Seasonal effects of protein levels on common carp (*Cyprinus carpio*) body composition

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Abstract

This study evaluated the growth performance and fed utilization of common carp (*Cyprinus carpio*) fed floating diets with varying protein levels across summer (June-August) and winter (December-February). During these periods, fish were acclimated to temperatures of 25.3°C in summer and 18.7°C in winter. Four dietary treatments with protein levels of 30%, 28%, 26%, and 35% were tested on 900 juvenile carp per group. Significant differences ($P < 0.05$) were found in daily feed consumed (DFC), total feed consumed (TFC), daily protein consumed (DPC), and total protein consumed (TPC) across treatments and seasons. Higher protein diets (28% and 35%) led to better growth in both seasons. The protein efficiency ratio (PER) and net protein utilization (NPU) were highest at 26% protein, indicating optimal protein utilization. Specific growth rate (SGR), metabolic growth rate (MGR), and condition factor (K-factor) were significantly higher with the 28% protein diet in summer, aligning with improved feed efficiency ratio (FER) and protein efficiency ratio (PER). These results highlight the importance of protein optimization for season-specific growth, driven by temperature-dependent metabolic processes, essential for sustainable carp aquaculture.

Keywords: Mosul Dam, feed consumed, *Cyprinus*, Protein utilization.

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Introduction

The worldwide demand for sustainable and financial farming practices has grown substantially towards optimizing farmed fish diet management (Bohnes *et al.,* 2022). The common carp (*Cyprinus carpio*) is one of the most frequently grown fish and has been recognised for its hardiness and economic significance (Souza *et al.,* 2022). Being ectothermic animals, fish require specialised physiological functions that require more significant protein amounts than terrestrial animals (Kaushik & Seiliez, 2010). Various conditions, including life stage, species, and climatic conditions, such as temperature, influence the amount of protein in the fish diet (Nilsson *et al.,* 2010). Temperature considerably impacts animal metabolism, which fluctuates with the seasons (Pang *et al.,* 2011). Throughout the summer, warmer water temperatures often enhance metabolism rates, resulting in more feed intake and quicker development (Sinclair *et al.,* 2016). Conversely, in the winter, lower water temperatures reduce metabolic activity, resulting in decreased feed intake and slower development (Islam *et al.,* 2022).

These seasonal metabolic changes require modifications in dietary protein levels to enhance growth efficiency and feed usage (Gaylord & Gatlin, 2001). According to findings, carp require more significant amounts of protein throughout the summer to promote enhanced metabolism and accelerate development rates (Al Sulivany *et al.,* 2024). Siddiqui (2023) observed that standard carp-fed diets with 35% protein had significantly higher growth rates and better feed conversion rates (FCR) than those with lower protein content. Moreover, Zhang *et al.* (2022) demonstrated that sustaining a 30-35% dietary protein content improved common carp's growth rate and nutrient absorption, especially during summertime.

However, the protein needs of the fish during the winter season is still poorly recognised. Lower protein levels may be sufficient to meet the fish's nutritional requirements due to lowered metabolic rates and feed consumption during colder months (NRC, 2011). Lin *et al.* (2021) investigated the effects of various protein levels on the development and feed utilisation of common carp in winter. Their findings indicate that a diet containing 25% protein was sufficient to maintain development and overall well-being, whereas higher protein levels did not provide additional benefits. This aligns with the research results of Kousoulaki *et al.* (2015), who hypothesised that decreased dietary protein intake may be acceptable for fish during reduced physiological activities.

Over-feeding protein can cause nitrogen excretion, which contributes to water pollution and eutrophication (Quang *et al.,* 2022). Therefore, optimising protein levels to correspond with the fish's metabolic demands can help in modifying these environmental impacts. Economically, protein is the most expensive component of fish feed and reducing unnecessary protein intake during periods of low metabolic activity can significantly lower feed costs (Nogales *et al.,* 2019). This dual benefit of environmental sustainability and economic efficiency underscores the importance of season-specific feeding strategies in aquaculture. This study assesses the growth performance, feed utilisation parameters, and condition factors of common carp feeding diets containing different protein levels during climate changes. Additionally, it aims to determine which protein concentration is most beneficial for carp in the summer and which is optimal for the winter.

Materials and methods

Study Area: The selected site for floating cage fish farming is in Khanki Township, Sumel District, Duhok Governorate of the Kurdistan Region, Iraq. This area was chosen because the Tigris River runs through it, and the Mosul Dam (Chambarakat Dam) was constructed here. The reservoir created by the dam covers an estimated area of approximately 54,900 km^2 (Al Sulivany *et al.,* 2024).

Fish and Experimental Protocols:

The experimental protocols included two seasons (Winter and Summer). The initial phase of the experiment took place 90 days from June to the end of August, while the second phase occurred from December until the end of February. Healthy juvenile common carp of a similar age group (mean weight: 156.90± 10.34g; mean length: 24.38± 4.05cm) were obtained from a fish hatchery farm in Mosul. The fish were transported under appropriate morning conditions and placed in rectangular ponds in the Dam.

The water parameters in the aquaculture pond aquarium during both seasons were evaluated using a multimeter (Al Sulivany *et al.,* 2024). The parameters included temperature (25.3°C and 18.7°C), pH (7.9 and 7.7), electrical conductivity (453 µS/cm and 425 µS/cm), total dissolved solids (290 ppm and 272 ppm), turbidity (3.4 and 2.7), dissolved oxygen (5.9 mg/l and 7.5 mg/l), total hardness (238 mg/l and 275 mg/l), biological oxygen demand measured over five days (2.7 mg/l and 3.4 mg/l), total alkalinity (135 mg/l and 147 mg/l), and salinity (0.31 ppt and 0.26 ppt). The fish were re-examined and acclimated a day before being released into the water culture floating cages (4×4×3 meters). A continuous flow-through of water was maintained during the experimentation period. In each experimental design, fish were separated into four groups, each floating cage housing nine hundred fish. The groups were designated as follows: the first group (T1), second group (T2), third group (T3), and fourth group (T4). Each group was fed a commercial extruded floating diet with different crude protein concentrations: T1 received 30%, T2 received 28%, T3 received 26%, and T4 received 35%. The fish diet was purchased from the aquatic feed production complex in Arak, Iran, through the *Kimiyagran-e-taghziyeh* company (Table. 1). To ensure its quality, the feed underwent analysis using a specialised instrument from Perten, a company associated with PerkinElmer. The feeding regimen lasted for 90 days; during the study, the fish were supplied with a daily feed ration equivalent to 3% of their estimated weight, calculated based on the total biomass of the fish. The feeding protocol employed in this study is based on the work by (Soliman *et al.,* 2022). After a feeding period, a random sample of twenty fish was selected to measure their length and weight.

Table 1. The nutritional composition of the diets for the four fish groups during the summer and winter seasons.

Fish diet	T1	T2	T3	T4
Protein (%)	30	28	26	35
Carbohydrate (%)	40	45	45	45
Fat (%)	6	6	6	6
Fiber (%)	8	8	8	8
Ash (%)	10	10	10	10
Moisture (%)	10	10	10	10

Body Weight and Length Measurement

The length of the carp was measured using a roller, and its body weight was determined using a digital balance.

Assessing Growth Performance in Carp

Daily weight gain (DWG): Calculated as the difference in weight between two consecutive days:
DWG (g)= Weight on Day 2 - Weight on Day 1 (Hassan *et al.*, 2021).
Total weight gain (TWG): The cumulative weight increase over a given period:
TWG (g/days) = Final Weight - Initial Weight (Hassan *et al.*, 2021).

Weight growth rate (WGR): Calculating the percentage increase in weight over a specified period relative to its initial weight:

WGR (%) = ((Final Weight - Initial Weight) / Initial Weight) * 100 (Guo *et al.*, 2021).
Relative growth rate (RGR): The proportional increase in the size relative to time:
RGR (%) = (ln (Final Length) – ln (Initial Length)) / Time (Lieke *et al.,* 2021).

Metabolic growth rate (MGR): The relationship between an individual fish's growth and its metabolic rate:
MGR (gkg$^{0.8}$ day^{-1}) = (TWG) / [{(IW/1000)$^{0.8}$ + (FW/1,000)$^{0.8}$}] /2 (White *et al.*, 2022).
Specific growth rate (SGR): The percentage increase in the body weight per unit of time:
SGR (%) = ((ln (Final Weight) – ln (Initial Weight)) / Time) * 100 (Mizory *et al.,* 2023).

Assessing Fish Feeding Efficiency

Total feed consumed (TFC): The quantity consumed by a group of fish during a specific time.
TFC (g/Time) = Number of fish × Average feed intake per fish × Time (Truong *et al.,* 2021).

Daily feed consumed (DFC) (g/Time) = Total feed consumed/Time (Truong *et al.,* 2021).
Total protein consumed (TPC): Quantifies the overall protein intake over time.
TPC(g/Time) = Total feed consumed × Protein content of the diet (Colombo *et al.*, 2023).
Daily protein consumed (g/Time) = Total consumed/Time (Colombo *et al.*, 2023).
Feed efficiency ratio (FER): It measures fish growth by the amount of feed consumed:
FER (%) = Weight gain/Feed consumed (Gabriel *et al.*, 2021).
Protein efficiency ratio (PER) Signifies the growth in body mass relative to protein intake:
PER (%) = Weight gain / Protein Consumed (Singh *et al.*, 2011).
Net protein utilization (NPU): A metric for the efficiency of protein utilization:
NPU (%) = Weight gain/ Total protein Intake (Singh *et al.*, 2011).
The protein productive value (PPV): An indicator evaluating the efficiency of protein utilization.
PPV (%) = (Weight gain / Total protein consumed) * 100 (El-Dahhar *et al.,* 2016).

Statistical Data Assessment

Statistical analysis was performed with the Graph Pad Prism software (version 8) from Graph Pad Prism Software in Finland. Morphological performance and feed utilization

Were evaluated using an Unpaired (non-parametric)-t-test. The outcomes were presented as means ± standard error of means.

Results

The findings regarding the impact of varying dietary concentrations on common carp (*Cyprinus carpio*) during both the summer and winter seasons are presented in Table 2 and Figure 1, denoted as A, B, C, D, E, F, G, and H. The statistical data showed that during the summer, the daily feed consumed (DFC) was significantly higher at 0.46±0.009 g for T1, 0.6±0.009g for T2, and 0.55±0.008g for T4, compared to winter, where the values were 0.4±0.007g, 0.46±0.006g, and 0.43±0.007g respectively. The total feed consumed (TFC) also reflected similar trends, with higher consumption in summer across all diets. In particular, T2 recorded 53.9±0.8g in summer versus 41.3±0.5g in winter.

The daily protein consumed (DPC) and total protein consumed (TPC) followed a significant pattern, with the highest values observed in T4 during the summer at 0.2±0.003g and 0.19±0.002g, respectively. Feed efficiency ratio (FER) and protein efficiency ratio (PER) remained relatively consistent across seasons. However, they showed significant differences, such as a PER of 1.33±0.007 for T2 in summer compared to 1.32±0.0001 in winter. Net protein utilization (NPU) and protein product Value (PPV) displayed minor variations without significant seasonal impact.

Table 2: The feed efficiency of the fish-fed diet with different protein levels over 90 days during the summer and winter seasons.

	T1 (30%)		T2 (28%)		T3 (26%)		T4 (35)	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
DFC	0.46**±0.009	0.4±0.007	0.6***±0.009	0.46±0.006	0.44±0.01	0.38**±0.005	0.55±0.008	0.43±0.007
TFC	41.5**±2.1	35.7±1.8	53.9***±0.8	41.3±0.5	39.8**±2.0	34.4±0.7	49.2***±1.7	38.9±2.1
DPC	0.14*±0.003	0.12±0.002	0.16**±0.003	0.13±0.001	0.11*±0.002	0.09±0.0002	0.2**±0.003	0.15±0.004
TPC	0.46*±0.05	0.39±0.06	0.17**±0.02	0.13±0.04	0.12*±0.002	0.098±0.001	0.19**±0.002	0.15±0.002
FER	0.33**±0.008	0.32±0.0009	0.33**±0.0008	0.32±0.0009	0.33**±0.0006	0.321±0.0006	0.34**±0.0007	0.32±0.0008
PER	1.34±0.007	1.34±0.003	1.33*±0.007	1.32±0.001	1.42±0.01	1.43±0.003	1.1±0.005	1.05±0.002
NPU	48±0.2	48.26±0.02	47.75±1.5	47.72±3.3	51.23±2.3	51.43±2.1	37.85±1.5	38.2±1.5
PPV	0.042±0.0002	0.04±0.0004	0.04±0.003	0.04±0.0003	0.04±0.006	0.04±0.005	0.03±0.0002	0.03±0.0002

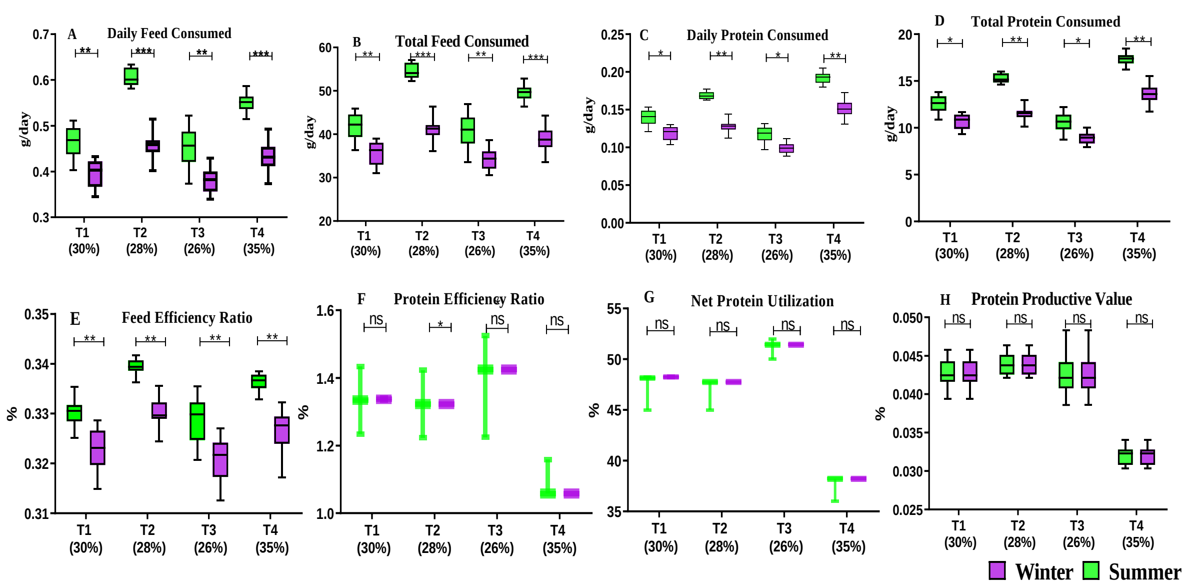

Figure 1. demonstrates the impact of varying protein concentrations in fish diets on feeding efficiency. A: represents daily feed consumption, B: Total feed consumption, C: Daily protein consumption, D: Total protein consumption, E: Feed efficiency ratio, F: Protein efficiency ratio, G: Net protein utilization, and H: Protein productive value. The data presented are expressed as

mean with standard error of the mean. (P < 0.05-0.01-0.001) Significant differences were observed.

The growth performance data are presented in (Table 3 and Figure 2; A, B, C, D, E, F, and G) and during the summer and winter, significant variations in growth performance parameters were recorded. In the summer, the daily weight gain (DWG) was significantly higher (*P<0.001*), with T2 showing 18.28±0.29g compared to 13.63±0.2g in winter. Similarly, total weight gain (TWG) for T2 was 1645±26.25g in summer versus 1231±18.15g in winter (*P<0.001*). The weight gain rate (WGR) followed this trend, with T2 at 1088±20.08g in summer and 827.6±15.01g in winter.

The relative growth rate (RGR) for T2 also displayed a significant increase during summer (*P<0.001*) compared to winter. Metabolic growth rate (MGR) and specific growth rate (SGR) showed similar patterns, with T2 recording 20.08±0.13% and 3.23±0.007% in summer, respectively, compared to lower winter values. Fulton condition factor (F-factor) presented less variation but remained slightly higher in winter for all treatments.

Table 3: The growth performance of an experimental fish-fed diet with different protein levels during the summer and winter seasons.

	T1 (30%)		T2 (28%)		T3 (26%)		T4 (35)	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
DWG	13.68**±0.3	11.58±0.22	18.28***±0.29	13.63±0.2	13.08**±0.37	11.07±0.19	16.52***±0.27	12.76±0.24
TWG	1231**±27.29	1044±21.08	1645***±26.25	1231±18.15	1177**±34.04	991±17.19	1486***±24.95	1157±23.73
WGR	815.3**±17.5	688.3±15.88	1088***±20.08	827.6±15.01	785.7**±25.2	656.6±14.04	978.7***±19.15	760.3±16.4
RGR	44.07**±0.39	41.06±0.44	49.27***±0.41	44.51±0.36	43.39**±0.75	40.23±0.55	47.29***±0.44	42.82±0.42
MGR	18.02**±0.55	16.84±0.43	20.08***±0.13	18.07±0.11	17.7**±0.21	16.52±0.13	19.34***±0.13	17.53±0.14
SGR	3.11**±0.009	3.05±0.008	3.23***±0.007	3.11±0.005	3.1**±0.011	3.04±0.006	3.189***±0.007	3.08±0.008
FCF	3.6±0.18	3.64±0.23	5.4*±0.54	4.8±0.23	3.48±0.13	3.37±0.07	4.44*±0.32	3.53±0.23

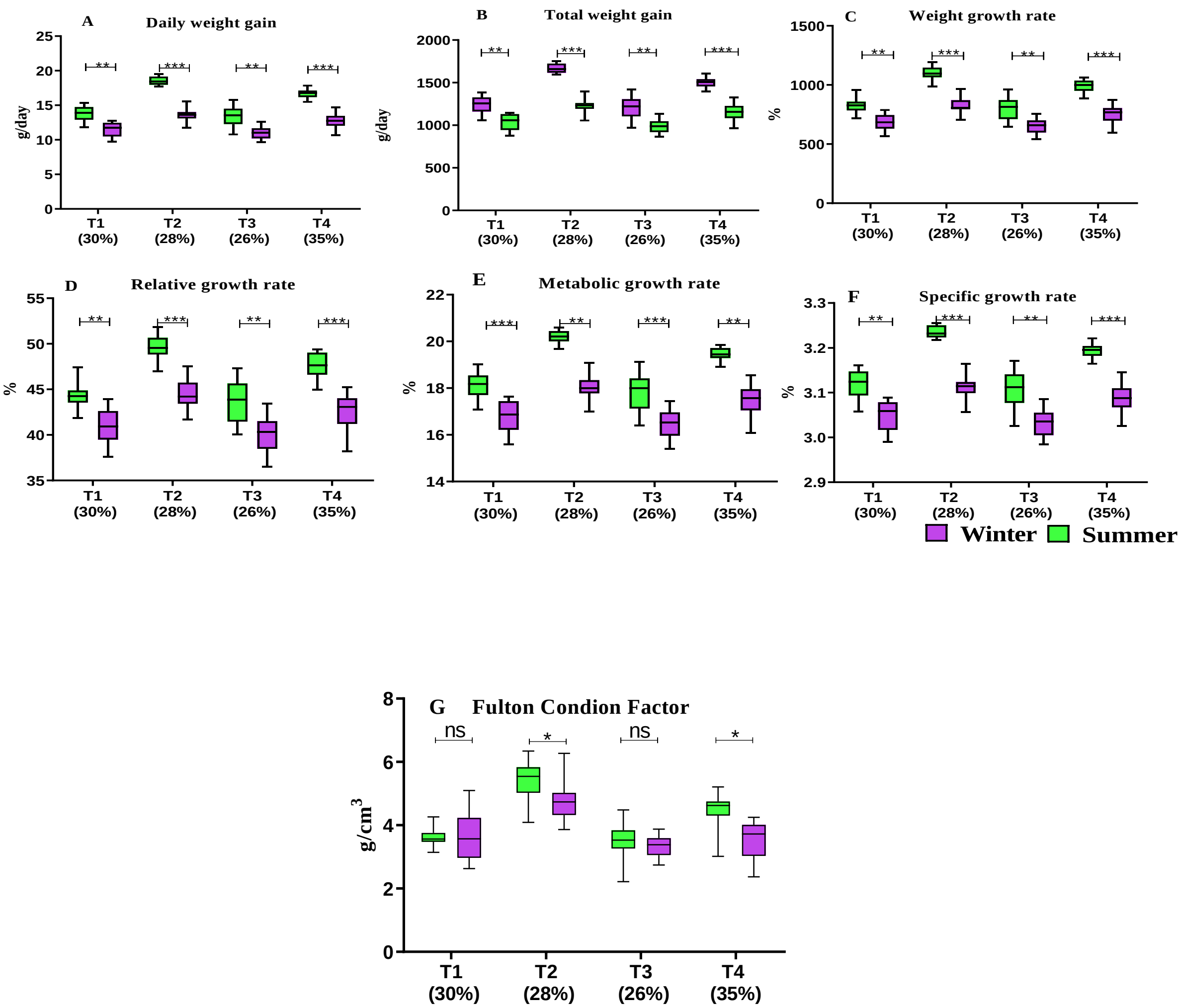

Figure 2 demonstrates the impact of varying protein concentrations in fish diets on Growth performances. A: Daily weight gain, B: Total weight gain, C: Weight growth rate, D: Relative growth rate, E: Metabolic growth rate, F: Specific growth rate, and G: Fulton condition factor. The data presented are expressed as mean with standard error of the mean. ($P < 0.05$-0.01-0.001) Significant differences were observed.

Discussion

The daily feed consumed (DFC) and total feed consumed (TFC) varied significantly with changing protein levels and seasons. T2 showed the highest DFC in summer, followed by T4, indicating a greater feed intake at higher protein concentrations. Conversely, in winter, T2 and T4 also showed higher DFC but lower absolute values than in summer, reflecting reduced metabolic activity during colder months. These findings are consistent with studies conducted by (Zeng *et al.,* 2023; Radhakrishnan *et al.,* 2020), who found that higher dietary protein intake in warmer temperatures leads to increased feed consumption. The notable differences in DPC and TPC highlight the significance of maintaining optimal protein levels to maximise growth during periods of high metabolic demand. This is supported by the findings of Li *et al.* (2015), who emphasised the significance of dietary protein in improving fish growth and health. These researchers propose that different fish species employ unique metabolic strategies when exposed to temperature fluctuations, and the exact mechanisms underlying these adaptations warrant additional investigation.

The feed efficiency rate (FER) and protein efficiency rate (PER) remained relatively stable across treatments, with slight improvements observed for T4 in the summer. This indicates better feed conversion efficiency at higher protein levels.

Physiologically, warmer temperatures optimise enzymatic activities related to digestion, such as proteases, amylases, and lipases, and function more efficiently at higher temperatures, resulting in better breakdown and absorption of nutrients. This enhanced nutrient absorption supports better growth rates and feed utilization (Kuiper, 2020). Moreover, studies have shown that the thermoregulatory behaviour of carp, where they seek out optimal temperature zones, aligns with increased metabolic and digestive efficiency during the summer months (Thirukanthan *et al.,* 2023).

These findings underscore the importance of maintaining optimal protein levels to enhance protein utilization, as previously highlighted by Khan and Maqbool (Khan & Maqbool, 2017) in their study on fish nutrition. Furthermore, fish's growth hormone (GH) and insulin-like growth factor (IGF) system, which are critical for growth and metabolism, are also temperature-sensitive. Elevated summer temperatures can upregulate the GH/IGF axis, increasing protein synthesis and growth rates (Lee *et al.,* 2001). This hormonal regulation further supports the observed increase in FER and PER during warmer periods. The notable differences in net protein utilization (NPU) among the treatments, particularly in the summer season, emphasize that higher protein levels do not necessarily lead to improved protein utilization.

These findings align with the observations made by Luo *et al.* (2022), who also observed optimal protein utilization at moderate dietary protein levels in various fish species. In addition, the protein productive value (PPV) demonstrated relatively consistent values across different treatments and seasons, with only minor fluctuations. During both summer and winter, T1 and T2 exhibited similar PPV values, indicating that PPV may be less responsive to variations in dietary protein levels compared to other measures. This is supported by the research conducted by Zakeri and Minabi (2013), who found that PPV is influenced by several factors, including feed composition and fish metabolism.

The daily weight gain (DWG) and total weight gain (TWG) of common carp exhibited significant variations across different protein concentrations and seasons. During both seasons, the highest DWG and TWG were observed in T2, with significant differences (p

< 0.01 for T1 and T3, $p < 0.001$ for T2 and T4) in the summer. These findings are consistent with recent studies that suggest optimal protein levels (around 28%) enhance carp growth performance (Ahmed *et al.,* 2020; Robinson *et al.,* 2022). The slow growth observed in fish with lower protein levels may be attributed to their inability to effectively utilize dietary protein after reaching optimal intake (Phillips, 1972).

Conversely, excessive protein in their diet could hinder growth rates due to the energy required for protein breakdown rather than tissue development. The reduced weight gain associated with excessive protein intake may also result from inadequate energy available for growth and a deficiency in non-protein energy required for processing high-protein feeds. The growth rate of weight (WGR) showed significant variation across different protein levels in the summer season. Notably, T2 demonstrated the highest WGR during this period, displaying significant disparities. These findings correspond with previous studies that underscore dietary protein levels' impact on aquaculture growth rates (Hossain *et al.,* 2022).

These results suggest that various fish species adopt distinct metabolic strategies in response to temperature fluctuations, necessitating further investigation into the specific mechanisms involved. The relative growth rate (RGR) demonstrated similar significant trends. The highest RGR was observed in T2 during summer, with significant differences ($p < 0.001$). This observation is supported by recent research, which confirms that intermediate protein levels (around 28%) promote optimal fish growth (Wang *et al.,* 2023). Metabolic growth rate (MGR) varied significantly across diets and seasons. The highest MGR was observed in T2 during summer. These findings are consistent with the understanding that adequate protein intake is crucial for metabolic efficiency and growth in fish. This could be attributed to the heightened expression of IGF-1 at 28°C. Numerous studies have demonstrated the close relationship between IGF-1 and fish growth, highlighting its significant role in promoting growth development (Huang and Chang, 2016). Similarly, research by Qiang *et al.* (2012) indicated that plasma IGF-1 levels and IGF-1 mRNA expression in Nile tilapia increase with rising temperatures, correlating with enhanced growth performance. Moreover, studies have shown that within a specific temperature range, fish exhibit increased digestive enzyme activity (Miege *et al.*, 2010) and improved protein synthesis efficiency (Katersky & Carter, 2007).

The specific growth rate (SGR) results were consistent with other growth indicators. During the summer, SGR values exhibited variation, with T2 demonstrating the highest SGR and significant differences observed ($p < 0.001$). These findings are supported by studies emphasising dietary protein intake's role. These studies have shown that increasing dietary protein rates enhances SGR in Nile tilapia (Ali *et al.,* 2018). The authors demonstrated that fish fed with higher protein diets exhibit improved growth development, attributed to enhanced protein utilization and increased availability of essential amino acids for anabolic processes. Furthermore, Schrama *et al.* (2018)

focused their research on carp and found that diets with higher protein content significantly enhance SGR compared to diets with lower protein levels. The Fulton condition factor (FCF) was highest in T2 and showed significant differences ($p < 0.05$) during the summer, indicating improved condition and growth efficiency with a 28% protein diet. These results align with recent studies suggesting that optimal protein levels not only support better growth rates but also improve overall fish health and disease resistance (Paul *et al.*, 2023). This explained the higher BWG, TWG, WGR, RGR, MGR, SGR, and FCF during summer. In general, the appropriate dietary protein also promoted the expression of IGF-1, consistent with the report of (Amer *et al.*, 2020) on the Mirror carp.

Conclusion

This study demonstrated that during the summer season, higher protein diets, particularly around 28%, resulted in significantly improved metrics such as daily weight gain, total weight gain, and condition factor. These findings underscore the importance of matching protein intake to the fish's metabolic needs, balancing growth promotion with efficient feed utilization. Moreover, the study highlights the economic and environmental benefits of precise nutritional management in aquaculture, contributing to sustainable practices and enhanced profitability.

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Conflict of interests

The authors confirm that there are no conflicts of interest concerning the publication of this article.

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التأثيرات الموسمية لمستويات البروتين على تركيب جسم اسماك الكارب الشائع (*Cyprinus carpio*)

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المستخلص

قيمت الدراسة الحالية أداء النمو وكفاءة التغذية لأسماك الكارب الشائع (*Cyprinus carpio*) المغذى بأعلاف طافية بمستويات بروتين مختلفة خلال فصل الصيف (حزيران- أب) والشتاء (كانون الأول -شباط). خلال هاتين الفترتين، تم تكييف الأسماك مع درجات حرارة 25.3 درجة مئوية في الصيف و18.7 درجة مئوية في الشتاء. تم اختبار أربع معاملات غذائية بمستويات بروتين 30٪، 28٪، 26٪، و35٪ على 900 من صغار الكارب لكل مجموعة. وجدت فروق معنوية ($P < 0.05$) في الاستهلاك اليومي للغذاء (DFC)، والاستهلاك الكلي للغذاء (TFC)، والبروتين المستهلك يومياً (DPC)، والبروتين المستهلك إجمالياً (TPC) عبر المعاملات والمواسم. أدت الأعلاف ذات البروتين الأعلى (28٪ و35٪) إلى نمو أفضل في كلا الموسمين. كان معدل كفاءة البروتين (PER) والاستخدام الصافي للبروتين (NPU) في أعلى مستوياته عند 26٪ بروتين، مما يشير إلى الاستخدام الأمثل للبروتين. كان معدل النمو النوعي (SGR)، ومعدل النمو الأيضي (MGR) ومعامل الحالة (K-factor) أعلى بشكل ملحوظ مع النظام الغذائي البروتيني بنسبة 28٪ في الصيف، متوافقاً مع تحسن معدل كفاءة التغذية (FER) ومعدل كفاءة البروتين (PER). تبرز هذه النتائج أهمية تحسين البروتين للنمو الموسمي المنظم بالعمليات الأيضية المعتمدة على درجة الحرارة وهو أمر أساسي للتربية المستدامة لأسماك الكارب.

الكلمات المفتاحية: سد الموصل، العلف المستهلك، السيبرينوس، استغلال البروتين.

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