# Evaluation of protein enriched co-products originating from wheat fermentation in diets of common carp Cyprinus carpio to examine effects on growth response, mineral retention, haematological status and intestinal integrity.

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# Evaluation of protein enriched co-products originating from wheat fermentation in diets of common carp *Cyprinus carpio* to examine effects on growth response, mineral retention, haematological status and intestinal integrity

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## Data availability statement (DAS)

The authors confirm that the data supporting the findings of this study are available within the article; also raw data were generated when the lead author conducted his PhD (Omar 2011). Derived data supporting the findings of this study are available from the corresponding author elharoun@gmail.com upon reasonable request.

## Ethical statement

All work with fish conformed to local ethical approval and conducted under the auspices of the codes of practice of the Institutional Animal Care Committee and licenses and UK law under the Animal Scientific Procedures Act of 1986.

## Abstract

Six experimental isonitrogenous (380 g/kg crude protein) and isolipidic (80 g/kg) diets were formulated for juvenile carp *Cyprinus carpio*. The fish meal protein component of a basal diet (control treatment) was effectively replaced by yeast protein concentrate unrefined ($YPC_U$), yeast protein concentrate refined ($YPC_R$), yeast protein concentrate potable alcohol ($YPC_{PA}$) at 300 g/kg of total dietary protein originating from a novel wheat fermentation process. Distillers dried grains with solubles (DDGS) at two levels (150 and 300 g/kg) of dietary protein were also tested separately. After an 8-week feeding trial, carp fed $YPC_U30$ recorded the highest ($P \leq 0.05$) growth performance and feed efficiency. The apparent net protein utilization of phosphorus, sodium, magnesium and chromium for all diets tested showed significant differences of retention efficiencies. The control fed carp had ($P<0.05$) significantly lower phosphorus and magnesium efficiency than the carp fed other diets. Histological examinations showed the density of microvilli in the region of fish fed YPCU30, YPCPA30, DDGS15, and DDGS30 was decreased compared with the control fed fish with no significant differences were found among the treatments ($P \geq 0.05$). Significant differences in the number of hepatocytes were observed between fish fed YPCR30 or YPCPA30 and DDGS30. Furthermore, no significant ($P \geq 0.05$) differences were obtained in the number of hepatocytes in the fish fed YPCU30 and DDGS15 diets with fish fed the control diet. Hepatic structure showed that the liver of the fish fed the control, YPCU and YPCR diets appeared to be healthy with no signs of pathological change. In conclusion, fermented wheat derived yeast protein concentrate and distillers dried grains with solubles are promising ingredients in carp diets to reduce feed costs and achieve aquaculture sustainability



**Keywords:** Common carp, yeast enriched co-product, Wheat fermentation, Distillers dried grains; Growth performance; Haematology; Liver and intestine histology. Hepatic enzyme activities


## Introduction

Feed formulation receives the most attention in the production of fish for intensive aquaculture, the key aspect being to provide a balanced diet that meets the full nutritional requirements based on reliable and quality ingredients. Fish meal (FM) has typically been the main source of dietary protein in the commercial production of fish as reported over the years (Edwards et al., 2004). This is due to its high protein content, excellent amino acid profile, as well as its high nutrient digestibility (Gatlin et al., 2007). However, being too reliant on any one available ingredient imposes increased risks associated with supply, price and quality fluctuations as reported by Glencross et al., 2007. In fact, aquafeed production costs are very high (over 50%) in some aquaculture practices. In order to decrease dietary costs, increase profitability and obtain good growth, expensive ingredients may be substituted with lower cost ingredients especially for fish at a lower trophic status. Animal protein, plant protein and single cell protein sources (SCP) are good candidates to use as alternative non-conventional protein sources in aquafeeds (Gatlin et al., 2007; Hassaan et al 2018; Goda et al 2019; 2020 a, b; Davies et al 2019; Anwar et al 2020; Hassaan et al 2020, Hassaan et al ., 2021; El-Nokrashy et al 2021). Soybean meal (SBM) has been a common source of plant protein used in the formulation of aquaculture feeds, as a substitute for FM as reported by many workers (Patnaik et al., 2005). Generally, SBM has been popular due to its cost efficiency, availability, and for its relatively high protein content and good balance of amino acids (Carter and Hauler 2000). However, the increasing cost of feedstuffs including SBM can place limitations on its overall use as a feed in the global expansion of aquaculture. This highlights the need to find alternative protein sources to meet an expansive global demand as described by the recent overview of the fish in: fish out concept by Kok et al. (2020) for many farmed fish species. The use of Distillers Dried Grains and solubles (DDGS) and yeast has been the focus of much interest in feed development for several species due to their growing availability from defined sources. As industrial waste streams from ethanol manufacturing, these products may provide a cost-effective potential source of protein in aquaculture feeds (Muzinic et al., 2004). DDGS is potentially a good source of alternative protein in aquaculture (Chevanan et al., 2009). The essential amino acids lysine

and methionine are however lower in DDGS than FM, and this may limit its inclusion in fish diets (Shelby et al., 2008). Distillers dried grains with solubles (DDGS) have been used as alternative protein sources in a variety of marine species including sea bass (Goda et al 2020, 2019a, b). The inclusion of DDGS in fish diets has already been recommended at varying substitution levels in a number of different species such as rainbow trout (Oncorhynchus mykiss), Channel catfish (Ictalurus punctatus), Nile tilapia (Oreochromis niloticus), sunshine bass (Morone chrysops x Morone saxatilis) and common carp Cyprinus carpio Linnaeus, 1758 (Robinson and Menghe 2008; Zerai et al., 2008; Shelby et al., 2008). Recently Davies et al. (2020) confirmed that a commercial US product of high protein DDGS was an effective ingredient in diets for farmed Atlantic salmon, *Salmo salar*. Normally protein in DDGS is contributed from around 50% yeast (Belyea et al., 2004). Recent novel technologies that separate the YPC (Yeast Protein Concentrate) from first generation (DDGS) products obtained from biofuel and potable alcohol production have been advocated. Wheat or corn is fermented in the bio-refinery to produce ethanol using live yeast strains. The residual wheat/corn protein (gluten) in the mass after distillation may undergo further processing to produce a valuable feed ingredient that can be used as a protein replacement in animal nutrition, particularly aquaculture. This can also be recombined with extracted yeast to vary the final protein content of various products. The novel separated yeast co-product fraction contains approximately above 340 g/kg protein, and is termed yeast protein concentrate (unrefined $YPC_U$). In order to produce high protein yeast based product, the $YPC_U$ is washed with water and the new dry yeast protein can increase to (550 g/kg protein). This product is termed yeast protein concentrate refined (washed) ($YPC_R$). The third type of yeast concentrate used in this study derived from whisky distillery using a novel way to refine the yeast from the remaining DDGS in alcohol production in Scotland. This is termed yeast protein concentrate potable alcohol ($YPC_{PA}$) with a protein level of 530 g/kg. These are described in detail by the review of Scholey et al. (2012). The DDGS that are used in this study are relatively rich in protein content (320 g/kg) as described by Omar (2011). The aim of the investigation was to evaluate the efficacy of various novel yeast protein

concentrates ($YPC_U$, $YPC_R$ and $YPCP_A$) and DDGS derived from bio-fuel production and other related industrial streams. Their nutritional value was evaluated in a series of experimental diets for juvenile common carp *Cyprinus carpio* during the course of a short-term feeding trial to assess their effects on the growth performance, body composition, mineral analysis, liver function, intestinal histology, haematology and selected metabolic enzyme activity of this important farmed species. Carp remains one of the most important freshwater farmed species globally and is omnivorous with an ability to utilise a variety of feed ingredients of plant origin.

## 2 Materials and methods

### 2.1 Diet preparation

Six isonitrogenous (380 g/kg) and isolipidic (80 g/kg) diets were formulated by partial replacement of FM protein with three different types of fermented wheat derived yeast protein concentrates (*S. cerevivisiae*) which was substituted at 458.02 g $kg^{-1}$ (300 g/kg) yeast protein concentrate unrefined ($YPC_U$), 214.42 g $kg^{-1}$ (300 g/kg) yeast protein concentrate refined ($YPC_R$) and 286.81 g $kg^{-1}$ (300 g/kg) yeast protein concentrate potable alcohol ($YPCP_A$). Distillers dried grains and solubles (DDGS) were also used to formulate two more diets with combination of ($YPC_R$) 100 g $kg^{-1}$ (10%) in each, 150 g $kg^{-1}$ (15%). (Details provided in Omar (2009) & Omar et al. (2012)

Dietary ingredients were mixed in a Hobart food mixer (Hobart Food Equipment, Australia, model no: HL1400 – 10STDA) with warm water until a soft slightly moist consistency was achieved. This was then cold press extruded (La Monferrina P6, La Monferrina, Asti, Italy) and air dried at 40C to produce a 2 mm pellet. Dietary chemical composition, ingredient sources, nutrient specifications and details of formulations of diets are is shown in Table 1.

### 2.2 Fish holding facility

Common carp (*Cyprinus carpio*) were provided by Bowlake fish farm, Hampshire, UK. Carp fry were transported to the Aquaculture Aquarium Facility. After 4 weeks acclimation and on-growing, 25 fish (15.21 ±0.07 g) were randomly distributed into 80 L fibreglass tanks, each provided with 99% re-circulated aerated freshwater at a rate of 300 L $h^{-1}$ (see section 2.4).

Each treatment was conducted in triplicate groups. Fish were fed the experimental diets at 4% tank biomass per day (equal rations at 09.00, 13.00 and 17.00 h) for 8 weeks. Daily feed was corrected on a weekly basis following batch weighing after a 24 h starvation period. A 12 h light/12 h dark photoperiod was maintained throughout the trial duration.

### 2.3 Water quality

The water quality variables was measured as follows; water temperature, 24.42 ± 0.93 $^{o}$C; dissolved oxygen, 7.07 ± 0.40 mg $L^{-1}$; total ammonia nitrogen (TAN), 0.06 ± 0.03 mg $L^{-1}$; nitrite, 0.07 ± 0.05 mg $L^{-1}$; nitrate, 32.96 ± 19.78 mg $L^{-1}$; and pH, 7.01 ± 0.30 (adjusted with $NaHCO_3$ as necessary).

### 2.4 Growth parameters and chemical analysis

Specific growth rate (SGR), Final weight (FW), weight gain (WG), survival rate, feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), apparent net protein utilisation (ANPU) and condition factor (K) were assessed.

Apparent net mineral utilisation was calculated by the following equation;

Apparent net mineral utilisation (ANMU %) = FBW= final body weight *[Min Conc.] -IBW= Initial body weight *[Min Conc.]/ Feed Intake *[Min Conc.] * 100

### 2.5 Chemical composition analysis of the diets and fish carcasses

Diets and fish samples (initial and final) from the feeding trial were analysed according to AOAC (2002) standard methods for proximate composition. Fish sampled for whole body analysis (18 fish sampled at the start and 4 fish per tank at the end of trial) were ground and homogenized in a blender prior to chemical assays. Two samples per tank (2 fish per sample pooled together) were analysed to minimize the standard deviation between the samples. Amino acids analysed (except tryptophan) carried out by Sciantec Analytical Services Ltd. Laboratories (Yorkshire; UK) and shown in Table 2. Mineral compositions were conducted using spectrophotometer (ICP-MS) on a dry basis are presented in Table 3 after digestion in Analar Grade Nitric acid (6N).

### 2.6 Histology

### 2.6.1 Light microscopy

Histological appraisal of the liver from 6 fish per experimental group was conducted at the end of the trial using light microscopy as described previously by Omar et al (2012) and Bowyer et al (2019).

A photograph of sections of each fish liver at 40X magnification was taken with an Olympus E-620 digital camera mounted on a Vanox Olympus research microscope model AHBT.

Using the software Image J 1.43, numbers of nuclei were then counted in a standardized area per photograph. This was done by firstly calibrating exact sizes of photographs; then using a standardized 15x15 μm square located at the same coordinates in each image, all nuclei present inside the square, but not touching the square perimeter, were marked and a total number was calculated by the software.

### 2.6.2 Electron Microscopy

Samples for SEM were taken in the anterior and posterior region of gut from six separate fish per treatment and processed according to Bowyer et al. (2019). Microvilli densities were assessed as described by Omar (2011) and Bowyer et al. (2019). Samples from posterior region of the gut were observed from six fish per treatment for TEM and microvilli density was measured.

### 2.7 Haematological parameters

At the end of the trial, fish were sacrificed and blood collected from 10 fish per treatment. Hematocrit determination was assayed using heparinized capillary tubes. Total blood haemoglobin concentration was determined using Drabkins spectrophotometric method. Erythrocyte counts were performed by diluting 20 μL of fresh blood with 1 mL of Dacies solution, and counts were performed with a Neubauer haemocytometer (Dacie and Lewis, 2001). A glass pipette were used and to ensure that the blood cells were re-suspended, a small quantity of the blood cell suspension were introduced on the platform of the haemocytometer at the edge of the cover slip to be drawn into counting area by capillary action. Then, total erythrocyte evaluations are carried out in the five small squares in the centre of the grid under a light

microscope. The volume counted per square = 0.2 × 0.2 × 0.1= 0.004 $mm^{-3}$. Blood smears were prepared by adding a drop of blood onto a slide and allowed to air dry. Smears were fixed in 95% methanol and slides were stained using 6% Giemsa (BDH) for 20 min and washed twice for one min in distilled water. Slides were air – dried and mounted with cover slips using DPX (BDH). Images were taken with a DCMI30 digital camera (Brunel microscopes Ltd, Wiltshire, UK) using scopPhoto (ScopeTeck®, China) and a Medilux-12 light microscope (Kyowa).

Total leukocyte counts (neutrophil, monocyte, thrombocyte and lymphocyte) were performed with a digital imaging system scored blindly as total number of leukocytes per 1000 blood cells as described by Merrifield, et al. (2010)

**2.8 Haematological indices**

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from RBC, Hct, and Hb according to the following formulae: MCV = (PCV x 1000)/RBC, MCH = Hb/RBC and MCHC = (Hb × 10)/Hct (Lee et al., 1998; Al-Dohail et al., 2011).

**2.9 Hepatic enzyme assays**

At the end of the experiment six fish per treatment were euthanized and livers were frozen. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were assayed using the micro plate reader (Molecular Devices) (Omar et al. 2012). The total protein content protein of the supernatant was determined. These techniques were described according to a modified Bradford (1976) assay as described previously by Omar (2012) for expression of Specific Enzyme Activity Units.

**2.10 Statistics**

Statistical analysis (i.e. growth parameters, body composition, enzyme, histology) was carried out using One-Way ANOVA (SPSS 17.0) with a fibreglass tank of fish being the

experimental unit (n = 3). *Post hoc* LSD test was used to determine significant differences between means Percentage data was arcsine transformed prior to subsequent analysis and significance was accepted at the $P < 0.05$ level.

## 3 Results

### 3.1 Growth parameters and feed efficiency

Growth performance and feed utilization data of common carp fed the six experimental diets are presented in Table 4. During the growth trial, all the fish readily accepted the experimental diets. The survival rate recorded during the experimental period was 100% for all experimental diets except DDGS30 which was 98%. There were significant differences between the final weights of the fish fed YPC$_U$30 inclusion diet compared to fish fed control, YPC$_R$30, DDGS15 and DDGS30 inclusion diets ($P \leq 0.05$). Fish fed YPCP$_A$30 inclusion diet exhibited significantly lower final weight compared to all other fish groups ($P \leq 0.05$) (Table 4). Furthermore, the weight gain of fish fed YPC$_U$30 was significantly ($P \leq 0.05$) higher than fish fed the control, YPC$_R$30, DDGS15 and DDGS30 diets. Fish fed with YPCP$_A$30 inclusion experienced significantly lower weight gain compared to control diet and replacement level diets (Table 4). The highest specific growth rate (SGR) was observed in the group YPC$_U$30 (2.45) which had higher value than any other group, followed by groups DDGS15 (2.32), control (2.31), DDGS30 (2.28) and YPC$_R$30 (2.28). The lowest feed conversion efficiency was obtained in the group YPC$_{PA}$30 (2.13±0.001) which was significantly lower than all other groups ($P \leq 0.001$). Although trends towards elevated FCE were observed in all fish fed inclusion diets, significant elevations were observed in fish fed YPC$_U$30. While fish fed YPC$_{PA}$30 FCE was significantly ($P \leq 0.05$) decreased among the treatments (Table 4). Compared to the control group, the FCR was significantly improved with YPC$_U$30 inclusion; however, YPC$_{PA}$30 inclusion obtained worst (i.e. highest) FCR within the experimental group ($P \leq 0.001$). PER for YPCU30 fed carp was improved significantly from those carp fed YPCPA30 and DDGS30 ($P < 0.05$). On the other hand, protein efficiency ratio (PER) of fish fed diets control, YPCR30 and DDGS15 did not differ significantly from YPCU30 fed fish ($P \leq 0.05$). Apparent net protein utilisation

(ANPU) was statistically highest for the YPCU30 and lowest for YPCPA30 inclusion diet. In addition, ANPU of the fish fed DDGS15, DDGS30 and Control differ significantly from the ANPU of fish fed YPCU30 and YPCPA30 ($P \leq 0.05$). Furthermore, ANPU of fish fed YPCR30 was significantly lower from ANPU of fish fed YPCU30 DDGS15, DDGS30 and control but is not differ significantly from the ANPU of fish fed YPCPA30 ($P \geq 0.05$).The condition factor (K) of fish fed YPCR30 and YPCU30 are not statistically different from fish fed YPCPA30, DDGS15, DDGS30 and Control diets ($P \geq 0.05$) but they are significantly different from each other ($P=0.039$).

Body composition data of carp fed various types of dietary YPC and DDGS are summarized in Table 5 Moisture content was increased significantly ($P \leq 0.05$) only with the YPCU30 and YPCPA30 dietary inclusion compared to the control diet. Significant differences were apparent for whole-body protein which tended to increase significantly ($P \leq 0.05$) in fish fed YPCU30 dietary inclusion compared to the fish fed fish meal diet. Fish fed other experimental diets did not display any differences in body protein content. A similar tendency was found for PER which was found to be maximum at YPCU30 inclusion in the diet. Compared to the control, lipid contents decreased significantly ($P \leq 0.05$) in fish fed on experimental diets, except for fish fed YPCR30 which was not significantly different ($P \geq 0.05$).Whole-body ash was significantly higher in fish fed YPCU30 (11.83±0.373), YPCPA30 (11.10±0.492) and DDGS30 (10.68±0.004) compared with fish fed control diet (9.50±0.439) ($P=0.005$). However, there was no significant differences in the fish fed YPCR (9.44±0.576) and DDGS15 (10.00±0.025) compared with fish fed the control diet (9.50±0.439) ($P \geq 0.05$). Body gross energy level was comparable in fish fed all diets. No significant ($P \geq 0.05$) differences were apparent with regards to nitrogen free extracts (NFE) contents.

Carcass mineral levels can be viewed in Table 6. A clear trend of elevated carcass calcium levels was observed with dietary inclusions of various types of YPC leading to significant increases ($P \leq 0.05$) with $YPC_U30$ and $YPC_{PA}30$. Similar results were observed with respect to phosphorous whereby significantly higher levels were observed in all YPC fed groups,

except the DDGS15 fed group. Additionally, higher levels of carcass sodium and magnesium were observed in fish fed $YPC_U30$ and $YPC_{PA}30$ diets. Potassium levels were significantly ($P<0.05$) higher only in fish fed $YPC_U30$, and iron levels were significantly ($P\leq0.05$) lower only in fish fed $YPC_{PA}30$. Copper levels were significantly ($P\leq0.05$) higher in carp fed $YPC_{PA}30$ than fish fed other dietary groups. Zinc levels were significantly ($P\leq0.05$) lower in fish fed $YPC_U30$, DDGS15 and DDGS30 diets and no significant differences were apparent in fish fed DDGS30 and $YPC_{PA}30$. Chromium and manganese levels were not affected by dietary inclusion of $YPC_U$, $YPC_R$, $YPC_{PA}$ and DDGS. The Apparent Net Mineral Utilisation (ANMU) is presented in Table 7. The Net apparent mineral utilisation ANMU of phosphorus, sodium, magnesium and chromium for all diets tested showed significant differences of retention efficiencies between treatments. The control fed carp had ($P\leq0.05$) significantly lower phosphorus and magnesium retention efficiency than the carp fed other diets.

Also the retention of sodium and chromium in fish fed various types of YPC and DDGS were significantly higher than fish fed control diet except $YPC_{PA}30$ dietary inclusion which was significantly ($P\leq0.05$) lower than carp fed the control diet. The highest value for ANMU of copper was obtained in fish fed $YPC_U30$ inclusion and $YPC_{PA}30$ fed fish lowest, significant differences were found between treatments ($P\leq0.05$).

$YPC_{PA}30$ had a significantly higher potassium value of ANMU in comparison to other dietary treatments ($P\leq0.05$). Fluctuating values for ANMU of iron were observed in fish fed all dietary treatments compared to the iron value in fish fed the control diet, which were significantly different ($P\leq0.05$). Control fed fish had significantly higher zinc ANMU ($P\leq0.001$) compared to all other dietary groups.

The results of the histological examinations of the microvilli density and microvilli lengths are presented in Table 8. The relative density of microvilli in the posterior region of fish fed $YPC_U30$ (4.35±0.82 AU), $YPC_{PA}30$ (4.13±0.53 AU), DDGS15 (3.62±0.95 AU), DDGS30 (3.45±0.66 AU) decreased compared with the control fed fish (4.34±0.42 AU) but with no significant differences found among the treatments ($P\geq0.05$), However, there was a significant

difference in the fish fed $YPC_R30$ (2.44±1.15 AU) compared to the control fish (4. 34±0.42 AU) (Figure 2, Table 8). There were no significant (*P≥0.05*) differences in the anterior region (Figure 3, table 8).

For liver morphology, the number of hepatocytes (field of view 225 $\mu m^2$) in the liver of fish fed YPCPA30 were not significantly different to those fish fed the control diet (*P≥0.05*). Significant differences in the number of hepatocytes were observed between fish fed with $YPC_R30$ or $YPC_{PA}30$ and DDGS30, which they had a fewer number of hepatocyte compared to the fish fed with the control diet (*P≤0.05*). Furthermore, no significant (*P<0.05*) differences were obtained in the number of hepatocytes in the fish fed $YPC_U30$ and DDGS15 diets with fish fed the control diet.

Hepatic structure showed that the liver of the fish fed the control, $YPC_U$ and $YPC_R$ diets appeared to be healthy with no signs of pathological change. The liver from fish fed the $YPC_{PA}$ appeared to be abnormal as vacuolisation and relatively disorganised hepatocyte structures were observed. Vacuolization was also noticed, but not as prominently as carp fed the DDGS15 and DDGS30 diets (Figure 1; Table 8). Microvilli length from the posterior region increased from 0.98±0.32 μm in carp fed control diet to 1.45±0.13 μm in carp fed $YPC_U30$ diet.

Although, the microvilli length in the posterior region of carp fed $YPCR30$ (1.19.± 0.03 μm) and DDGS30 (1.19±0.28 μm) was longer than microvilli length of carp fed control diet (0.98±0.32) but no significant (*P≥0.05*) differences were observed (Figure 4, Table 8). The microvilli length of fish fed $YPC_{PA}30$ diet (0.94±0.38 μm) was comparable with carp fed control diet (0.98±0.32) (*P≤0.05*).

Haematological measurements for the different groups of fish are shown in Table 9. No significant (*P≥0.05*) differences were observed in the haematocrit (Hct), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC) or mean corpuscular haemoglobin (MCH) from fish fed the different experimental diets. The erythrocyte levels (RBCC) of fish fed DDGS30 and $YPC_U30$ diets were significantly higher than of fish fed the $YPC_{PA}30$ diet (*P*≤0.05). There were no statistically significant differences in RBCC of fish fed control,

DDGS15 and $YPC_R30$ inclusion diets compared to the other groups ($P \geq 0.05$) (Table 8). The total number of leucocytes (WBC) was significantly higher ($P \leq 0.001$) in fish fed the $YPC_U30$ diet (88.25 ± 7.30 per 1000 blood cells) than in the fish fed the DDGS15 (61.38± 13.29) and YPCPA (61.38±11.27) diets; fish fed control and $YPC_R30$ (79.00± 9.20) diets were not different to fish fed the $YPC_U30$ (88.25±7.30) diet. While, fish fed DDGS30 (68.50± 8.99) were significantly lower than those fish fed $YPC_U30$ (88.25±7.30) and control (81.00) ± 9.58) diets ($P < 0.05$). The mean corpuscular volume (MCV) of fish fed the control and $YPC_{PA}30$ diets were significantly ($P \leq 0.05$) higher than fish fed $_{YPCU30}$ diet ($P$=0.012). Furthermore, there were no significant ($P \geq 0.05$) differences between fish fed $YPC_R30$, DDGS15 and DDGS30 compared to the fish fed the control and $YPC_{PA}30$ or YPCU30 diets.

Hepatic ALAT activities of fish fed experimental diets (various types of YPC and DDGS inclusion) revealed no significant differences between dietary treatments ($P \geq 0.05$) (Table 9). ASAT activities decreased with dietary inclusion of various types of YPC and DDGS and compared to the control and were statistically ($P \leq 0.05$) lower in all inclusion dietary treatments ($YPC_U30$, $YPC_R30$, $YPC_{PA}30$, DDGS15 and DDGS30) (Table 9).

## 4 Discussion

This study showed that the growth performance and feed utilization of fingerling common carp could be maintained or improved with bioethanol and whisky derived co-products such as yeast enriched protein concentrates and distillers dried grains from the industrial wheat fermentation process. This is consistent with several findings in fish such as Nile tilapia (Lim et al., 2001; Shelby et al., 2008). Previous research considers that high levels of yeast may invoke negative effects as described by Attack and Matty (1979) who found depressed feed intake in rainbow trout fed a 40 g/kg brewer's yeast diet compared to a control diet. In contrast, Rumsey et al. (1992) observed that there was no negative effect on feed intake when rainbow trout were fed a diet where 500 g/kg of FM was replaced with brewer's yeast. Kukačka and Mareš (2008) showed that the substitution with 15 g/kg corn DDGS generated better growth than 30 g/kg corn DDGS in the diets of common carp but no adverse effects were found in the fish fed 30% DDGS compared to the control fish fed diet. Nile tilapia has shown no reduction in growth and feed

393 utilization effects when fed up to a 500 g/kg substitution of FM with brewers waste (Zerai et al.,
394 2008). However, Nile tilapia fed biofuel derived DDGS (more similar to that used in this study
395 produced significantly lower weight gain than those fed FM, although a 175 g/kg DDGS diet
396 gave similar feed conversion ratio and protein efficiency ratio to fish fed FM diet (Schaeffer et
397 al., 2010). More recently, Davies et al. (2020) reported the performance of Atlantic salmon,
398 *Salmo salar* fed NexPro® protein: A novel next-generation protein ingredient derived from dry-
399 mill bio-ethanol. They found that growth was not impaired at around 30% inclusion of this
400 fermented corn protein complex with no reduction in FCR or protein retention efficiency.
401 With regard to the amino acid profile diets fed to carp, it was noticed that methionine
402 content was appreciably lower for the DDGS30 inclusion (0.68) falling below NRC (2011) carp
403 requirements of 0.8. DDGS contains more fibre and has potentially an inferior digestibility for
404 protein that was not tested in the study. Threonine was also lower than requirement for carp
405 especially at DDGS15 and DDGS30 inclusion thus becoming second limiting essential amino
406 acid factor. Lysine was only limiting for the $YPC_U30$ diet whilst all other treatments mostly
407 exceeded essential amino acid requirements for carp.
408 The results from this trial supported a previous study undertaken by Oliva-Teles and
409 Gonçalves (2001), who indicated that protein retention efficiency (ANPU) of juvenile sea bass
410 (*Dicentrarchus labrax*) fed diets containing yeast was superior to bass fed a fish meal based diet.
411 On the contrary, in rainbow trout the inclusion of mixed single cell protein (including yeasts) for
412 casein in semi-purified diets, though not affecting growth negatively did affect apparent net
413 protein utilisation ANPU (Murray and Marchant 1986). However, as in the current study, these
414 workers recorded that the ANPU increased in trout with the increasing the levels of yeast extract
415 (Rumsey et al., 1992). Rumsey et al., (1991) stated that some of the non-protein nitrogen (NPN)
416 may be used as a source of N non-essential amino acids. This may support the good performance
417 in terms of protein assimilation for tilapia if this species can reflect the excellent profile protein
418 and amino acid profile of the formulated diets for Nile tilapia. Such a concept is not known for
419 carp but may be likely due its long intestine and potential for hind gut microbial fermentation.

Results for whole body composition are in general agreement with the findings in tilapia galilee reported by Abdel-Tawwab et al. (2010). In contrast, the lipid content of the carcass in carp was lower in fish fed the experimental diets in comparison to the control diet, except YPCR30. An interesting result was the statistically greater ash content of carp fed the YPCU30, YPCPA30 and DDGS30 compared to those fish fed with control, YPCR30 and DDGS15 diets similar to the findings reported by Abdel-Tawwab et al. (2010).

More detailed research has been conducted to investigate the mineral requirements of cultured fish of a number of species (Papatryphon et al., 1999; Roy and Lall 2006). It is well known that fish may derive minerals either from diet or the surrounding water. The characteristic concentration and functional forms of minerals and trace elements need to be maintained within narrow ranges for essential metabolic activities in cells and tissues and organs. In the present investigation, it appears that increased inclusion of different YPC sources and DDGS can elevate the tissue retention levels of specific minerals due to the increased dietary contribution of phosphorus, magnesium, potassium, sodium, chromium, copper, iron, manganese and zinc from this source and reduction in the overall ash content as fish meal is reduced. This is particularly evident for the macro-elements phosphorus, sodium and magnesium and for the trace element chromium (Roy and Lall 2006). Ahmed et al. (2012) were reported the benefits of chromium enriched yeast for mirror carp with increased whole body retention and modulating effects on carbohydrate metabolism. An improved retention of phosphorus has environmental consequences and may lead to the reduced need to supplement diets with inorganic phosphorus sources (Nwanna et al., 2010).

Biochemical markers relating to metabolism are useful indicators of general health and function in the animals. ALAT and ASAT have an important role in amino acid synthesis in higher vertebrates including fish due to their roles in deamination and transamination. Goda et al 2019a and Goda et al. 2019b provided such metabolic data indicative of the health of seabass fed High Protein Distillers Grains (HP-DDG) under experimental conditions with similar findings to this study with but some improvement of blood indices. Excess deposition of energy as

447 glycogen or lipid can enhance the activities of several key enzymes involved in glycolysis,
448 lipogenesis as well as protein and amino acid synthesis and degradation. Also ALAT and ASAT
449 has been a useful indicator of tissue injury or hepatotoxicity in human and animals and as bio-
450 marker of adaptive reactions (Samsonva et al. 2003).

451 Gaye-Siessegger et al. (2007) found that three purified diets differing only in their non-
452 essential amino acid composition had observed the higher effect on the ASAT activity but the
453 ALAT activity remained unaffected of Nile tilapia. Sugita et al. (2001) tested the response of
454 enzyme activities linked with metabolic regulation in hepatopancreas and muscle of carp. In this
455 study, ALAT activity was unaffected by the substitution of fish meal in the diets with various
456 types of YPC and DDGS. In contrast however, ASAT activity was significantly decreased in
457 carp fed diets with YPC and DDG at all levels (YPCU30%, YPCR30%, YPCPA300 g/kg,
458 DDGS150 g/kg and DDGS300 g/kg of inclusion, respectively) compared to carp fed the control
459 diet. In contrast to this finding, Abdel Tawwab et al., (2010) found that a diet containing live
460 baker's yeast did not affect either ALAT or ASAT of Galilee tilapia *Sarotherodon galilaeus* (L.).
461 Also Carver and Walker (1995) and Sato et al. (1995) recorded that different dietary yeasts had
462 no significant effects on ALAT and ASAT activities in the human and rat.

463 The findings in the current trial indicate that dietary yeast inclusion may influence the
464 liver metabolism of carp fed dietary yeast compared to fish fed a fish meal based control diet but
465 not adversely. Reduction in ASAT activity could have been associated with a decreased need for
466 pyrimidine biosynthesis which involves aspartate as a substrate. Various types of YPC and
467 DDGE are rich sources of purines and pyrimidine bases within nucleic acids and may have
468 reduced demand within the hepatocytes of carp fed different types of YPC and DDGE thus
469 sparing the need for *de novo* synthesis.

470 Haematological analysis often provides valuable information for health assessment and
471 subsequent management of cultured fish (Hoseinifar et al., 2011). In the present study
472 haemotocrit, haemoglobin, MCH and MCHC levels were all not affected by any of the
473 experimental diets. These results are in agreement Welker et al. (2007) who observed no

474 negative effects on the haematological parameters Haemotocrit (Htc) and Haemoglobin (Hb)
475 when Channel catfish (*Ictalurus punctatus)* fed on 0.2% dietary whole cell brewer‟s yeast **(***S.*
476 *cerevisiae*) compared to control fed fish, total leukocyte counts were significantly reduced in fish
477 fed DDGS (150-300 g/kg) dietary inclusion, and in the fish fed YPCR30. However carp
478 receiving YPCPA30 remained unaffected, whilst fish fed YPCU30 had elevated levels. Reque et
479 al. (2010) observed that the total leukocyte count for Nile tilapia was not affected by 2% dietary
480 yeast inclusion. The levels of red blood cells counts (RBCC) in the fish fed YPCU30 and
481 DDGS30 were significantly elevated compared to those fish fed FM based diet. This result is
482 consistent with the findings of Abdel-Tawwab et al. (2008) who reported that the
483 supplementation of the diets with 0.1–0.5 % commercial baker's yeast (*S. cerevisiae*)
484 significantly increased RBCC in Nile tilapia. The results from the current study may indicate a
485 general improvement of fish health when fed an YPC and/or DDGS dietary inclusion over a
486 period of growth and development in young fish.

487 Morphological examination of the liver and gastro intestinal tract gastrointestinal tract
488 was also undertaken in this trial. Histological analysis of the liver showed a significant
489 difference in the number of nuclei between carp fed YPCR30 and DDGS30 with those fed fish
490 meal only**.** In this study it is uncertain why nuclei numbers were affected. Evidence indicated
491 that DDGS30 samples had disruptions in hepatic cell layers which may have affected hepatocyte
492 number. However this was not found in the YPCR30 fish although these did have similar overall
493 nuclei counts. The observed reduction in the hepatocyte number in fish fed YPCR may possibly
494 be due to removal of the valuable nutritional components during the washing process. This result
495 is supported by a study conducted by Rumsey et al. (1990) that observed lower growth
496 performance and nutritional status in lake trout (*Salvelinus namaycush*) fed washed brewer's
497 yeast compared to fish fed unwashed yeast. Unfortunately there is not enough information
498 available on washed yeasts as novel proteins, particularly in terms of their effects on histology.
499 Further study needs to be done in the future to indicate health implications and morphology and
500 structure of liver of fish fed novel proteins such as yeasts and single cell protein (SCPs). With

the known limitations of substitution of FM with DDGS it can be proposed that DDGS at 300 g/kg inclusion may result in effects due to higher fibre levels in the DDGS. The liver is a useful indicator of health and nutrition status (Wold et al., 2009; Berntssen et al., 2010), and it is suggested that fish fed YPCR30 and DDGS30 had fewer hepatocytes compared to fish fed other experimental diets. However, it is possible that pathological changes may still occur and determining this requires more extensive of liver histology investigations.

The gastrointestinal tract of carp was also studied in this trial to evaluate the effect of diets on morphology and ultrastructural changes. SEM (scanning electron microscopy) analyses in the present study revealed that various types of yeasts and distillers dried grain with solubles (DDGS) inclusion exerted no major change on microvilli density in the anterior intestinal region.

Microvilli density in the posterior intestinal region was however affected, fish fed YPC had a higher dense microvilli structure which can be a possible explanation for better growth performance and food utilisation of carp in general receiving yeast protein concentrate (YPC). The possibility of the nucleotide fraction in yeast exerting positive functional effects in this way were reported by Bowyer et al. (2019a) in seabass fed a nucleotide commercial product. Bowyer et al. (2019b) also tested a solid state (SSF) fermentation product as a dietary supplement for tilapia showing clear benefits to gut integrity using the same assessment criteria. Their observations compared to this investigation and gut morphology correlated to fish performance.

It was also found in the current trial with carp that TEM (transmission electron microscopy) analyses of the intestine revealed that various types of yeasts and DDGS30 can increase the microvilli length, especially in the case of fish fed YPCU30 diets. Similar increases in microvilli density have been observed with 2% mannan oligosaccharide (MOS) dietary supplementation have been reported for cobia larvae (Salze et al., 2008), and also Dimitroglou et al. (2009) who observed the same results with gilthead sea bream fed a mannan-oligosaccharide (MOS) as a feed additive derived from yeast.

## 5 Conclusions

Results of this trial indicated no significant difference in weight gain of carp fed DDGS up to at least 300 g/kg compared to that of fish fed the FM based diet. Novel wheat fermented protein rich in yeast protein proved effective in carp. YPCU appeared to be the most successful ingredient within the different yeast protein concentrates tested against FM replacement. In addition, liver enzyme analysis suggested that the different yeast protein concentrates and DDGS may offer better performance in carp perhaps due to their additional micro-nutrients and functional properties as dietary ingredient. Using YPC and DDGS will help to reduce the cost of cultured finfish via increased productivity, profitability whilst meeting sustainability criteria. This also has importance in meeting with the cyclic economy and use of bio-resources for aquaculture feeds in the future.

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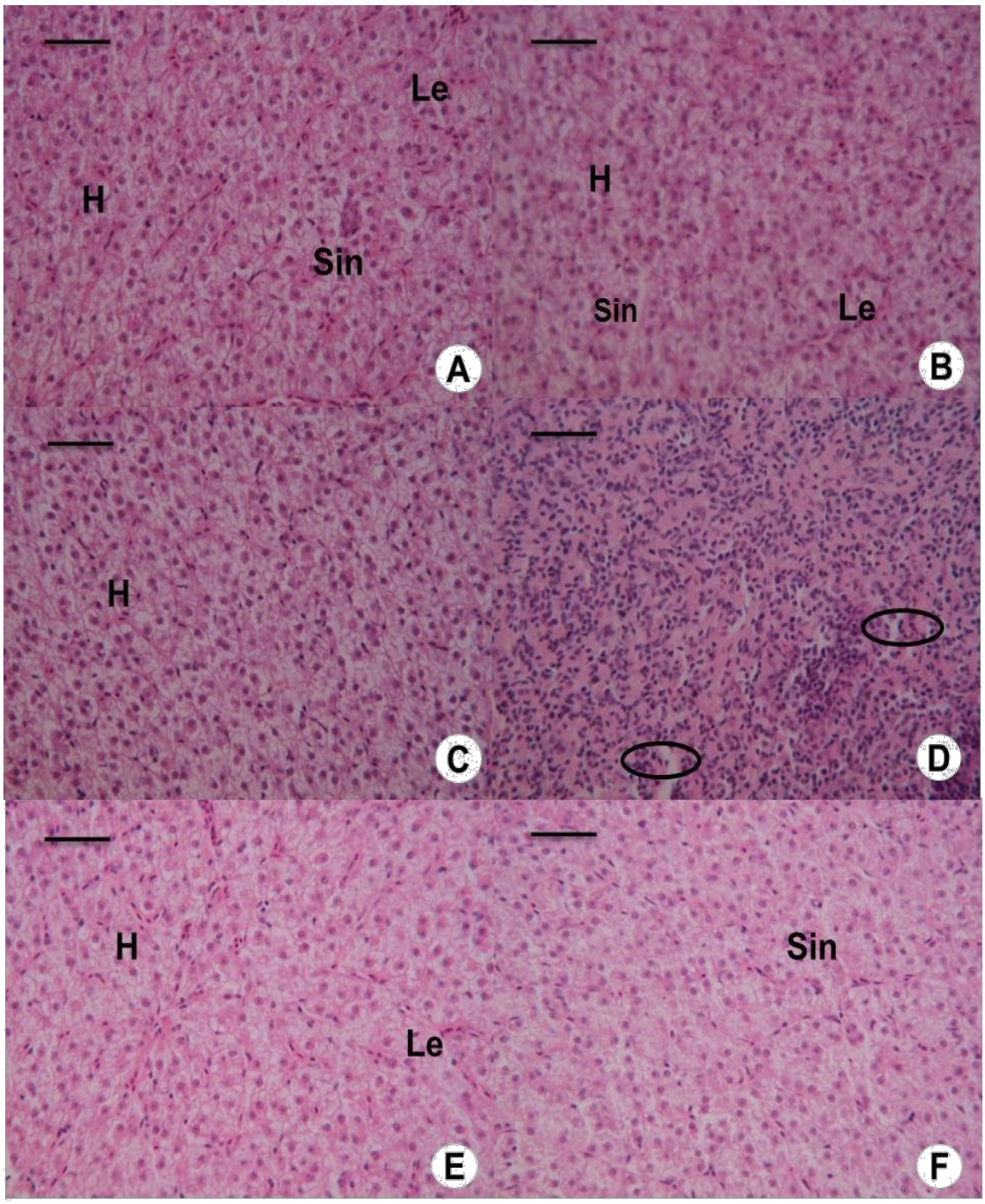


**Figure .1** Photomicrograph of liver section of carp stained with haematoxylin and eosin. Fish were fed (A) fishmeal (B) YPCU30, (C) YPCR30, YPCPA30, (E) DDGS15 and (F) DDGS30. (Scale bar = 50 µm). Hepatocytes, Le: Leukocytes, Sin: Sinusoid and Circle areas the slight necrosis or hypertrophy of liver cells (hepatocytes).

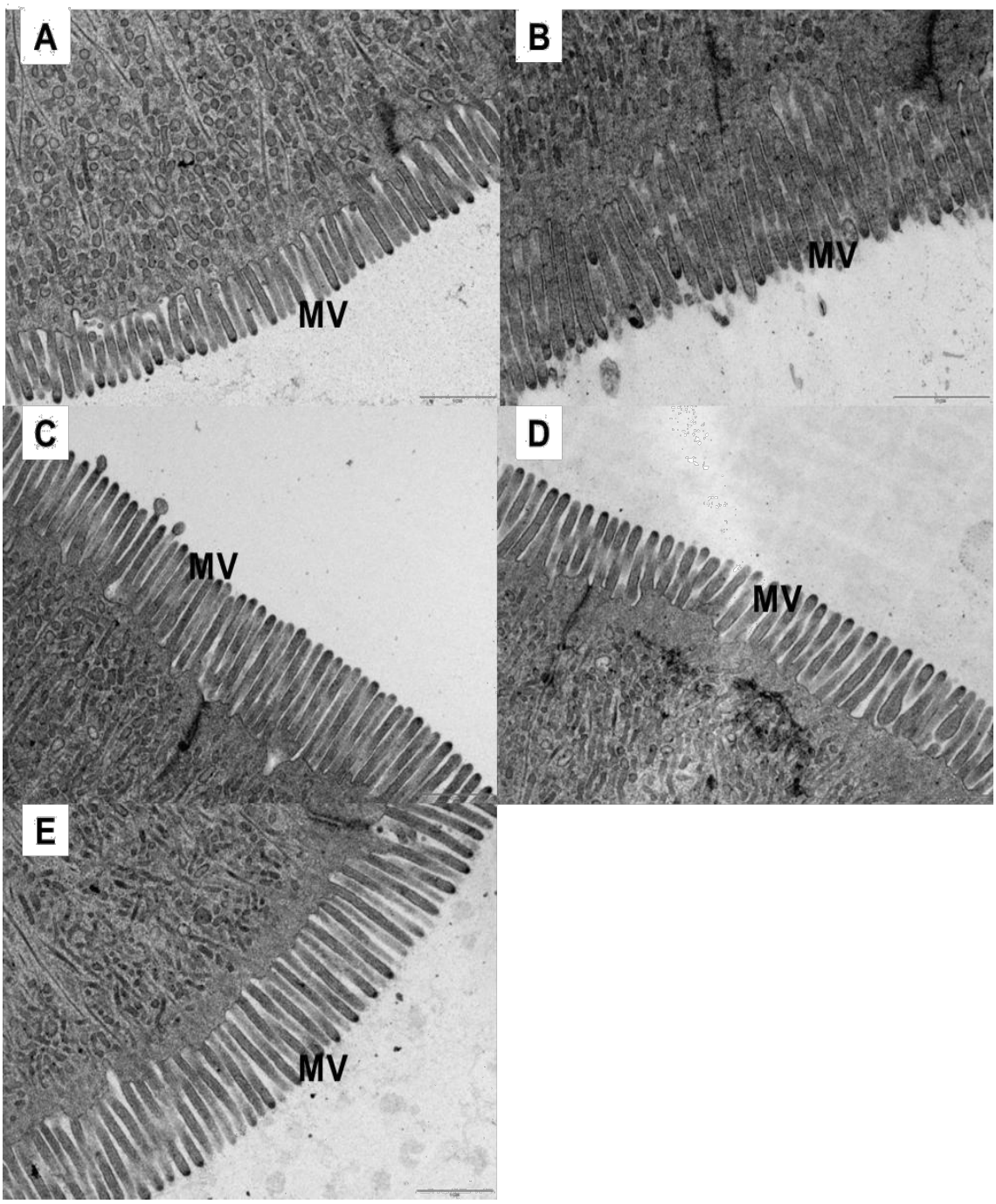


**Figure .2** Comparative TEM micrographs of the posterior intestine of (A) control fed fish, (B) YPCU30 fed fish, (C) YPCR30 fed fish, (D) YPCPA30 fed fish and (E) DDGS30 fed fish. Microvilli length appear longer and healthier significantly in the group YPCU compare to the other treatments. (Scale bar = 1 μm). MV: Microvilli**.**


**Figure .3** Comparative SEM micrographs of anterior intestine of carp fed (A) control fed fish, (B) YPCU fed fish (C) YPCR fed fish (D) YPCPA fed fish, (E) DDGS15 fed fish and (F) DDGS30 fed fish. There are no distinctive differences of the microvilli density between the treatments. (Scale bar = 1 μm)

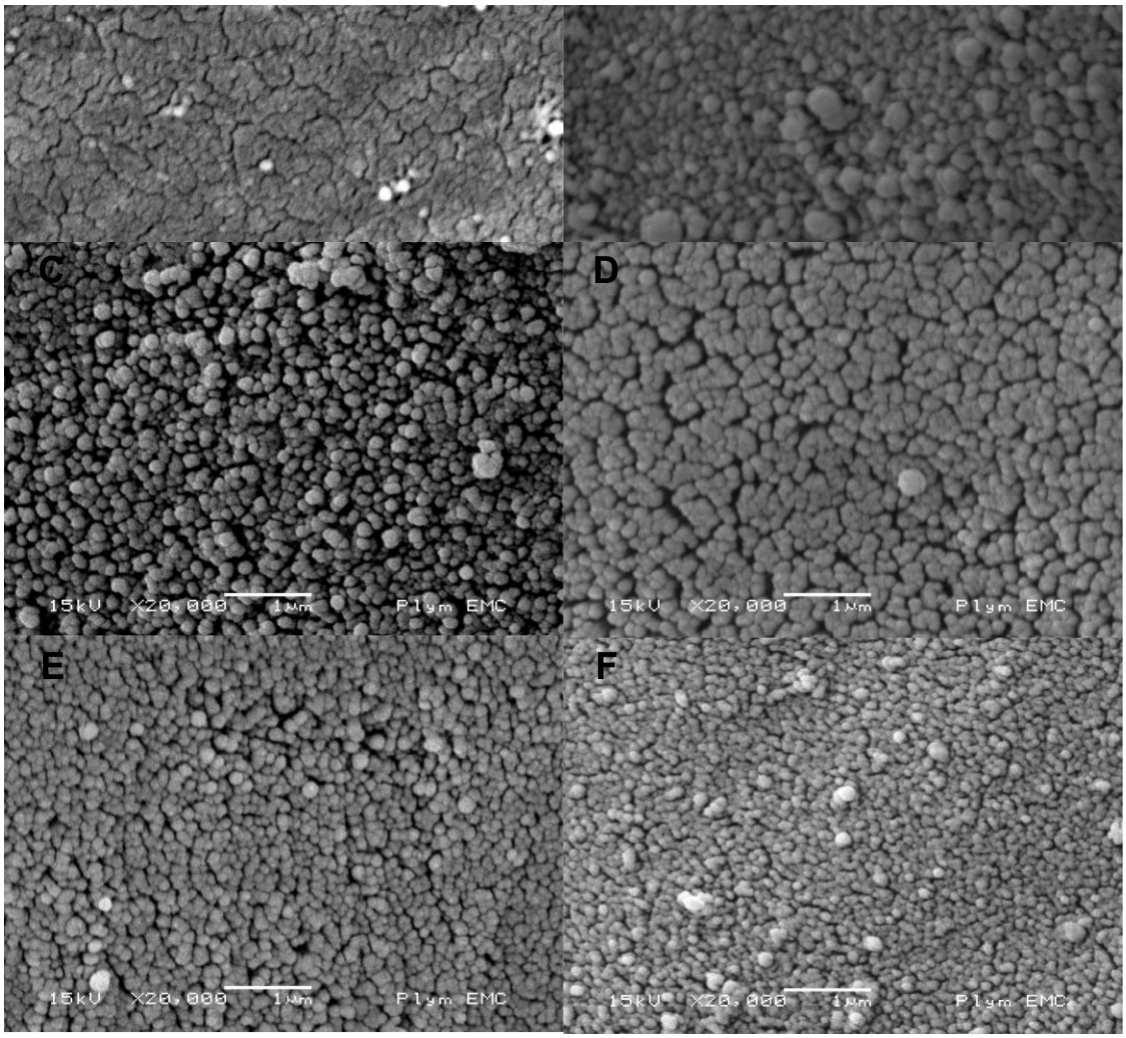


**Figure .4** Comparative SEM micrographs of posterior intestine of carp fed control fed fish, (B) YPCU fed fish, (C) YPCR fed fish (D) YPCPA fed fish, (E) DDGS15 fed fish and (F) DDGS30 fed fish. There are significant differences of the microvilli density in YPCR fed fish compared to other treatment groups.
(Scale bar = 1 μm)

**Table 1** Dietary formulations, proximate composition and energy content of the experimental diets (g/kg)

| Ingredient g kg$^{-1}$ | Control | YPC$_U$30 | YPC$_R$30 | YPC$_{PA}$30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|
| Herring Meal LT92[1] | 419.54 | 250 | 250 | 250 | 297.65 | 243.03 |
| Wheat Carrier Flour[2] | 508.02 | 214.6 | 457.07 | 371.24 | 380.06 | 276.58 |
| Yeast (YPCU)[3] | - | 458.02 | - | - | - | - |
| Yeast Washed (YPCR)[3] | - | - | 214.42 | - | 100 | 100 |
| Scottish Yeast (YPCPA)[3] | | - | - | 286.81 | - | - |
| DDGS[3] | - | - | - | - | 150 | 300 |
| Fish Oil[4] | - | 20 | 20 | 20 | 5 | 10 |
| Vegetable Oil [5] | 27.44 | 12.38 | 13.51 | 26.95 | 22.29 | 15.39 |
| Vitamin and mineral Premix[6] | 20 | 20 | 20 | 20 | 20 | 20 |
| Wheat gluten {Viten)[7] | 20 | 20 | 20 | 20 | 20 | 30 |
| Molasses[8] | 5 | 5 | 5 | 5 | 5 | 5 |
| Proximate composition (g/kg) | | | | | | |
| Protein | 373.7 | 379 | 385.9 | 384.6 | 385.6 | 405.1 |
| Lipid ) | 87.5 | 94.6 | 90.8 | 101 | 93.1 | 91.9 |
| Ash (%) | 95.7 | 81.2 | 74 | 76 | 77.7 | 75.3 |
| NFE (%) [9] | 390.7 | 379.5 | 392.1 | 365.3 | 380.5 | 377.7 |
| Gross Energy(Mj kg$^{-1}$) | 19.69 | 18.55 | 20.22 | 18.93 | 19.26 | 20.23 |

[1]Scottish Fish meal, United Fish Products Ltd, UK.

[2] Ewos-Bathgate Scotland,

[3] Details provided in Omar (2009) & Omar et al. (2012). Materials kindly arranged by Dr. P.V. Williams

[4] Epanoil, Sevenseas Ltd, UK.

[5] Corn oil.

[6] Premier Nutrition vitamin/mineral premix: 121 g kg$^{-1}$ Calcium, Vit A 1.0 µg kg$^{-1}$, Vit D3 0.1 µg kg$^{-1}$, Vitamin E (as alpha tocopherol acetate) 7.0 g kg$^{-1}$, Copper (as cupric sulphate) 250 mg kg$^{-1}$, Magnesium 15.6 g kg$^{-1}$, Phosphorous 5.2 g kg$^{-1}$.

[7]Roquette Frêres, France.

[8]Holland and Barret Ltd UK

[9] nitrogen free extract (NFE) is DM – CP – Fat – ash – crude fiber

[9] Nitrogen-free extracts (NFE) = dry matter – (crude protein + crude lipid + ash).

YPCU = Yeast Protein Concentrate Unrefined, YPCR = Yeast Protein Concentrate Refined, YPCPA = Yeast Protein Concentrate Potable Alcohol, DDGS = Dried Distiller‟s Grain and Solubles.

**Table 2. Amino acid profile for experimental diets (**g/kg, DM basis**) (*n*=3)**

| | Control | YPCU30 | YPCR30 | YPCPA30 | DDGS15 | DDGS30 | Carp requirement ** |
|---|---|---|---|---|---|---|---|
| Essential AA | | | | | | | |
| Arginine | 2.15 | 2.00 | 2.03 | 2.08 | 1.90 | 2.04 | 1.6 |
| Histidine | 0.95 | 0.97 | 0.99 | 0.99 | 1.06 | 0.94 | 0.8 |
| Iso-Leucine | 1.60 | 1.58 | 1.49 | 1.81 | 1.74 | 1.53 | 1.0 |
| Leucine | 2.74 | 2.68 | 2.74 | 2.86 | 2.66 | 2.70 | 1.30 |
| Phenylanine | 1.55 | 1.68 | 1.72 | 1.7 | 1.65 | 1.73 | 2.50[a] |
| Lysine | 2.33 | 1.87 | 2.04 | 2.61 | 2.19 | 1.82 | 2.20 |
| Threonine | 1.47 | 1.34 | 1.59 | 1.72 | 1.44 | 1.18 | 1.50 |
| Valine | 2.03 | 1.92 | 1.84 | 2.23 | 2.21 | 1.67 | 1.40 |
| Methionine | 1.16 | 1.02 | 0.97 | 1.04 | 1.02 | 0.68 | 0.8 |
| Tryptophan | ND | ND | ND | ND | ND | ND | 0.30 |
| Non-Essential AA | | | | | | | |
| Alanine | 2.10 | 1.85 | 1.84 | 2.30 | 1.90 | 1.55 | ND |
| Aspartic acid | 3.10 | 2.77 | 2.82 | 3.37 | 2.79 | 1.85 | ND |
| Cysteine | 0.53 | 0.58 | 0.67 | 0.62 | 0.71 | 0.65 | ND |
| Glutamine | 5.85 | 7.69 | 7.75 | 5.64 | 7.01 | 8.17 | ND |
| Glycine | 2.04 | 1.84 | 1.86 | 1.96 | 1.94 | 1.81 | ND |
| Proline | 1.57 | 2.16 | 2.25 | 1.47 | 1.41 | 2.46 | ND |
| Serine | 1.48 | 1.58 | 1.74 | 1.89 | 1.73 | 1.43 | ND |
| Tyrosine | 0.97 | 1.05 | 1.01 | 1.09 | 1.07 | 1.05 | ND |

*Not determined, **% values obtained from references as cited by NRC (2011),[a] with 1% Iso-leucine, YPCU = Yeast Protein Concentrate Unrefined, YPCR = Yeast Protein Concentrate Refined, YPCPA = Yeast Protein Concentrate Potable Alcohol, DDGS = Dried Distiller"s Grain and Solubles.

**Table 3. Mineral analysis for experimental diets.** (DM basis) (*n*=3)

| Mineral | Control | YPC$_U$30 | YPC$_R$30 | YPC$_{PA}$30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|
| g kg$^{-1}$ | | | | | | |
| Ca | 12.06±3.18 | 8.33±0.14 | 8.75±0.50 | 8.22±0.53 | 9.11±0.16 | 8.28±0.21 |
| K | 8.26±1.98 | 9.70±0.59 | 7.39±0.13 | 6.26±0.08 | 7.65±0.06 | 7.61±0.09 |
| Mg | 2.55±0.59 | 2.65±0.16 | 2.22±0.08 | 1.94±0.04 | 2.33±0.04 | 2.36±0.02 |
| Na | 6.34±1.53 | 4.32±0.12 | 4.45±0.06 | 6.31±0.07 | 5.30±0.06 | 5.08±0.01 |
| P | 11.68±2.99 | 11.08±0.26 | 9.79±0.37 | 10.00±0.37 | 9.99±0.13 | 9.69±0.14 |
| mg kg$^{-1}$ | | | | | | |
| Cr | 2.57±0.66 | 0.92±0.19 | 0.76±0.56 | 1.22±0.43 | 1.33±1.08 | 0.82±0.43 |
| Cu | 17.22±3.73 | 13.00±1.73 | 15.53±1.00 | 33.08±0.50 | 16.58±0.53 | 16.10±0.79 |
| Fe | 215.75±59.94 | 197.90±18.94 | 202.08±6.49 | 172.68±36.38 | 198.11±3.80 | 200.89±20.87 |
| Mn | 61.99±15.20 | 78.77±0.41 | 62.53±2.53 | 63.04±1.58 | 63.47±2.17 | 63.98±0.20 |
| Zn | 106.47±30.22 | 95.64±4.91 | 88.39±3.47 | 83.76±0.95 | 94.03±1.40 | 114.93±29.74 |

Data are presented as mean ± S.D, YPC$_U$ = Yeast Protein Concentrate Unrefined, YPC$_R$ = Yeast Protein Concentrate Refined, YPC$_{PA}$ = Yeast Protein Concentrate Potable Alcohol, DDGS = Dried Distiller‟s Grain and Solubles.

**Table .4 Growth performance and feed utilization of common carp fed the experimental diets for 8 weeks. (*n*=3)**

| | Control | YPCU30 | YPCR30 | YPCPA30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|
| Initial weight (g) | 15.26 ±0.08 | 15.20 ± 0.00 | 15.14 ± 0.03 | 15.18 ± 0.03 | 15.18 ± 0.08 | 15.28 ± 0.06 |
| Final Weight (g) | 55.52 ±0.34$^{b}$ | 60.00 ± 1.92$^{a}$ | 54.40 ± 0.34$^{b}$ | 49.92 ± 0.06$^{c}$ | 55.68 ± 0.85$^{b}$ | 55.57 ± 1.08$^{b}$ |
| Weight Gain (g) | 40.26 ±0.25$^{b}$ | 44.80 ±1.92 $^{a}$ | 39.26 ± 0.37$^{b}$ | 34.74 ± 03$^{c}$ | 40.50 ± 0.76$^{b}$ | 40.29 ± 1.14$^{b}$ |
| Specific growth rate (% day$^{-1}$) | 2.31 ± 0.001$^{b}$ | 2.45 ± 0.057$^{a}$ | 2.28 ± 0.014$^{b}$ | 2.13 ± 0.001$^{c}$ | 2.32 ± 0.017$^{b}$ | 2.28 ± 0.002$^{b}$ |
| Feed conversion efficiency | 87.22 ±0.34$^{ab}$ | 91.34 ± 0.76$^{a}$ | 86.56 ± 1.24$^{ab}$ | 83.43 ± 0.07$^{b}$ | 87.52 ± 0.70$^{ab}$ | 89.26 ± 6.32$^{ab}$ |
| Feed conversion ratio | 1.33 ± 0.001$^{b}$ | 1.23 ± 0.027$^{a}$ | 1.35 ± 0.010$^{bc}$ | 1.43 ± 0.001$^{d}$ | 1.33 ± 0.000$^{b}$ | 1.37 ± 0.021$^{c}$ |
| Protein efficiency ratio | 2.33 ± 0.009$^{ab}$ | 2.41 ± 0.020$^{b}$ | 2.24 ± 0.032$^{ab}$ | 2.17 ± 0.002$^{a}$ | 2.27 ± 0.018$^{ab}$ | 2.20 ± 0.156$^{a}$ |
| Apparent net protein utilization (%) | 31.38 ±0.50$^{bc}$ | 35.97 ± 0.93$^{a}$ | 30.49 ± 0.13$^{cd}$ | 29.54 ± 1.07$^{d}$ | 32.41 ± 0.42$^{b}$ | 32.37 ± 0.75$^{b}$ |
| Condition factor (K) | 1.41 ± 0.03$^{ab}$ | 1.48 ± 0.10$^{a}$ | 1.34 ± 0.07$^{b}$ | 1.39 ± 0.03$^{ab}$ | 1.46 ±0.12$^{ab}$ | 1.41 ± 0.09$^{a}$ |
| Survival (%) | 100 ± 0.00 | 100 ± 0.00 | 100 ± 0.00 | 100 ± 0.00 | 100 ± 0.00 | 98 ± 2.00 |

Data are presented as mean ± S.D., Data in the same row with different superscript are significantly different ($P<0.05$), YPCU = Yeast Protein Concentrate Unrefined, YPCR = Yeast Protein Concentrate Refined, YPCPA = Yeast Protein Concentrate Potable Alcohol, DDGS = Dried Distiller‟s Grain and Solubles.

**Table 5** Whole body proximate composition (g/kg, DM basis) of the initial fish and fish fed experimental diets for 8 weeks. $n = 3$

| Whole Body composition | Initial | Control | YPCU30 | YPCR30 | YPCPA30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|---|
| Dry matter | 241.7 ± 0.43 | 255.4 ± 0.41$^{ab}$ | 238.2 ± 0.14 $^{c}$ | 259.7 ± 0.01 $^{a}$ | 241.0 ± 0.69$^{bc}$ | 251.5 ± 1.05$^{abc}$ | 246.6 ± 0.55$^{abc}$ |
| Protein | 560.0 ±1.82 | 573.7 ± 1.32$^{a}$ | 607.2 ±0.42$^{b}$ | 569.4 ± 0.59$^{ac}$ | 602.3 ± 2.26$^{ab}$ | 590.6 ±0.17$^{abc}$ | 600.4 ± 1.58$^{abc}$ |
| Lipid | 261.5 ± 0.59 | 288.7± 0.69$^{c}$ | 238.7 ±0.07$^{a}$ | 302.2 ± 0.18$^{c}$ | 239.1± 1.02$^{a}$ | 267.6 ± 0.04$^{b}$ | 256.2 ± 1.59$^{ab}$ |
| Ash | 113.9 ± 0.15 | 94.8 ± 0.44$^{a}$ | 118.0 ± 0.37$^{c}$ | 94.3 ± 0.57$^{a}$ | 110.7 ± 0.48$^{ab}$ | 99.9 ± 0.03$^{cd}$ | 106.6 ± 0.00b$^{bc}$ |
| NFE | 64.6±1.41 | 42.8± 2.45 | 36.1±0.72 | 34.1±0.16 | 47.9 ± 1.73 | 41.9 ± 0.16 | 36.8 ± 0.02 |
| Gross energy | 247.1±0.20 | 255.1±0.01 | 240.4 ±0.52 | 252.6±0.13 | 244.6±0.24 | 250.8±0.08 | 247.6±0.31 |

* Dry matter basis, Data are presented as mean ± S.D., Data in the same row with different superscript are significantly different ($P<0.05$), Nitrogen-free extracts (NFE) = dry matter – (crude protein + crude lipid + ash), YPCU = Yeast Protein Concentrate Unrefined, YPCR = Yeast Protein Concentrate Refined, YPCPA = Yeast Protein Concentrate Potable Alcohol, DDGS = Dried Distiller‟s Grain and Solubles.

**Table 6 Mineral analyses for common carp (whole body) fed on experimental diets for 8 weeks. (*n*=6)**

| Mineral | Initial | Control | YPCU30 | YPCR30 | YPCPA30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|---|
| g $kg^{-1}$ | | | | | | | |
| Ca | 24.10±1.82 | 17.89±0.51$^{a}$ | 25.15±1.34$^{c}$ | 17.82±0.38$^{a}$ | 21.71±0.58$^{b}$ | 18.02±0.02$^{a}$ | 19.54±0.54$^{a}$ |
| K | 7.81±0.24 | 8.94±0.24$^{ab}$ | 9.90±0.03$^{c}$ | 8.93 ± 0.21$^{ab}$ | 9.42±0.31$^{bc}$ | 8.80±0.26$^{a}$ | 8.98±0.11$^{ab}$ |
| Mg | 1.08±0.06 | 0.88±0.01$^{a}$ | 1.11±0.02$^{d}$ | 0.89±0.01$^{a}$ | 1.02±0.01$^{c}$ | 0.88±0.18$^{a}$ | 0.96±0.00$^{b}$ |
| Na | 3.11±0.14 | 2.99±0.05$^{ab}$ | 3.35±0.08$^{c}$ | 2.82±0.01 $^{a}$ | 3.13±0.19 $^{b}$ | 2.86±0.03 $^{a}$ | 2.90±0.00 $^{a}$ |
| P | 16.86±0.96 | 14.11±0.52$^{a}$ | 18.46±0.50$^{d}$ | 14.28±0.05a$^{b}$ | 16.46±0.25$^{c}$ | 14.16±0.37$^{a}$ | 15.19±0.49$^{b}$ |
| mg $kg^{-1}$ | | | | | | | |
| Cr | 0.38±0.13 | 0.72±0.17 | 0.44±0.03 | 0.49±0.30 | 0.38±0.18 | 0.47±0.13 | 0.40±0.04 |
| Cu | 9.41±0.45 | 7.92±0.90$^{ab}$ | 8.57±0.07$^{abc}$ | 7.25±0.69 $^{a}$ | 9.93±0.56 $^{c}$ | 9.36±0.45$^{bc}$ | 8.45±1.16$^{abc}$ |
| Fe | 90.70±1.18 | 73.34±1.09 | 75.97±2.08 | 72.89±0.55 | 71.03±0.62 | 75.30±7.05 | 71.19±1.56 |
| Mn | 7.22±0.43 | 2.79±0.25 | 3.08±0.30 | 2.73±0.12 | 2.88±0.15 | 2.61±0.15 | 2.96±0.26 |
| Zn | 282.97±0.43 | 202.96±11.85$^{c}$ | 172.46±7.37$^{a}$ | 195.95±1.47$^{bc}$ | 224.53±0.29$^{d}$ | 179.43±3.16$^{ab}$ | 188.08±0.52$^{abc}$ |

Data are presented as mean ± S.D., Data in the same row with different superscript are significantly different ($P<0.05$).

**Table 7.** Mineral Retention for common carp fed experimental diets for 8 weeks. (*n*=6)

| Mineral | Control | YPC$_U$30 | YPC$_R$30 | YPC$_{PA}$30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|
| Macro Mineral | | | | | | |
| Ca | 94.89±0.40$^{d}$ | 95.72±1.68$^{d}$ | 88.44±0.51$^{b}$ | 84.80±0.04$^{a}$ | 91.39±0.14$^{c}$ | 85.96±2.07$^{ab}$ |
| K | 23.32±0.0$^{b}$ | 22.54±0.42$^{a}$ | 26.34 ± 0.17$^{d}$ | 29.24±0.02$^{e}$ | 24.54±0.02$^{c}$ | 23.66±0.57$^{b}$ |
| Mg | 6.37±0.03$^{a}$ | 8.54±0.18$^{d}$ | 7.69±0.06$^{c}$ | 9.52±0.01$^{e}$ | 7.06±0.01$^{b}$ | 7.60±0.18$^{c}$ |
| Na | 9.57±0.039$^{b}$ | 16.41±0.339$^{e}$ | 12.72±0.092$^{d}$ | 8.94±0.005$^{a}$ | 10.75±0.003$^{c}$ | 10.75±0.260$^{c}$ |
| P | 23.24±0.09$^{a}$ | 35.34±0.73$^{d}$ | 28.45±0.22$^{c}$ | 29.48±0.02$^{c}$ | 27.11±0.02$^{b}$ | 28.86±0.70$^{c}$ |
| Micro Mineral | | | | | | |
| Cr | 6.74±0.029$^{b}$ | 10.23±0.203$^{e}$ | 14.82±0.089$^{f}$ | 5.74±0.003$^{a}$ | 7.87±0.008$^{c}$ | 9.46±0.229$^{d}$ |
| Cu | 8.88±0.036$^{b}$ | 13.12±0.305$^{e}$ | 8.75±0.071$^{b}$ | 5.56±0.003$^{a}$ | 11.57±0.002$^{d}$ | 9.63±0.233$^{c}$ |
| Fe | 6.45±0.026$^{b}$ | 7.37±0.183$^{d}$ | 6.89±0.054$^{c}$ | 6.55±0.005$^{b}$ | 7.24±0.006$^{d}$ | 6.09±0.148$^{a}$ |
| Mn | 0.41±0.001$^{cd}$ | 0.43±0.026$^{d}$ | 0.39±0.008$^{c}$ | 0.28±0.001$^{a}$ | 0.31±0.007$^{b}$ | 0.40±0.010$^{cd}$ |
| Zn | 33.96±0.14$^{d}$ | 28.88±0.98$^{b}$ | 39.14±0.35$^{e}$ | 43.01±0.03$^{f}$ | 31.16±0.10$^{c}$ | 25.41±0.62$^{a}$ |

Data are presented as mean ± S.D., Data in the same row with different superscript are significantly different ($P<0.05$), YPC$_U$ = Yeast Protein Concentrate Unrefined, YPC$_R$ = Yeast Protein Concentrate Refined, YPC$_{PA}$ = Yeast Protein Concentrate Potable Alcohol, DDGS = Dried Distiller‟s Grain and Solubles.

**Table 8.** Microvilli morphology and liver structure of common carp fed on experimental diets. ($n$ =6)

| Variable | Intestine | Control | YPCU30 | YPCR30 | YPCPA30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|---|
| Hepatocytes | | 182.3±20.32 $^{ab}$ | 175.2±10.80 $^{ab}$ | 158.7±14.02 $^{a}$ | 230.2 ±109.03 $^{b}$ | 173.2±27.32 $^{ab}$ | 151.8±28.59 $^{a}$ |
| Microvilli density* | Anterior | 1.75±0.45 | 1.76±0.37 | 1.90±0.12 | 1.91±0.45 | 1.75±0.34 | 1.51±0.19 |
| | Posterior | 4.34±0.42$^{b}$ | 4.35±0.82$^{b}$ | 2.4±1.15$^{a}$ | 4.13±0.53$^{b}$ | 3.62±0.95$^{ab}$ | 3.45±0.66$^{ab}$ |
| Microvilli length (μm) | Posterior | 0.98±0.32$^{a}$ | 1.45±0.13$^{b}$ | 1.2±0.03$^{ab}$ | 0.94±0.05$^{a}$ | - | 1.31±0.38$^{ab}$ |

* Arbitrary unit.

Data are presented as mean ± S.D.

Data in the same row with different superscript are significantly different ($P<0.05$).

**Table 9.** Haematological parameters and liver enzymes of common carp after 8 weeks of feeding on experimental diets. ($n$=10)

| | Control | YPCU30 | YPCR30 | YPCPA30 | DDGS15 | DDGS30 |
|---|---|---|---|---|---|---|
| Haematocrit (%) | 44.8 ± 2.62 | 41.60 ± 3.73 | 43.40 ± 3.95 | 43.50 ± 4.88 | 44.30 ± 5.01 | 44.90 ± 3.45 |
| Haemoglobin (g $dL^{-1}$) | 6.93± 0.70 | 6.78 ± 0.44 | 7.13 ± 0.44 | 6.61 ± 0.32 | 6.68 ± 0.96 | 7.14 ± 0.75 |
| RBC ($10^6$ µL) | $1.27 \pm 0.04^{ab}$ | $1.36 \pm 0.04^{b}$ | $1.29 \pm 0.17^{ab}$ | $1.23 \pm 00.19^{a}$ | $1.31 \pm 0.12^{ab}$ | $1.39 \pm 0.14^{b}$ |
| Leukocytes counts* | $81.00 \pm 9.58^{cd}$ | $88.25 \pm 7.30^{d}$ | $79.00 \pm 9.20^{bcd}$ | $71.88 \pm 11.27^{abc}$ | $61.38 \pm 13.29^{a}$ | $68.50 \pm 8.99^{ab}$ |
| MCV (fl) | $381.22 \pm 29.42^{b}$ | $327.10 \pm 28.99^{a}$ | $368.51 \pm 72.87^{ab}$ | $389.29 \pm 70.00^{b}$ | $366.45 \pm 47.48^{ab}$ | $350.18 \pm 33.24^{ab}$ |
| MCH (pg) | 54.59 ± 5.72 | 49.93 ± 3.03 | 56.08 ± 8.16 | 55.05 ± 8.30 | 51.21 ± 8.07 | 51.84 ± 7.77 |
| MCHC (g $dL^{-1}$) | 14.36 ± 1.47 | 15.39 ± 1.77 | 15.44 ± 1.87 | 14.31 ± 1.77 | 14.12 ± 2.38 | 14.82 ± 1.88 |
| ALAT (U $mg^{-1}$ protein) | 0.95 ± 0.27 | 0.90 ± 0.50 | 1.02 ± 0.76 | 0.79 ± 0.34 | 0.84 ± 0.16 | 1.15 ± 0.68 |
| ASAT (U $mg^{-1}$ protein) | $3.84 \pm 1.21^{b}$ | $2.17 \pm 1.17^{a}$ | $1.76 \pm 0.76^{a}$ | $1.68 \pm 0.97^{a}$ | $2.17 \pm 1.40^{a}$ | $2.35 \pm 0.88^{a}$ |

Data are presented as mean ± S.D, Data in the same row with different superscript are significantly different ($P<0.05$), * Number of leukocytes per 1000 blood cells, RBC-Red Blood cells, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Haemoglobin. MCHC-Mean Corpuscular Haemoglobin Concentration, YPCU = Yeast Protein Concentrate Unrefined, YPCR = Yeast Protein Concentrate Refined, YPCPA=Yeast Protein Concentrate Potable Alcohol, DDGS = Dried Distiller‟s Grain and Solubles