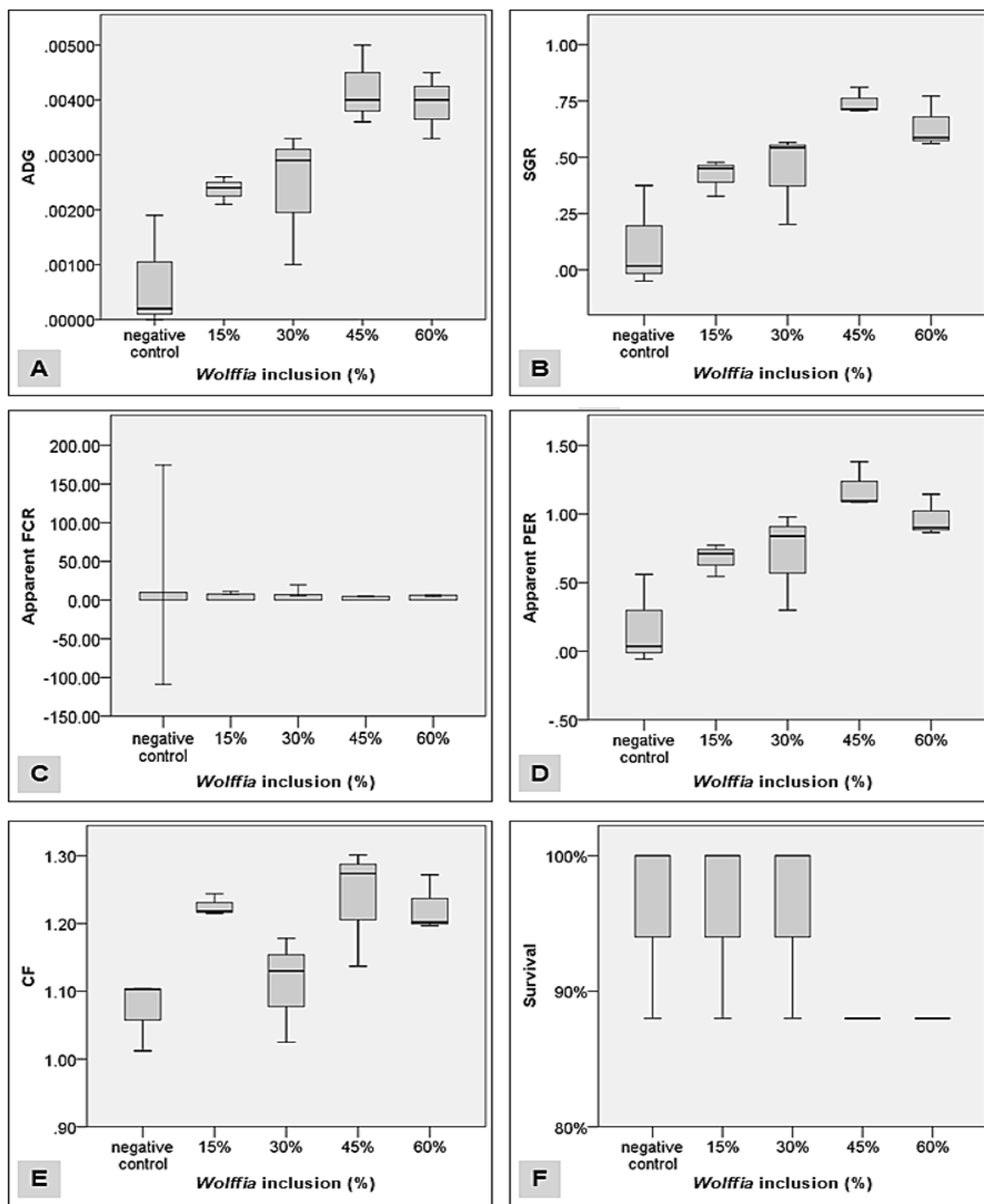


Figure 1 Growth performance, feed utilization, and survival of guppies fed 0–60% *Wolffia* spp. based on the base feed weight for 6 weeks. Data are shown as box-and-whisker plots ($n = 3$), with the horizontal line indicating the median, the box representing the interquartile range, and whiskers showing the minimum and maximum values: (A) average daily gain (ADG), (B) specific growth rate (SGR), (C) apparent feed conversion ratio (FCR), (D) apparent protein efficiency ratio (PER), (E) condition factor (CF), and (F) survival rate.

CONCLUSIONS

This preliminary study indicates that incorporating dried *Wolffia* spp. at levels up to 45% (base feed weight basis; 31.03% of total diet) is associated with positive trends in growth performance and feed utilization in guppies. However, as the experimental diets were non-isoproteinous, the observed growth response may reflect the overall increase in dietary protein content associated with higher *Wolffia* inclusion, rather than a specific functional effect of *Wolffia* spp. independent of nutrient levels. Given these exploratory findings, further research using nutritionally balanced, isoproteinous diets is warranted to more clearly characterize the role of *Wolffia* spp. as an aquafeed ingredient.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research.

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