



Evaluating Distiller's Dried Grains with Solubles as a Partial Substitute for Soybean Meal in the Nile Tilapia (*Oreochromis niloticus*) Diets: Growth Performance, Physiological Induces, and Intestinal Histomorphometry

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ABSTRACT

Feed costs constitute over 60% of aquaculture production expenses, driving the need for economical protein alternatives. This study evaluated the partial replacement of dietary soybean meal with distiller's dried grains with solubles (DDGS) on growth performance, physiological parameters, and intestinal histomorphometry of the Nile tilapia (*Oreochromis niloticus*). Over 90 days fish (3.56±0.5 g) were fed one of five isonitrogenous (25.7% CP) and isolipidic (7.9%) diets: a soybean-based control or diets replacing 10, 20, 30, and 40% of its protein with DDGS. The 10% DDGS replacement diet yielded optimal final body weight (19.87±0.31 g), specific growth rate (1.92±0.02% day⁻¹), feed conversion ratio (1.05±0.02) and protein efficiency ratio (3.70±0.06) compared to all other treatments ($P<0.05$). This group also demonstrated enhanced health, indicated by significantly improved immune markers (elevated white blood cells and globulins) and superior intestinal morphology (longer villi, higher goblet cell density), which facilitated superior nutrient absorption. While higher DDGS inclusions (20-40%) maintained growth comparable to the control, the 30% level emerged as the most economically efficient, reducing feed cost per kg of fish gain by 6.36%. In conclusion, a 10% DDGS replacement is recommended to maximize tilapia health and growth, whereas a 30% replacement offers the best economic return, providing flexible strategies for sustainable and cost-effective aquaculture.

INTRODUCTION

The global aquaculture industry has experienced remarkable growth, establishing itself as an economically vital sector. This expansion is reflected in production data; from 2010 to 2020, world fisheries and aquaculture output surged by 32.4%, reaching 177.8 million metric tons. To meet future demand, the total production of aquatic animals is

projected to reach 202 million tons by 2030, requiring an additional 24 million tons to maintain current per capita consumption (FAO, 2022). Within this global context, Egypt has emerged as a key player, now being the largest fish producer in Africa and the seventh globally, a position largely driven by its robust aquaculture since 2010 (FAO, 2022). Tilapia ranks among the world's most significant and widely cultured fish species. Originally native to Africa, its desirable traits including rapid growth, high disease resistance, broad environmental adaptability, and low reproductive and dietary demands have facilitated its introduction to nearly 100 countries since the 1950s. Of these species, the Nile tilapia (*Oreochromis niloticus*) is the most extensively farmed (Wang, 2014). Egypt is the world's third-largest producer of the Nile tilapia, after China and Indonesia. As the industry expands, traditional extensive culture systems are increasingly being replaced by semi-intensive and intensive ones. This shift is creating a critical demand for large quantities of high-quality tilapia formulated feed (FAO, 2022).

Feed constitutes the largest operational cost in aquaculture, accounting for over 60% of total production expenses, with protein being a primary cost driver. Consequently, developing cost-effective protein sources is crucial for reducing feed costs and facilitating the industry's sustainable expansion. Soybean meal (SBM) has emerged as the most extensively researched plant-based in aquaculture due to its wide availability, consistent quality, high protein content with a balanced amino acid profile, and relatively low cost (Hussain *et al.*, 2024). As a result of extensive research demonstrating its efficacy in replacing fishmeal, SBM has become a key ingredient in commercial tilapia feeds in Egypt (Ragab *et al.*, 2023).

Distiller's dried grains with soluble (DDGS), a by-product of the ethanol production, is often a more cost-effective protein source than soybean meal (SBM) on a per-unit basis, making it a promising alternative for the Nile tilapia feed (Diógenes, 2018). With an annually production exceeding 40 million metric tons of DDGS in the United States alone, represents a highly available resource for the aquafeeds industry (Lim *et al.*, 2011). Its nutritional profile typically includes 26-35% crude protein (depending on the grain source), 10% crude fat and approximately 11% crude fiber. A significant advantage is its lacks anti-nutritional factors, which are common in other plant proteins (Yan, 2019). Furthermore, DDGS provides high nutritional value, containing vitamin B complex and yeast. These components are potential sources of nucleotides and beta-glucans that may enhance the fish immunity (Hussain *et al.*, 2024). Therefore, this study aimed to evaluate the effects of partially replacing dietary SBM with varying inclusion levels of DDGS on the growth performance, feed utilization, physiological induces, and intestinal histopathological of the Nile Tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

1. Experimental location and duration

This study was carried out over a 90-days period (August 9th to November 7th, 2023) at El-Qanater Fish Research Station, affiliated with the National Institute of Oceanography and Fisheries (NIOF), in Qaliubiya Governorate, Egypt.

2. Experimental fish

A total of 300 mono-sex Nile Tilapia (*Oreochromis niloticus*) fingerlings with an average initial body weight of 3.56 ± 0.5 g/fish were obtained from the hatchery of El-Qanater Fish Research Station, National Institute of Oceanography and Fisheries (NIOF). Prior to the experiment, fish were acclimated to the experimental conditions for 14 days and fed a control diet to apparent visual satiation.

3. Experimental facilities

A total of 300 fish were randomly allotted to five treatments, each with three replicates (15 fiberglass tank total). The fiberglass tank (86×53×33 cm), each contained 150 liters of water (0.15 m³) and was stocked at a density of 20 fish per tank (This is equivalent to 133 fish/m³). All tank received continuous aeration and a constant supply of underground water sources. Water quality, including temperature, dissolved oxygen, pH, and total ammonia, was monitored weekly using YSI Professional Plus meter (YSI 1, USA). Moreover, fish were weighed biweekly. Tank maintenance involved siphoning feces twice weekly, which removed half of the water volume each time, complemented by a complete water change every week.

4. Experimental design and diets

This experiment investigated the replacement of soybean meal (42% crude protein) with DDGS (27% crude protein) by substituting the protein on an equivalent protein basis. Five experimental diets were formulated to be isonitrogenous (25.7% crude protein) and isolipidic (7.9% crude fat) (Table 1). Specifically, five dietary treatments were formulated: a control diet with no DDGS, and four test diets where 10% (T₁₀), 20% (T₂₀), 30% (T₃₀), and 40% (T₄₀) of the protein derived from soybean meal was replaced with protein from DDGS.

The experimental diets were formulated at the Fish Nutrition Laboratory, National Institute of Oceanography and Fisheries (NIOF), El-Qanater, Qaliubiya Governorate, Egypt. The DDGS was supplied by MIRASCO EGYPT Company, sourced from the United States. All dry ingredients were thoroughly mixed with oil and water manually, and the resulting dough was formed into small balls. These balls were then processed through a commercial meat grinder to create pellet-like extrusions. The pellets were air-dried, manually crumbled into smaller pieces, and stored at 4°C until use. The chemical composition (%) of the formulated diets was analyzed according to **AOAC (2000)**. Throughout the experiment, fish were hand-fed the experimental diets twice daily (9:00 and 14:00 h), six days per week. The daily feeding rate was initially set at 5% of the total

biomass and reduced to 4% at the start of the second month. Feed levels were adjusted bi-weekly in response to biomass sampling.

5. Growth indices and feed utilization

The mean final body weight (FBW) for each experimental treatment was determined by dividing the total fish weight in each aquarium by the number of fish. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), energy retention (ER), feed efficiency (FE), economical conversion rate (ECR), and survival (%) were calculated using the following equations, according to **Goda *et al.* (2019)**:

- $WG = \text{final body weight (g)} - \text{initial body weight (g)}$
- $SGR = 100 \times [(\ln \text{ final body weight (g)} - \ln \text{ initial body weight (g)}) / \text{duration of feeding (day)}]$
- $FCR = \text{feed intake (g)} / \text{weight gain (g)}$
- $PER = \text{weight gain (g)} / \text{protein intake (g)}$
- $PPV = (\text{protein gain (g)} / \text{protein intake (g)}) \times 100$
- $ER = (\text{final energy in fish (kJ)} / \text{initial energy in fish (kJ)})$
- $ECR = \text{cost of diet (\$ kg}^{-1}\text{)} \times \text{feed conversion ratio (FCR)}$
- $FE = \text{Weight gain (g)} / \text{dry feed fed (g)}$
- $\text{Survival (\%)} = 100 \times (\text{initial number of fish} / \text{final number of fish})$

6. Sampling and chemical analysis

The proximate composition of the experimental diets and fish samples were analyzed for their proximate composition according to the standard methods of **AOAC (2000)**. Fish samples (four fish/replicate) were taken from each treatment replicate, then pooled and frozen at -20°C until subsequent chemical analysis. All samples (diets and fish) were dried at 60°C for 48 hours, finely ground using an electrical mill, and stored at 4°C until analysis. The analyzed components included dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash.

7. Blood samples and hematological analysis

7.1. Blood sampling and serum separation

Blood samples (six samples/group) were collected from the caudal vertebral vein according to **Feldman *et al.* (2000)**. The erythrocytic and leukocytic counts were determined according to the method described by **Stoskopf (1993)** using a hemacytometer and Natt-Herrick solution.

7.2. Hematological parameters

Hemoglobin concentration was determined using the cyanmethemoglobin method with Drabkin's solution according to **Stoskopf (1993)**. The cyanmethemoglobin method converts all hemoglobin derivatives to methemoglobin using ferricyanide and cyanide ions. Methemoglobin is a stable red compound that can be measured calorimetrically. The micro-hematocrit method was used for estimation of packed cell volume (PCV%) according to **Dacie and Lewis (1991)**.

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The differential leukocytic count (DLC) was determined from thin blood films that air-dried, fixed with methanol for 3-5 minutes, and stained with Giemsa stain for 8-10 minutes. After then rinsing with distilled water and drying, white blood cells were differentiated by counting 100 cells per smears according to **Stoskopf (1993)**. The absolute DLC (heterophil, lymphocyte, monocyte, eosinophil, and basophil) was calculated according to **Thrall (2004)** using the following formula: Absolute DLC = (number of each white cell × number of total leukocytic count) / 100).

7.3. Biochemical parameters

Serum total proteins were determined calorimetrically according to **Cannon et al. (1974)** by commercial kits (Spectrum, Egyptian Company for Biotechnology, Egypt). Albumins were determined calorimetrically according to **Doumas et al. (1971)** by commercial kits (Bio diagnostic Co., Egypt). Moreover, globulin content was calculated mathematically. Activities of aspartate aminotransferase (AST) were determined calorimetrically according to **Reitman and Frankel (1957)** using commercial kits (Bio diagnostic Co., Egypt). Furthermore, alanine aminotransferase (ALT) was determined calorimetrically according to **Reitman and Frankel (1957)** using commercial kits (Bio diagnostic Co., Egypt). Creatinine was determined according to **Bartles et al. (1972)** and **Larsen (1972)** by commercial kits (Bio diagnostic Co., Egypt). Urea was determined according to **Fawcett and Scott (1960)** by commercial kits (Bio diagnostic Co., Egypt).

8. Histological examination

Samples for histopathological examination originated from the intestine of fish from different groups. Intestinal samples were collected, then fixed in 10% neutral buffered formalin. After dehydration and clearance, the tissues were embedded in paraffin and sectioned at 5µm thickness. The serial sections were subjected to staining with hematoxylin and eosin according to **Bancroft and Layton (2013)**. Histomorphometry analysis was performed using ImageJ analysis software (National Institutes of Health, MD, USA). The intestinal villi length, width, and inter-villi space were measured by ImageJ analysis software and expressed as µm according to **Bancroft and Layton (2013)**.

9. Economic evaluation

Economic evaluation was calculated according to the following equations (**Abdel Rahman et al., 2010**):

- Feed cost per kg fresh fish (LE) = Cost/kg diet (LE) × consumed feed to produce 1 kg fish
- Relative feed cost/kg fresh fish = Values of feed cost/kg fresh fish / the minimum value of the same parameter
- Feed cost/1 kg gain (LE) = Feed intake per kg gain (FCR) × cost/kg diet (LE)
- ECR = cost of diet (\$ kg⁻¹) × Feed Conversion Ratio (FCR)

10. Statistical analysis

Statistical analysis was performed using the SPSS software package (SPSS v16.0

program). The obtained data were subjected to a one-way analysis of variance (ANOVA). Odd replicate values were omitted for data integrity. When the ANOVA showed significant differences effects ($P < 0.05$), Duncan's multiple range tests (**Duncan, 1955**) was applied for post-hoc comparison of means.

RESULTS AND DISCUSSION

1. Water quality

All water quality parameters (Table 2) were within the acceptable range for the Nile tilapia according to **Popma and Masser (1999)**. Water temperature ranged from 26.85 to 29.04°C (average $28.15 \pm 0.06^\circ\text{C}$), dissolved oxygen (DO) from 4.08 to 6.46 mg/L (average 5.54 ± 0.06 mg/L), pH from 8 to 8.43 (average 8.05 ± 0.01), and total ammonia nitrogen from 0.89 to 2.72 mg/L (average 1.49 ± 0.04 mg/L). The stability of these optimal water quality conditions confirmed that experimental was not a confounding variable, allowing for the clear attribution of effects on fish growth and physiology to the specific dietary treatments.

2. Growth performance

Significant differences in final weight (g/fish), were observed among all treatments ($P < 0.05$). As shown in Table (3), the lowest DDGS inclusion level (diet T₁₀) resulted in a statistically significant improvement in the growth indices, followed by fish fed the diets T₂₀ and T₃₀, which did not differ significantly from each other. The same trend was recorded for weight gain (WG), average daily gain (ADG), and specific growth rate (SGR). The lowest final weight was obtained from fish fed control diet and T₄₀ diet. The fact that growth performance metrics in the T₂₀, T₃₀, and T₄₀ groups did not differ statistically from the control group, while T₁₀ showed an improvement, indicating that the growth-promoting benefits of DDGS are optimized at the 10% inclusion level. This suggests that a moderate inclusion of actively enhances the nutritional value rather than merely serving as a substitute. Potential explanations include the provision of beneficial yeast components, nucleotides, or residual lipids present in DDGS, which might synergistically improve palatability or complement minor nutritional gaps in the SBM-dominant control diet, as suggested by **Abo-State *et al.* (2009)**. Residual yeast from the fermentation process is believed to improve fish development by accelerating digestive maturation and providing prebiotic/probiotic benefits. This is attributed to its rich content of nucleic acids, mannan oligosaccharides, and β -glucans. Approximately 50% of the protein in DDGS is derived from yeast (**Goda *et al.*, 2019**).

The condition factor (CF) is a morphological index used to evaluate the physiological durability, nutritional status, and overall health of fish by relating their weight to length. It is often correlated with fat reserves and body composition. The T₁₀ group showed a significantly lower CF compared to the control, suggesting a shift toward leaner body composition. While desirable CF ranges for *O. niloticus* in other studies may fall between 1.6 and 1.8 (**Ighwela *et al.*, 2011**), the values observed in current study

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(ranging from 2.27 to 2.81) are uniformly high, indicating very specific high-density or high-energy feeding during the trial. This finding implies that the T₁₀ diet results in a leaner fish product with less fat deposition in the liver and viscera, a desirable outcome for aquaculture production (Schaeffer *et al.*, 2009). Soltan *et al.* (2015) reported that the Nile tilapia fry fed diets containing 10% and 20% DDGS + enzymes replacing a mixture of soybean meal and yellow corn had the highest significant ($P<0.05$) final body weight and average daily gain, while fish fed 40% DDGS showed the lowest growth performance values, possibly due to the lower lysine amino acid content in the 40% diet. The present finding that a 10% inclusion level is optimal differs from reports in other species. Goda *et al.* (2019, 2020) observed that replacing up to 50% of SBM with high-protein DDGS enhanced growth in European bass (*Dicentrarchus labrax*). While, Diógenes *et al.* (2019) found that DDGS could replace SBM at up to 35% in gilthead sea bream (*Sparus aurata*) without impairing growth. This discrepancy underscores the importance of species-specific dietary formulations.

3. Feed utilization

The dietary replacement of soybean meal with DDGS significantly affected key feed utilization parameters (Table 4). Although feed intake (FI) was not significantly different ($P<0.05$) among groups, the numerically highest FI was recorded for the T₁₀ diet, potentially indicating enhanced palatability. The Nile tilapia fed T₁₀ diet had a significantly better feed conversion ratio (FCR) compared to the control, highlighting its superior efficiency in converting feed to biomass. With higher replacement levels (T₂₀, - T₄₀), the FCR advantage was lost, with values reverting to those of the control. Similar results were obtained by Soltan *et al.* (2015), who found a significant decrease ($P<0.05$) in FCR when 10 and 20% of soybean meal and corn were replaced with DDGS in tilapia diets. The same trend was reported by Gabr *et al.* (2013) and Khadr *et al.* (2018b) for the Nile tilapia.

The optimal DDGS inclusion level appears to be highly species-specific, as evidenced by contrasting findings in the literature. Recently, Banik *et al.* (2025) reported that in carp (*Cirrhinus mrigala*), FCR was optimal only at a 6% replacement level and worsened with higher inclusions. Conversely, Goda *et al.* (2020) found that European sea bass (*Dicentrarchus labrax*) fed diets containing 30-50% HP-DDG exhibited superior FCR compared to control. The present study, which identified a 10% inclusion as optimal for Nile tilapia, further underscores the need for species-specific formulations.

Protein efficiency ratio (PER) and protein productive value (PPV) were significantly influenced by the dietary treatments. The T₁₀ diet resulted in the highest PER and PPV, with both values being significantly greater ($P<0.05$) than those of the control group. While the PER for the T₂₀, T₃₀, and T₄₀ groups was statistically similar to the control, their PPV values were significantly higher ($P<0.05$) than the control. Treatments T₂₀, T₃₀, and T₄₀ had intermediate PPV values. Our finding of superior PER and PPV at the 10% inclusion level is supported by previous work on tilapia. Studies by Gabr *et al.*

(2013) and **Khadr *et al.* (2018b)** also reported improved protein utilization metrics with moderate DDGS inclusion. The underlying mechanism is likely twofold: the concentrated amino acid content in DDGS and its lack of anti-nutritional factors, which facilitates more efficient protein digestion compared to soybean meal-based diets.

Energy retention (ER) exhibited a general increasing trend in DDGS diets compare to control, with the T₁₀ diet resulting in the highest ER, significantly higher than the control diet which yielded the lowest. This pattern contrasts with the findings of **Goda *et al.* (2011)**, who reported a decrease in ER at high inclusion levels (>40%) in their species, a result they attributed to an unbalanced amino acid profile limiting energy deposition. Regarding feed efficiency (FE), the T₁₀ diet produced the highest value, which was significantly superior to all groups except T₃₀. This aligns with **Goda *et al.* (2011)**, who also found enhanced FE in the Nile tilapia with up to 60% SBM replacement, and is supported by **Wesly *et al.* (2018)**, who identified 30% DDGS as a suitable inclusion level for maintaining feed utilization.

4. Proximate body composition of tilapia

The body composition of the Nile tilapia was significantly altered by DDGS inclusion (Table 5). The T₁₀ diet yielded the highest dry matter (DM) and crude protein (CP) content, with CP being significantly higher than the control and T₄₀. This suggests that a 10% replacement optimizes protein deposition. However, CP generally decreased at higher inclusion levels, aligning with previous studies where DDGS beyond 20-40% reduced body protein (**Labib *et al.*, 2009; Gabr *et al.*, 2013**). Conversely, the ether extract (EE) increased with DDGS inclusion level, with T₄₀ being significantly higher than T₁₀ and T₂₀, reflecting the higher lipid content of DDGS and resulting in fat deposition (**Welker *et al.*, 2014**). Ash content decreased as DDGS increased, likely due to a dilution effect from increased organic matter (protein and fat), despite DDGS being a good source of available phosphorus (**Hertrampf & Piedad-Pascual, 2012**). The results indicate that while DDGS is a viable alternative, its optimal inclusion for the Nile tilapia is limited, likely by its lower lysine content, with a 10% replacement rate providing the best balance for protein retention (**Lim *et al.*, 2007b; Abo-State *et al.*, 2009**).

5. Hematological parameters

The results of blood parameters are summarized in Fig. (1). The white blood cell (WBC) count, a key immune indicator, was significantly ($P<0.05$) highest in the T₁₀ group while the control diet yielded the lowest count. The T₂₀ group was not significantly different from T₁₀, and T₃₀ and T₄₀ were similar to the control, suggesting a positive immune effect only at lower DDGS inclusions. This boost is likely attributed to β -glucans and other bioactive compounds from the yeast in DDGS, which are known immune stimulants. In contrast, red blood cells (RBCs) and hemoglobin (Hb) showed no significant differences, indicating that DDGS substitution did not impair oxygen transport or cause anemia. The packed cell volume (PCV) was the highest in T₁₀ and T₂₀ and significantly lower in the control, further supporting the beneficial physiological status at

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moderate inclusion levels. The decline in immune enhancement at higher replacement levels (T₃₀, T₄₀) may be due to anti-nutritional factors or amino acid imbalances in DDGS. These findings align with previous studies confirming that moderate DDGS inclusion (up to 20-30%) can maintain or improve health indices in the Nile tilapia, without negative hematological effects (**Cheng et al., 2005; Lim et al., 2007c; Gabr et al., 2013; Khalil et al., 2013; Khadr et al., 2018a**). The immunostimulatory potential of DDGS is largely attributed to its yeast content (4-7% of composition) and associated bioactive compounds, particularly β -glucans, which can comprise up to 8% of DDGS (**Shurson, 2018**). These components are known to enhance non-specific immunity by binding to receptors on phagocytic cells, thereby potentiating their ability to engulf and destroy pathogens (**Hassaan et al., 2018, 2019; Ragab et al., 2024**). This mechanism is consistent with the findings of **Goda et al. (2019)**, who reported significantly elevated hematological indices (RBCs, WBCs, Hb, and PCV) in European sea bass fed a 50% high-protein DDG diet, underscoring the capacity of DDGS to bolster immune and physiological status.

6. Biochemical Parameters

6.1. Blood proteins

Serum protein analysis revealed that the T₁₀ diet significantly increased total protein and globulin concentration compared to the control group, while albumin remained stable across all treatments (Fig. 2). The elevated total serum protein at the T₁₀ level suggests improved overall protein utilization or nutritional status, potentially due to an optimal nutrient balance in the diet. An increase or decrease in total blood proteins, albumin and globulins has clinical relevance in fish (**Peres et al., 2015**). Crucially, the significant rise in globulins directly involved in immune function indicates a potentiated immune response at the 10% DDGS inclusion level. The stability of albumin confirms that fundamental physiological processes and liver synthesis function were uncompromised, even at the highest (40%) replacement level. These results demonstrate that a moderate 10% substitution of soybean meal with DDGS can enhance immune status without disrupting metabolic health. These findings are consistent with existing literature, though they highlight species-specific optimal inclusion levels. Our results align with **Banik et al. (2025)** in carp (*Cirrhinus mrigala*), where moderate DDGS levels (6 and 12%) significantly improved total protein and globulin without affecting albumin, and with **Salama et al. (2010)** and **Gabr et al. (2013)**, who reported no adverse health effects in tilapia at inclusions up to 30%. Conversely, **Goda et al. (2020)** demonstrated that high-protein DDGS (HP-DDGS) could be included at much higher levels (up to 50%) in European sea bass, enhancing all serum protein fractions. This contrast underscores that the immunostimulatory and nutritional benefits of DDGS are not only dose-dependent but also fundamentally influenced by the specific physiological needs and digestive capabilities of the target species.

6.2. Liver and kidney function parameters

Liver enzyme and kidney function markers are shown in Fig. (3). Aspartate

aminotransferase (AST), and alanine aminotransferase (ALT) activities were at their highest values in the T₃₀ group, with its AST being significantly greater than T₂₀, and T₄₀ in ALT. The control, T₁₀, and T₂₀ groups showed intermediate and statistically similar ALT values. Urea levels did not differ significantly among treatments, though T₃₀ and T₄₀ exhibited numerically higher values. Conversely, creatinine was significantly elevated in the T₃₀ group compared to T₁₀, T₂₀, and T₄₀, with the T₄₀ group recording the lowest value. The significant elevation of liver enzymes (AST, ALT) and creatinine specifically in the T₃₀ group suggests a potential for hepatorenal stress at this 30% DDGS inclusion level, possibly due to amino acid imbalances or other antinutritional factors (Yue & Zhou, 2008). This contrasts with the absence of adverse effects in the T₁₀ and T₂₀ groups, indicating a threshold effect. Our results present a nuanced picture within the existing literature. While they conflict with studies reporting no significant biochemical alterations in tilapia at various DDGS inclusion levels (El-Saidy & Gaber, 2003; Lim *et al.*, 2007a; Khadr *et al.*, 2018a), they align with findings in other species like European sea bass, where even high DDGS inclusions were safe (Goda *et al.*, 2020). This discrepancy underscores that the physiological impact of DDGS is not only dose-dependent but may also be influenced by specific dietary formulations, ingredient quality, or fish sub-populations, highlighting the need for careful, level-specific evaluation.

6.3. Digestive enzymes and thyroid hormones

Digestive enzyme activities (amylase, lipase, protease) did not differ significantly among treatments, although the highest numerical values were observed in the T₄₀ (amylase, protease) and T₃₀ (lipase) groups (Fig. 4). No significant differences were found for triiodothyronine (T3), though all DDGS groups showed slightly elevated levels compared to the control. In contrast, free thyroxine (FT4) levels varied significantly ($P < 0.05$), with the T₃₀ group exhibiting the highest mean value, which was significantly greater than T₂₀ and T₄₀. The control and T₁₀ groups showed intermediate FT4 values. The absence of significant changes in digestive enzyme activities suggests that the Nile tilapia effectively maintained digestive capacity even at a 40% DDGS inclusion level (Wilson 1994; Lim *et al.*, 2007a). The numerical increase in amylase, lipase, and protease in the T₃₀ and T₄₀ groups may indicate an adaptive physiological response to the higher fiber and altered nutrient profile of DDGS, rather than a dysfunction. This stability is a positive indicator of dietary tolerance and contrasts with studies in other species, like carp, where high DDGS significantly suppressed enzyme activity, potentially due to species-specific sensitivities or lysine deficiencies.

The endocrine data further clarifies this response. The stability of the active hormone T3 across all treatments indicates that core metabolic regulation was uncompromised. However, the significant peak in FT4 at the T₃₀ level may signal a transient metabolic adjustment or a stress response to a suboptimal nutrient balance (Power *et al.*, 2001; Banik *et al.*, 2025). The subsequent decline in FT4 at the T₄₀ level could reflect the onset of antinutritional effects that begin to interfere with thyroid

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function at the highest inclusion rate (Francis *et al.*, 2001; Refstie *et al.*, 2001; Chhron & Mediha 2008). Overall, while the digestive system demonstrates robust tolerance to DDGS inclusions up to 40%, the thyroid hormone profile suggests that the optimal inclusion level for the Nile tilapia, which supports both metabolic and digestive health, is below 30%.

7. Intestinal histomorphometry

Histomorphometry analysis revealed significant improvements in intestinal health with moderate DDGS inclusion (Table 6). The T₁₀ group produced the most favorable outcomes, exhibiting the longest villi length, the smallest inter-villi space, and the highest goblet cell density which significantly higher than all other treatments ($P < 0.05$). The control group had the shortest villi length. These morphological features are strongly associated with enhanced nutrient absorption capacity and strengthened intestinal barrier function. While villi width generally decreased with increasing DDGS levels, the structural integrity observed in the T₁₀ and T₂₀ groups indicates that a 10-20% replacement of soybean meal with DDGS optimally promotes intestinal health in the Nile tilapia.

This improvement is likely attributed to beneficial components in DDGS, such as yeast-derived β -glucans and B-vitamins, which can stimulate mucosal health. Interestingly, these results suggest that a moderate DDGS replacement may also mitigate the subclinical enteritis sometimes associated with high soybean meal (SBM) levels, as indicated by the poorer morphology in the control group (Aydın & Gümüş, 2016; Khojasteh *et al.*, 2018; Suehs & Gatlin, 2022; Eid *et al.*, 2024).

While studies on other species like sea bass (Goda *et al.*, 2020); rainbow trout (*Oncorhynchus mykiss*, Gümüş, 2020), and hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂, Zhu *et al.*, 2022) show intestinal improvements even at high (50%) HP-DDGS inclusion, the declining benefits in tilapia at T₃₀ and T₄₀ highlight a species-specific tolerance limit. This is likely due to the increasing load of fiber and non-starch polysaccharides in standard DDGS, which can increase gut viscosity and negatively impact morphology at higher inclusion levels. Consequently, a 10% replacement level represents the ideal balance for harnessing the gut-health benefits of DDGS in the Nile tilapia, without incurring the negative effects of its fibrous structure.

8. Histopathological findings

Histological examination revealed no pathological lesions in the intestine, liver, or pancreas across all treatments (Figs. 5-9). However, distinct and beneficial morphological changes were observed. The most significant improvement was in the T₁₀ group, which exhibited a marked increase in intestinal villi length and a notable shift in liver histology, characterized by decreased vacuolation and increased eosinophilic cytoplasm, indicating a transition from lipid storage to higher protein synthesis. These positive trends in villus length and reduced hepatic vacuolation were also present in the in the T₂₀, T₃₀, and T₄₀ groups, but to a lesser extent than in T₁₀. The control group showed normal but shorter

villi and the highest degree of hepatic vacuolation. All groups maintained normal pancreatic integrity.

9. Economic evaluation

The economic analysis confirmed that replacing SBM with DDGS significantly reduced production costs. While the T₄₀ diet had the lowest cost per kilogram of feed (27.51 LE/kg), the T₃₀ diet emerged as the most economically optimal, yielding the lowest feed cost per kilogram of fish gain (29.82 LE). This was due to its favorable combination of a lower ingredient cost and the highly efficient feed conversion ratio (FCR of 1.07). The T₁₀ diet, despite having the best FCR (1.05), was slightly less cost-effective than T₃₀ due to its higher diet cost.

All DDGS-based diets (T₁₀ to T₄₀) were more economical than the SBM-based control diet (Table 7), demonstrating that DDGS is a cost-effective alternative protein source that enhances the economic feasibility of tilapia production. These findings align with previous studies reporting improved net profit and economic efficiency with DDGS inclusion (Hoffman & Baker, 2011; Gabr *et al.*, 2013; El-Shinnawy *et al.*, 2015; Khadr *et al.*, 2018b). Furthermore, Eid *et al.* (2024) reported that the Nile tilapia fed fermented DDGS at levels of 25 and 50% of soybean meal had the best economic performance, specifically diet cost, feed cost to produce 1kg and relative feed cost compared to control.

CONCLUSION

This research conclusively demonstrates that distillers dried grains with solubles (DDGS) is a viable and sustainable alternative protein source for the Nile tilapia diets, with the optimal inclusion level dependent on producer priorities. A 10% replacement of soybean meal protein with DDGS was identified as the biological optimum, significantly enhancing growth performance, feed efficiency, immune status, and intestinal morphology. For operations where economic efficiency is the primary driver, a 30% replacement level is recommended, as it substantially reduces feed costs while maintaining acceptable growth and health, despite minor indications of physiological stress. These findings provide clear, evidence-based strategies for improving the economic viability and environmental sustainability of tilapia aquaculture by integrating a cost-effective ingredien

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List of Tables

Table 1. Ingredient and proximate composition of the experimental diets

Ingredients (g/kg)	control	T ₁₀	T ₂₀	T ₃₀	T ₄₀
Fish meal (60% cp)	50	50	50	50	50
Soy meal (42% cp)	300	270	240	210	180
Corn gluten (60% cp)	65	65	65	65	65
Yellow corn (7.5% cp)	300	285	270	255	240
DDGS (27% cp)	0	45	90	135	180
Wheat bran (14% cp)	198	198	198	198	198
Soy oil	50	50	50	50	50
Di Cal. Pho	9	9	9	9	9
Lysine	5	5	5	5	5
Methionine	5	5	5	5	5
Premix	10	10	10	10	10
Probiotic	7	7	7	7	7
Vitamin c	1	1	1	1	1
Total (g)	1000	1000	1000	1000	1000
Proximate composition (%)					
Moisture%	6.84	5.51	5.41	6.63	6.18
Protein%	26.0	25.9	25.7	25.6	25.4
Lipid%	7.6	7.8	7.9	8.1	8.3
Ash%	5.94	5.83	5.83	6.02	5.83
Total carbohydrates % ¹	60.46	60.47	60.57	60.28	60.47
Gross energy (kcal/kg) ²	4713.52	4727.24	4729.69	4730.81	4746.49
¹ Total carbohydrate content was determined by the difference; total carbohydrate = 100 – (% crude protein + % crude fat + % total ash).					
² Dietary gross energy was calculated using the conversion factors of 5.6, 9.45 and 4.2 kcal/g for protein, lipids and carbohydrates, respectively.					

Table 2. Average values, minimum and maximum of water quality parameters during the experiment

Parameters	Minimum	Maximum	Average	Standard error
Temperature (°C)	26.85	29.04	28.15	0.063
DO ¹ (ppm)	4.08	6.46	5.54	0.055
pH	8.00	8.43	8.05	0.007
TAN ² (mg /L)	0.89	2.72	1.49	0.044
¹ Dissolved Oxygen				
² Total ammonium nitrogen				

Table 3. Growth performance of the Nile Tilapia (*Oreochromis niloticus*) fed different experimental diets (means ± SE)

Treatments ¹	Control	T ₁₀	T ₂₀	T ₃₀	T ₄₀
IBW ²	3.57±0.03	3.54±0.05	3.55±0.03	3.53±0.03	3.54±0.05
FBW ³	18.23±0.40 ^b	19.87±0.31 ^a	18.78±0.51 ^{ab}	18.75±0.23 ^{ab}	18.44±0.19 ^b
WG ⁴	14.66±0.37 ^b	16.33±0.30 ^a	15.23±0.50 ^b	15.21±0.22 ^b	14.90±0.16 ^b
ADG ⁵	0.16±0.00 ^b	0.18±0.00 ^a	0.17±0.01 ^b	0.17±0.00 ^b	0.17±0.00 ^b
SGR ⁶	1.81±0.02 ^b	1.92±0.02 ^a	1.85±0.03 ^b	1.85±0.01 ^b	1.84±0.01 ^b
CF ⁷	2.81±0.14 ^a	2.27±0.05 ^b	2.34±0.06 ^{ab}	2.41±0.08 ^{ab}	2.74±0.26 ^{ab}
S% ⁸	91.67±4.41	93.33±4.41	91.67±3.33	100.00±0.00	93.33±1.67

Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.

¹ Control, T₁₀, T₂₀, T₃₀ and T₄₀ contained 0, 10, 20, 30 and 40% DDG instead of soybean meal, respectively.

² IBW: Initial body weight. ³ FBW: Final body weight. ⁴ WG: Weight gain. ⁵ ADG: Average daily gain. ⁶

SGR: Specific growth rate. ⁷ CF: Condition factor. ⁸ Survival %.

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Table 4. The feed utilization of the Nile Tilapia (*Oreochromis niloticus*) fed different experimental diets (means $\pm$ SE).

Treatments ¹	Control	T ₁₀	T ₂₀	T ₃₀	T ₄₀
FI ²	16.06 $\pm$ 0.35	17.07 $\pm$ 0.44	16.65 $\pm$ 0.69	16.37 $\pm$ 0.23	16.47 $\pm$ 0.46
FCR ³	1.10 $\pm$ 0.00 ^a	1.05 $\pm$ 0.02 ^b	1.09 $\pm$ 0.01 ^a	1.07 $\pm$ 0.00 ^{ab}	1.11 $\pm$ 0.02 ^a
PER ⁴	3.51 $\pm$ 0.02 ^b	3.70 $\pm$ 0.06 ^a	3.56 $\pm$ 0.03 ^{ab}	3.63 $\pm$ 0.01 ^{ab}	3.56 $\pm$ 0.06 ^{ab}
PPV ⁵	51.96 $\pm$ 0.20 ^c	57.94 $\pm$ 0.93 ^a	55.79 $\pm$ 0.51 ^{ab}	56.60 $\pm$ 0.21 ^{ab}	55.17 $\pm$ 1.01 ^b
ER ⁶	27.34 $\pm$ 0.09 ^d	33.34 $\pm$ 0.52 ^a	29.89 $\pm$ 0.30 ^c	31.22 $\pm$ 0.11 ^b	31.08 $\pm$ 0.57 ^b
FE ⁷	0.91 $\pm$ 0.00 ^b	0.96 $\pm$ 0.01 ^a	0.91 $\pm$ 0.01 ^b	0.93 $\pm$ 0.00 ^{ab}	0.91 $\pm$ 0.02 ^b

Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.

¹ Control, T₁₀, T₂₀, T₃₀ and T₄₀ contained 0, 10, 20, 30 and 40% DDG instead of soybean meal, respectively.

² FI: Feed intake. ³ FCR: Feed conversion ratio. ⁴ PER: Protein efficiency ratio. ⁵ PPV: Protein productive value. ⁶ ER: Energy retention. ⁷ FE: Feed efficiency.

Table 5. Chemical composition (%) of the Nile Tilapia (*Oreochromis niloticus*) fed different experimental diets (means $\pm$ SE)

Treatments ¹	Control	T ₁₀	T ₂₀	T ₃₀	T ₄₀
DM % ²	24.14 $\pm$ 0.54 ^b	26.57 $\pm$ 0.54 ^a	25.57 $\pm$ 0.39 ^{ab}	25.85 $\pm$ 0.95 ^{ab}	26.05 $\pm$ 0.40 ^{ab}
CP % ³	58.07 $\pm$ 0.48 ^c	62.59 $\pm$ 1.26 ^a	60.87 $\pm$ 0.30 ^{ab}	59.98 $\pm$ 0.39 ^{abc}	59.17 $\pm$ 0.74 ^{bc}
EE % ⁴	26.86 $\pm$ 0.31 ^{ab}	23.49 $\pm$ 1.38 ^c	24.94 $\pm$ 0.15 ^{bc}	26.29 $\pm$ 0.05 ^{ab}	27.57 $\pm$ 0.20 ^a
Ash %	13.78 $\pm$ 0.78 ^a	12.63 $\pm$ 0.13 ^{ab}	12.90 $\pm$ 0.15 ^{ab}	12.44 $\pm$ 0.44 ^{ab}	11.97 $\pm$ 0.54 ^b

Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.

¹ Control, T₁₀, T₂₀, T₃₀ and T₄₀ contained 0, 10, 20, 30 and 40% DDG instead of soybean meal, respectively.

² DM: Dry matter. ³ CP: Crude protein. ⁴ EE: Ether extract.

Table 6. Intestinal histomorphometry of the Nile Tilapia (*Oreochromis niloticus*) fed different experimental diets (means $\pm$ SE)

Treatments ¹	Control	T ₁₀	T ₂₀	T ₃₀	T ₄₀
Villi Length μ m	298.61 $\pm$ 10.79 ^d	610.33 $\pm$ 12.67 ^a	446.80 $\pm$ 21.44 ^b	341.78 $\pm$ 6.13 ^c	346.80 $\pm$ 11.03 ^c
Villi Width μ m	71.73 $\pm$ 6.64 ^a	63.64 $\pm$ 10.80 ^{ab}	58.39 $\pm$ 5.08 ^{ab}	46.48 $\pm$ 5.23 ^b	69.09 $\pm$ 2.25 ^a
Inter Villi Space μ m	84.09 $\pm$ 6.81 ^a	32.71 $\pm$ 1.40 ^c	47.96 $\pm$ 4.11 ^c	63.89 $\pm$ 6.99 ^b	72.38 $\pm$ 3.17 ^{ab}
Goblet cells/mm ²	113.33 $\pm$ 4.41 ^d	282.67 $\pm$ 8.11 ^a	173.67 $\pm$ 5.93 ^b	136.33 $\pm$ 4.33 ^c	142.33 $\pm$ 2.85 ^c

Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.

¹ Control, T₁₀, T₂₀, T₃₀ and T₄₀ contained 0, 10, 20, 30 and 40% DDG instead of soybean meal, respectively.

Table 7. Cost of feeds required for producing one Kg gain of *O. niloticus* fed different experimental diets

Diets ¹	Control	T10	T20	T30	T40
Cost /kg diet (L.E) ²	28.95	28.59	28.23	27.87	27.51
Relative to control %	100.00	98.76	97.51	96.27	95.03
Consumed feed to produce 1kg fish ³	1.10	1.05	1.09	1.07	1.11
Feed cost per kg fresh fish (L.E) ⁴	31.85	30.02	30.77	29.82	30.54
Relative % of feed cost/ kg fish ⁵	100.00	94.27	96.63	93.64	95.89
Decrease in feed costs %	0.00	5.73	3.37	6.36	4.11

¹ Control, T10, T20, T30 and T40 contained 0, 10, 20, 30 and 40% DDG instead of soybean meal, respectively.

² Cost of 1 kg ingredients used were 60 L.E for fish meal, 20 L.E for SBM, 14 L.E for YC, 13 L.E for wheat bran, 38 L.E for Corn gluten, 10 L.E for DDG, 50 L.E for soy oil, and 10 L.E for Vit & Min, 500 L.E for Lysine and Methionine 12 L.E for De-calcium phosphate; 400 L.E for probiotic, and 200 L.E Vita.C. Egypt Feed Ingredients price at start of 2024.

³ Feed intake/final weight Kg/Kg (FCR).

⁴ Feed cost per kg fresh fish (L.E) = Cost /kg diet (L.E) X consumed feed to produce 1kg fish (kg).

⁵ Respective figures for step 3/ highest figure in this step.

Note: 1\$= 30.75 L.E (Egyptian pound) at start of 2024.

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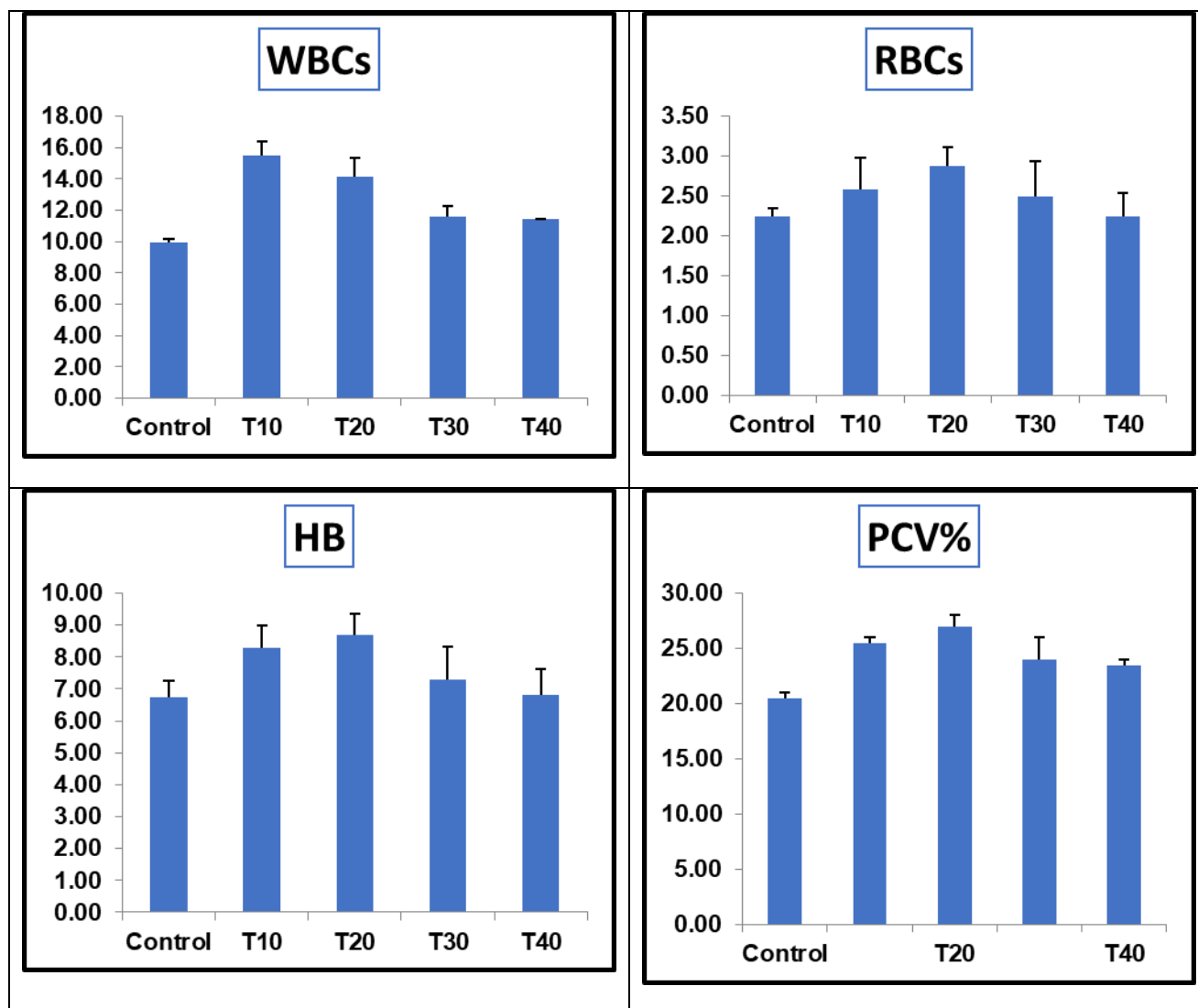


Fig. 1. Effect of partial replacement of soybean by various levels of distiller's dried grains (DDGS) on hematological parameters of the Nile Tilapia (*Oreochromis niloticus*).

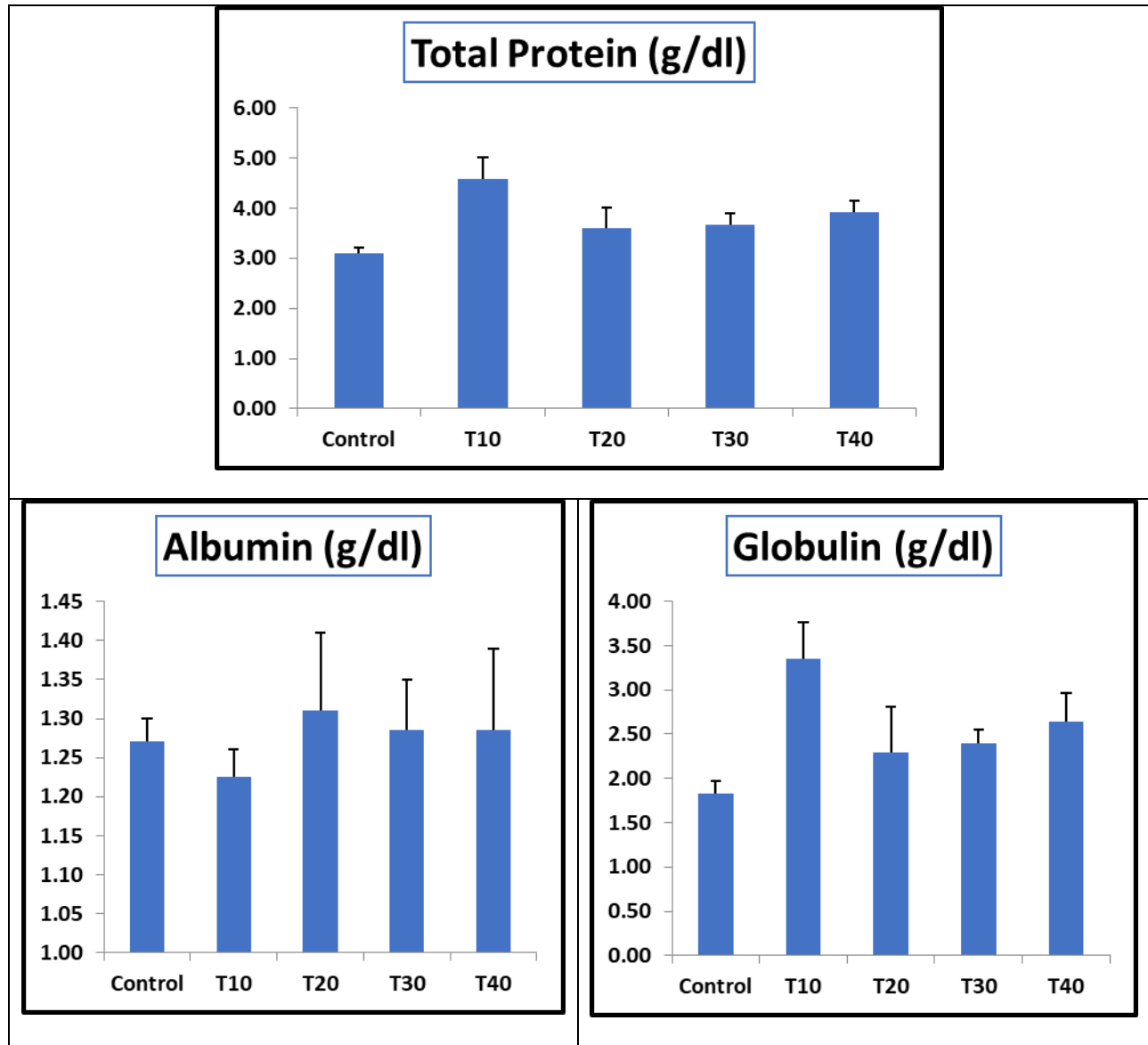


Fig. 2. Effect of partial replacement of soybean by various levels of distiller's dried grains (DDGS) on blood proteins of the Nile Tilapia (*Oreochromis niloticus*).

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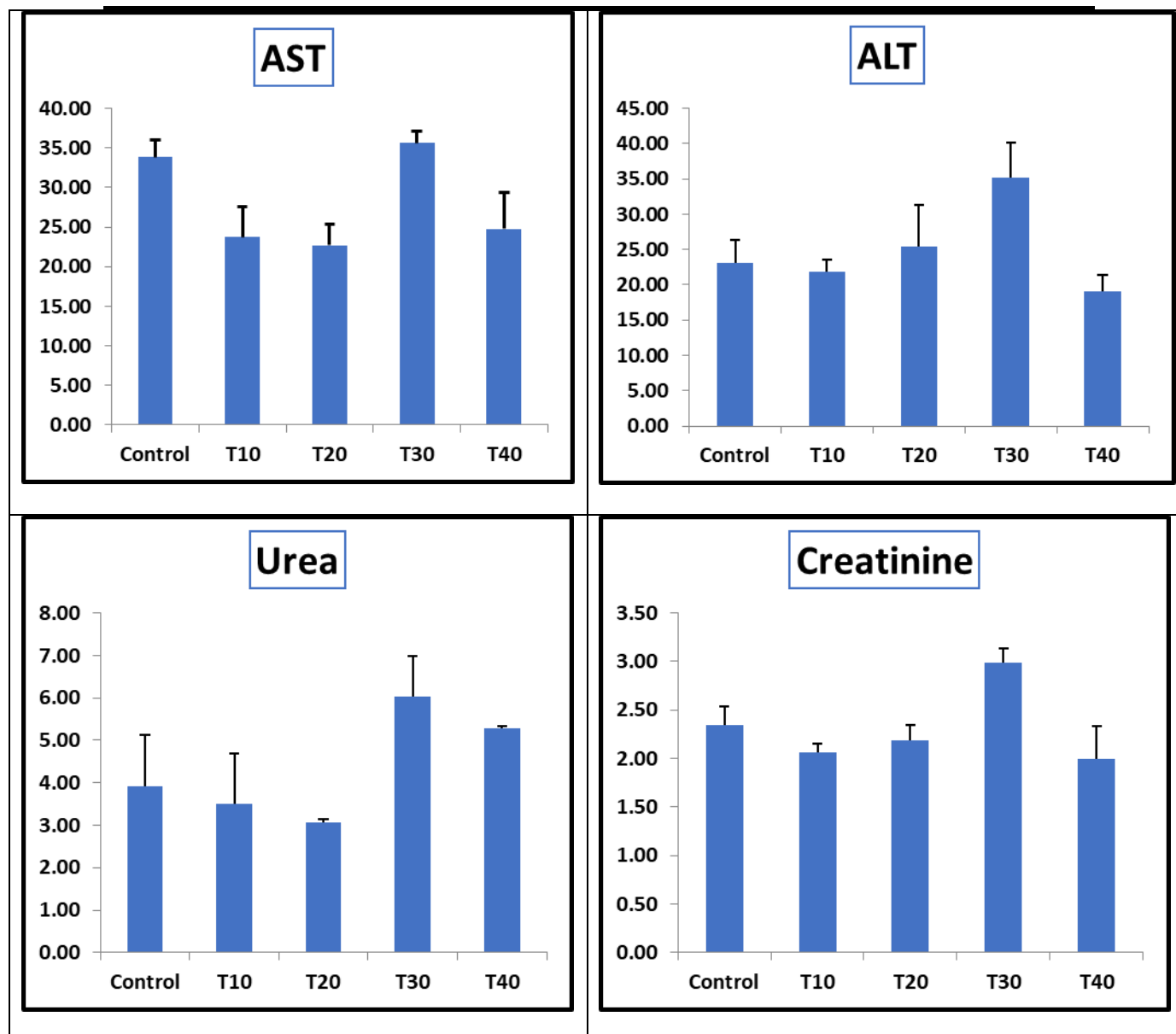


Fig. 3. Effect of partial replacement of soybean by various levels of distiller's dried grains (DDGS) on liver and kidney functions of the Nile Tilapia (*Oreochromis niloticus*).

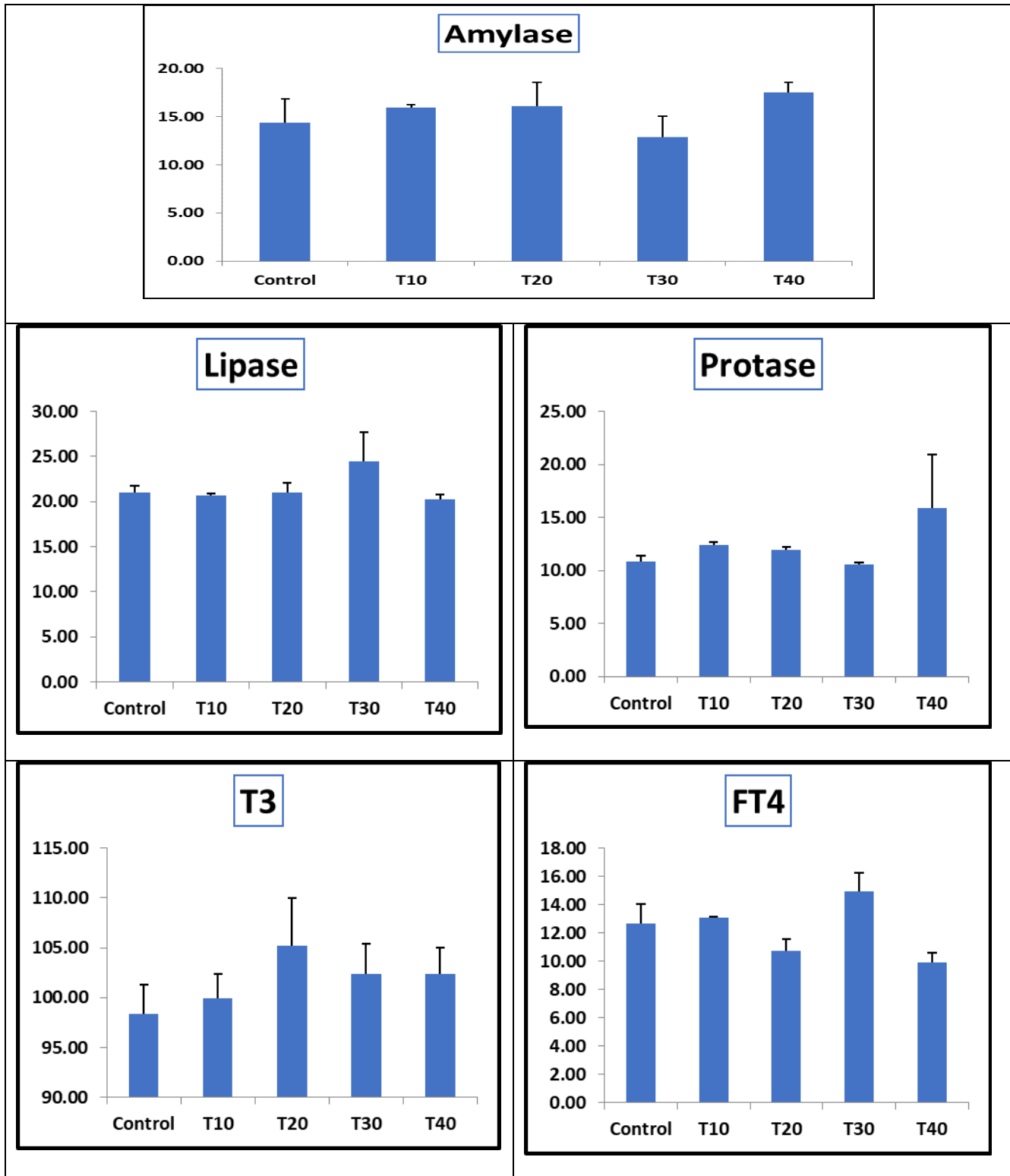


Fig. 4. Effect of partial replacement of soybean by various levels of distiller's dried grains (DDGS) on digestive enzymes and thyroid hormones of the Nile Tilapia (*Oreochromis niloticus*).

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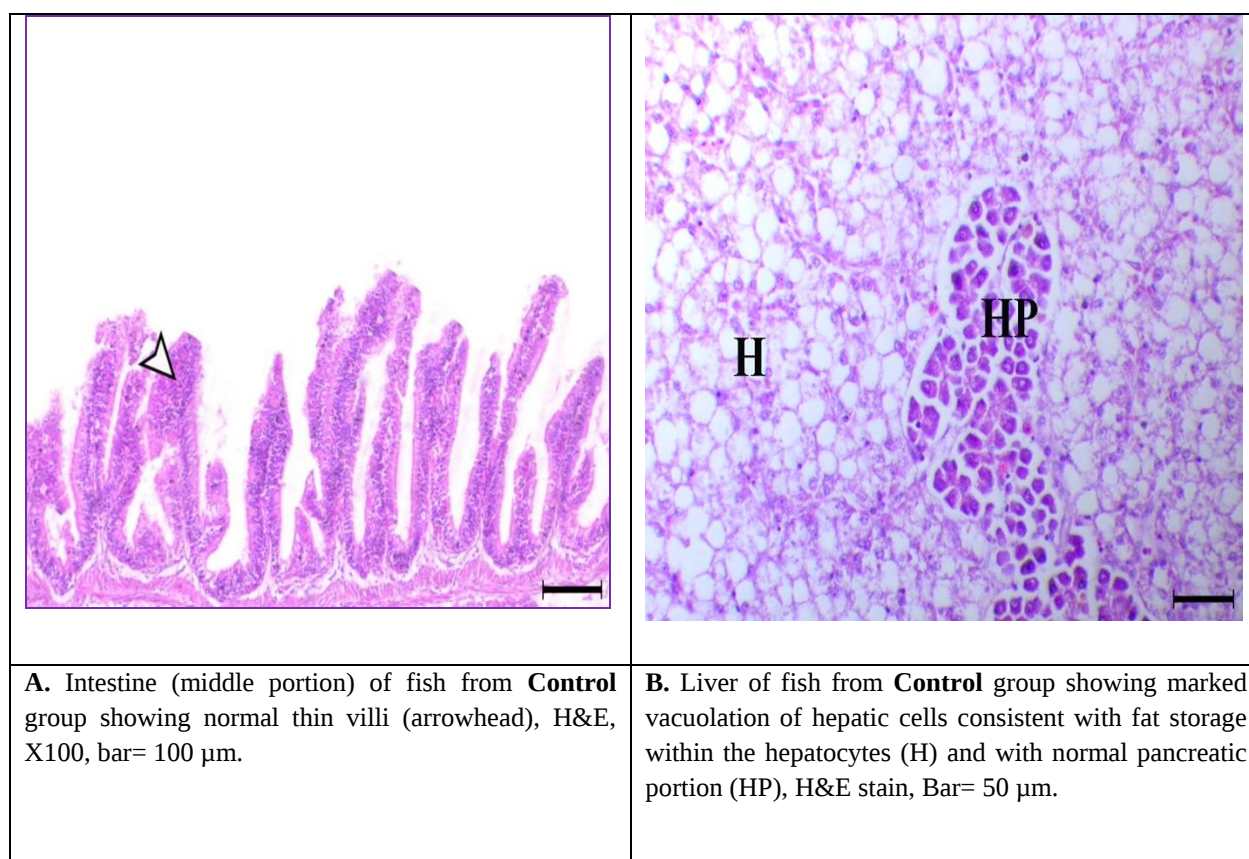


Fig. 5. **A.** Intestine and **B.** Liver of fish from control fed soy bean-based diet

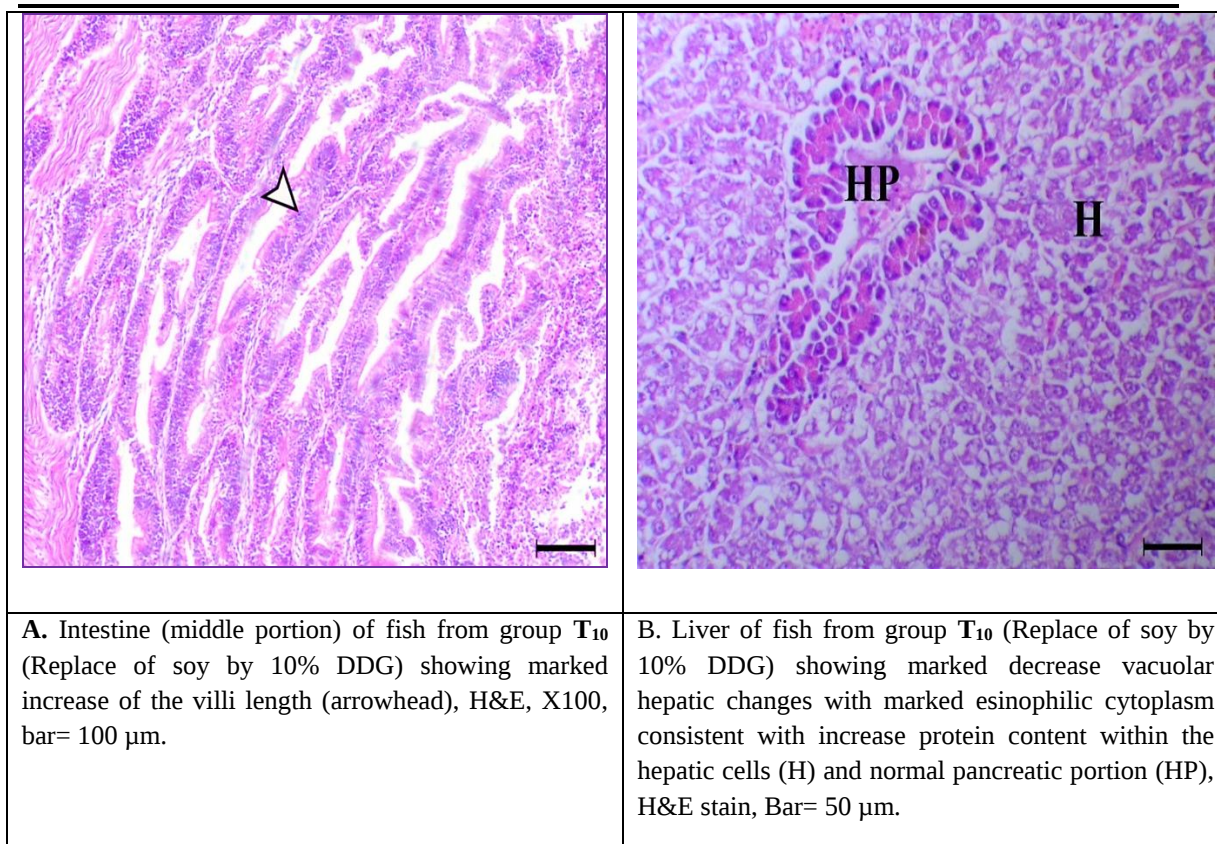


Fig. 6. A. Intestine and **B.** Liver of fish from group T₁₀ (Replace of soy by 10% DDGS)

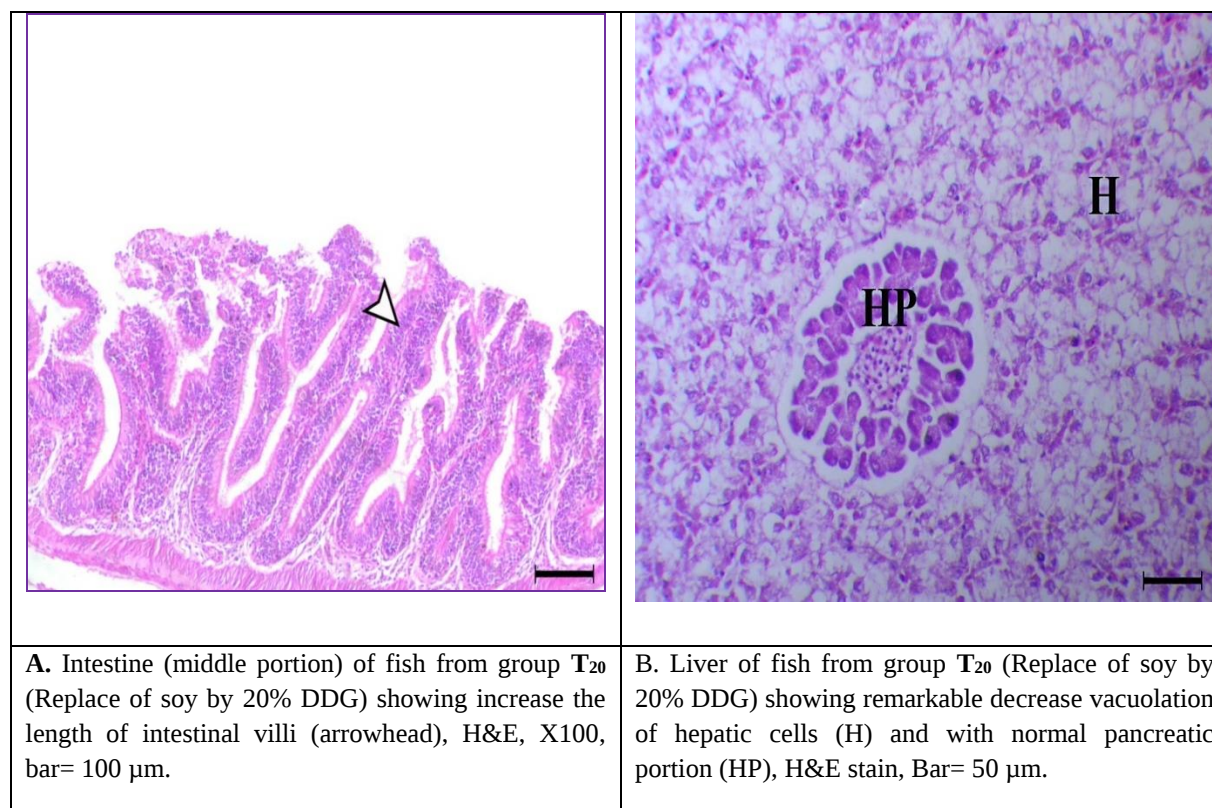


Fig. 7. A. Intestine and **B.** Liver of fish from group T₂₀ (Replace of soy by 20% DDGS)

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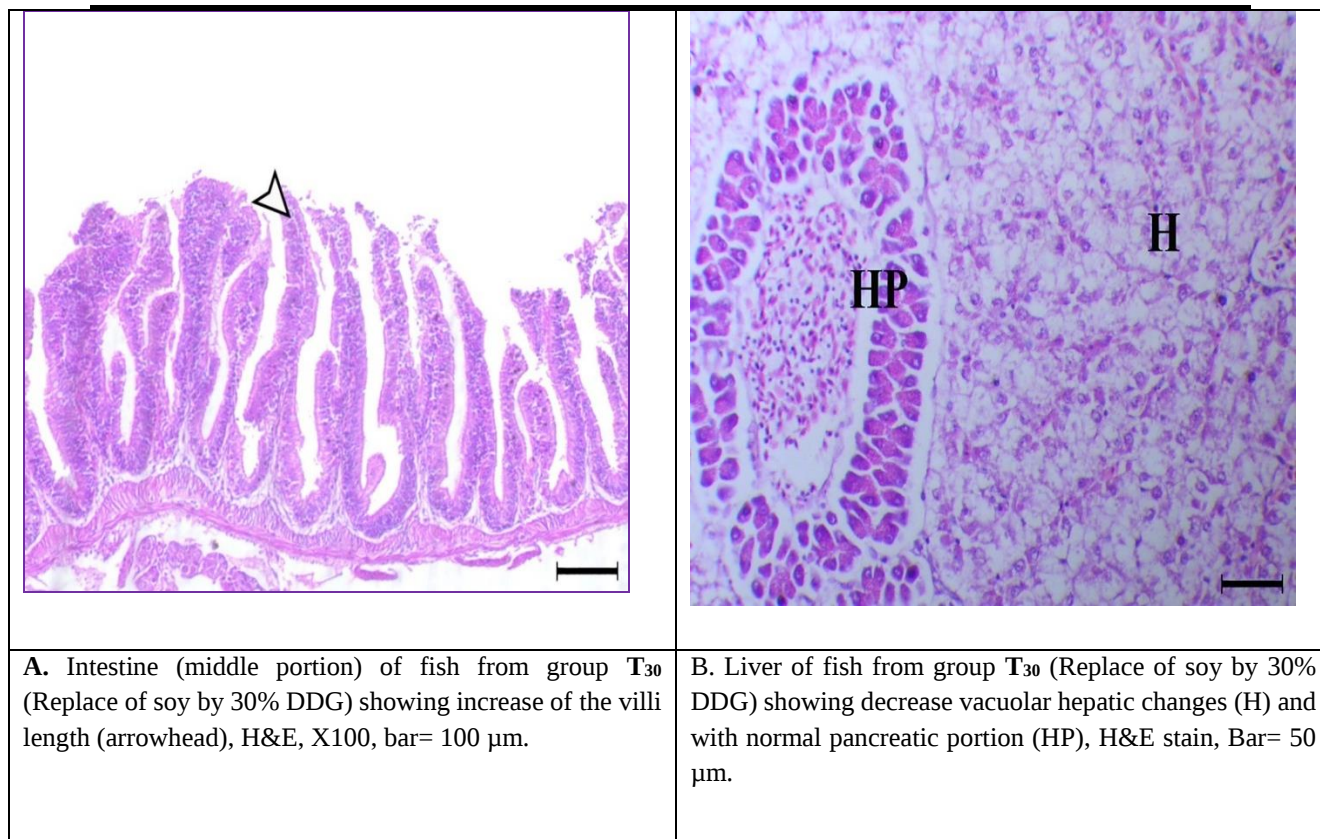


Fig. 8. **A.** Intestine and **B.** Liver of fish from group T₃₀ (Replace of soy by 30% DDGS)

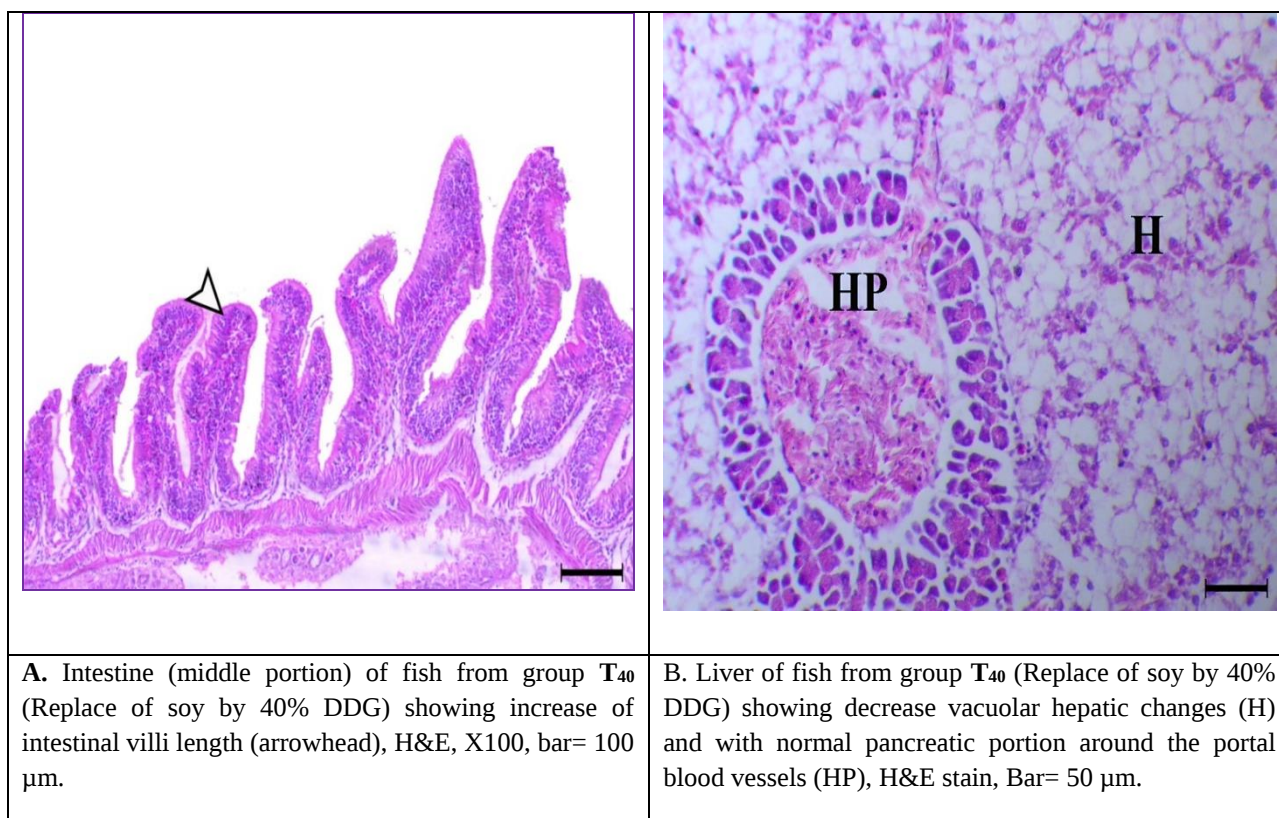


Fig. 9. A. Intestine and **B.** Liver of fish from group T₄₀ (Replace of soy by 40% DDGS)

الملخص العربي

تقييم الحبوب الجافة المقطرة كبديل جزئي لفول الصويا في علائق البلطي النيلي (*Oreochromis*

niloticus): أداء النمو، والمؤثرات الفسيولوجية، ومقياس النسيج المعوي

تشكل تكاليف الأعلاف أكثر من 60% من تكاليف إنتاج الأحياء المائية، مما يُعزز الحاجة إلى بدائل بروتينية اقتصادية. قُيِّمت هذه الدراسة الاستبدال الجزئي لكسب فول الصويا بالحبوب الجافة المقطرة (DDGS) على أداء النمو، والمعايير الفسيولوجية، ومقياس النسيج المعوي لأسماك البلطي النيلي (*Oreochromis niloticus*). على مدى 90 يوماً، تم تغذية الأسماك (0.5 ± 3.56 جم) على أحد الأنظمة الغذائية الخمسة المتساوية البروتين (25.7%) والمتساوية الدهون (7.9%): نظام غذائي كنترول أو أنظمة غذائية تعتمد على فول الصويا محل 10% و 20% و 30% و 40% من بروتينها بـ DDGS. أعطى النظام الغذائي البديل DDGS بنسبة 10% وزن الجسم النهائي الأمثل (19.87 ± 0.31 جم)، ومعدل النمو النوعي (1.92 ± 0.02 يوم⁻¹)، ونسبة تحويل العلف (1.05 ± 0.02) ونسبة كفاءة البروتين (3.72 ± 0.06) مقارنة بجميع المعاملات الأخرى ($P < 0.05$). وأظهرت هذه المجموعة أيضاً تحسناً في صحتها، وهو ما يشير إليه تحسن كبير في العلامات المناعية (ارتفاع خلايا

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الدم البيضاء والجلوبيولينات) وشكل الأمعاء المتفوق (الزغابات الأطول وكثافة الخلايا الكأسية الأعلى)، مما يسهل امتصاص العناصر الغذائية بشكل أفضل. وفي حين حافظت نسبة DDGS الأعلى (20-40%) على نمو مماثل للكنترول، فقد برز مستوى 30% باعتباره الأكثر كفاءة اقتصادياً، حيث قلل من تكلفة العلف لكل كيلو غرام من زيادة الأسماك بنسبة 6.36%. وفي الختام، يوصى باستبدال 10% من DDGS لتعزيز صحة ونمو أسماك البلطي، في حين يوفر الاستبدال بنسبة 30% أفضل عائد اقتصادي، مما يوفر استراتيجيات مرنة لتربية الأحياء المائية المستدامة والفعالة من حيث التكلفة.