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Original Article

Effects of thermal treatment of some dietary feed ingredients on their digestibility and growth of common carp, *Cyprinus carpio* fingerlings

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Abstract: The purpose of the current study was to determine the effects of thermal treatment (autoclaving) of several dietary feed ingredients, including fishmeal, soybean meal, wheat bran, yellow corn, and barley, on the apparent digestibility coefficients ADCs, as well as the growth and feed efficiency of common carp, *Cyprinus carpio* fingerlings. The feed ingredients were autoclaved for 10 min at 121ºC and 15 psi in a laboratory autoclave. Two experiments were conducted, the first consisted of 11 diets, reference, and 10 experimental diets (reference was mixed with each raw or autoclaved test ingredient in a ratio of 70:30) to determine the digestibility of feed ingredients. The second experiment consisted of 8 diets, a control diet of raw ingredients and seven experimental diets were formulated, five of them in which one of the raw feed ingredients was replaced with the autoclaved one, the sixth in which three raw ingredients (wheat bran, yellow corn, and barley) were replaced together with the autoclaved ones, and the seventh in which all the raw ingredients were replaced with the autoclaved ones. The results of the first experiment presented that autoclaving significantly enhanced ADCs of dry matter, protein, and energy, of all feed ingredients except fishmeal. The results of the second experiment similarly presented that the thermal treatment significantly enhanced ADCs in the diets containing autoclaved soybean meal or wheat bran, yellow corn, and barley or all ingredients compared control diet. The growth and feed efficiency were better significantly in autoclaved soybean meal or all ingredients diets compared control diet. It is advised that plant-based ingredients, especially soybean meal, be thermally-processed to improve their nutritional value and lessen their environmental impact.

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Introduction

Aquaculture is one of the fastest-growing food production activities in the world, as it plays an important role in many countries by providing food and employment opportunities (Kannadhason et al., 2011). Global production of fish and crustaceans from capture fisheries and aquaculture reached approximately 177.8 million tons (FAO, 2022). Increased growth in aquaculture increases the need for research to obtain feed information and thus meet the nutritional requirements of aquatic organisms (Godoy et al., 2016). The most important challenge facing the fish feed industry is manufacturing high-quality feed that meets the nutritional requirements of fish, reduces production costs, limits negative environmental impacts, and enhances product quality (Guo et al., 2011). Feed constitutes the main cost in aquaculture operations. The cost of feed constitutes at least 50% of the total production cost, and to achieve profits and success in fish farming, feed ingredients must be easily available and cheap (Falaye et al., 2014).

The nutritional value of feed ingredients can be determined by the digestibility of protein and energy (Mmanda et al., 2020). Exposing fish feed to certain levels of thermal treatment can lead to the breaking of weak bonds in protein molecules and thus enhance their digestibility by making them more responsive to the action of digestive enzymes (Opstvedt et al., 2003), whereas exposing it to higher levels of heat may lead to the opposite effect, which reduces digestibility as a result of the formation of bonds between amino acids and some other compounds, making them resistant to the action of digestive enzymes (Stanley, 1998). Therefore, thermal

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treatment, depending on its type and type of feed ingredients, can lead to an increase or decrease in digestibility, and it is known that the fate of the protein consumed by fish depends mainly on its digestibility and the nutritional value of the feed ingredients supplied and included in the manufacture of the feed (Khanom et al., 2017).

Extrusion or autoclaving are used to manufacture fish feed, in both cases, heat, moisture, and pressure are used. This leads to an increase in the efficiency of starch present in the feed, either due to gelatinization or due to reducing the complexity of the components, so the effect of thermal treatment on starch digestibility is considered positive (Stone et al., 2003). Thermal treatment leads to shortening the chain found in starch molecules, as well as destroying enzyme inhibitors, which increases digestibility (Kumar et al., 2006). The quality of dietary protein depends on its composition of amino acids, their digestibility, and availability. A deficiency in essential amino acids leads to misuse of dietary protein by turning it into a source of energy instead of growth. Thus, it reduces the nutritional value of the feed and leads to a decline in growth rates (Rollin et al., 2003). Thermal treatment may also weaken the quality and availability of some nutrients, depending on the technology, logistics, and conditions used, as some amino acids may become unavailable after thermal treatment, and the protein can undergo denaturation, dissolution, or bonding under the influence of heat and pressure during the extrusion process (Chen et al., 2021). Extrusion is considered in general, the prevailing method in manufacturing fish feed. It works to remove microbes, reduce the levels of antinutrients found in plant materials, and increase the availability of nutrients, especially the quantity of digestible energy which increases through increased starch gelatinization (Hernández et al., 2010). During feed extrusion processing, raw materials may undergo several chemical and structural transformations, including starch gelatinization, protein denaturation, amylopectin formation, and Millard reactions in the presence of sugars (Chu et al., 2015).

The process of determining the digestibility of nutrients in the feed is the first indicator of their nutritional value and quality (Luo et al., 2009). Determining digestibility allows a clearer estimate of the nutritional value of feed ingredients, as one might think one of the feed ingredients, based on its chemical composition, indicates that it is an excellent source of nutrients, but on the other hand, this feed ingredient may have little nutritional value, due to the difficulty of digesting and absorbing it by the target fish species (Khanom et al., 2017).

The properties of thermally-treated feed affect the nutritional value of the feed and the digestibility of nutrients, which may affect rates of fish growth (Weththasinghe et al., 2021). Extrusion and other thermal treatment techniques are commonly used to produce fish feed, physical properties are improved, such as stability in water, durability, hardness, and buoyancy, compared to non-thermally-treated feeds (Aas et al., 2009).

When manufacturing fish feed, thermal treatment is often carried out on the final mixture of the feed, which includes most of the feed ingredients involved in its preparation, whether using hot water or traditional or modern thermal treatments such as extrusion. The response of the feed ingredients to these thermal treatments varies and has a positive effect on some of them and maybe a negative on others. According to Gatlin et al. (2007), there are two main ways to increase the nutritional value of feed ingredients used in fish diets: Pre-heating the feed ingredients individually and heat-treating the entire mixture. Therefore, the current study aims to examine how some feed ingredients used in the production of locally produced common carp, *Cyprinus carpio* fish feeds respond to thermal treatment individually for each of them, and their impact on growth and feed efficiency.

Materials and Methods

The current study was conducted to study the effect of thermal treatment using an autoclave for each feed ingredient (fishmeal, soybean meal, wheat bran, yellow corn, and barley) individually before using it in the manufacture of diets. The study was conducted

Table 1. Chemical composition of feed ingredients used in the study (dry weight basis).

	Moisture (%)	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)	Energy (Kcal/100g)
Fishmeal	7.84±0.43	67.08±2.46	9.27±0.57	8.26±0.81	15.39±1.09	409.48±3.45
Soybean meal	7.15±0.61	46.41±2.64	1.74±0.31	45.63±2.78	6.23±0.45	382.84±0.47
Wheat bran	11.06±1.08	16.15±0.86	3.98±0.33	74.44±1.31	5.44±0.78	366.26±3.48
Yellow corn	12.65±1.12	8.89±0.91	4.96±0.35	83.85±1.61	2.31±0.34	374.76±4.50
Barley	12.91±1.36	9.93±0.62	2.14±0.30	84.86±0.79	3.07±0.13	359.03±2.49

in two stages by conducting two separate experiments. The first was intended to study the effect of thermal treatment on the apparent digestibility of feed ingredients, while the purpose of the second experiment was to determine the effect of including each of these feed ingredients after thermal treatment on the apparent digestibility coefficients ADCs of diets, growth rates, and feed efficiency.

Aquaculture system: The two experiments were conducted in the laboratory of the Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah.

In the first experiment, 11 rectangular plastic tanks (40x30x30 cm; length × width × height) with a capacity of 30 liters of water were used. The tanks were provided with submersible heaters to maintain the water temperature within the appropriate levels (≈ 25°C), the first experiment was extended for 9 weeks from 1/30/2022 to 3/31/2022. In the second experiment, 16 plastic tanks similar to that in the first experiment were used, extended for 60 days from 4/24/2022 to 6/22/2022.

Fish: Experiments were conducted using common carp fingerlings, brought from the Agricultural Research and Experimentation Station of the College of Agriculture in Al-Haritha through the Aquaculture Unit. The fish were acclimated to laboratory conditions for two weeks, during which they were fed experimental diets.

Feed ingredients: Five types of feed ingredients were used including fishmeal, soybean meal, wheat bran, yellow corn, and barley. In addition, other materials were used in the manufacture of the diets, namely carboxymethyl cellulose as a binder, sunflower oil, and a mixture of vitamins, minerals, and amino acids (Table 1).

First experiment (feed ingredients digestibility): The first experiment was conducted to study the effect of thermal treatment by autoclaving feed ingredients individually on their apparent digestibility.

Table 2. Percentages of feed ingredients used in manufacturing the reference diet.

Feed ingredients	%
Fishmeal	20
Soybean meal	28
Wheat bran	15
Yellow corn	15
Barley	15
Vit. Mix.	2
CMC	2
Sunflower oil	2
Cr_2O_3	1

Diets: The five feed ingredients (fishmeal, soybean meal, wheat bran, yellow corn, and barley) were prepared by grinding them well and sifting them with a sieve with small holes (0.4 mm). 100 grams of each of them were weighed and kept without any treatment for use later in the manufacture of diets, another 100 grams of each of them was subjected separately to thermal treatment by autoclaving at a temperature of 121°C and a pressure of 15 psi for 10 minutes. The thermally treated feed ingredients were dried again by exposing them to air at room temperature and then also preserved for later use in the manufacture of diets.

Eleven diets were used in the first experiment. The first is the reference diet, which was prepared according to the proportions mentioned in Table 2 and Table 3 shows its chemical composition. Ten other test diets were prepared (Table 4), each of them consisting of 70% of the reference diet and 30% of one of the five feed ingredients mentioned above (Bureau et al., 1999), in its raw (unautoclaved) state once and in its thermally treated (autoclaved) state again.

Experimental procedures: The first experiment was conducted to study the digestibility of feed ingredients

Table 3. Chemical composition of the reference diet (dry weight basis).

Moisture %	Protein %	Lipid %	Carbohydrate %	Ash %	Energy Kcal/100g
8.44±0.95	32.98±3.05	5.64±0.61	53.82±3.30	7.57±0.86	384.14±7.36

Table 4. Diets used in the first experiment.

	Diet	reference diet %	Feed ingredient %
1	Unautoclaved fishmeal diet UFD	70% of reference diet	30% of unautoclaved fishmeal
2	Autoclaved fishmeal diet AFD	70% of reference diet	30% of autoclaved fishmeal
3	Unautoclaved soybean meal diet USD	70% of reference diet	30% of unautoclaved soybean meal
4	Autoclaved soybean meal diet ASD	70% of reference diet	30% of autoclaved soybean meal
5	Unautoclaved wheat bran diet UWD	70% of reference diet	30% of unautoclaved wheat bran
6	Autoclaved wheat bran diet AWD	70% of reference diet	30% of autoclaved wheat bran
7	Unautoclaved yellow corn diet UYD	70% of reference diet	30% of unautoclaved yellow corn
8	Autoclaved yellow corn diet AYD	70% of reference diet	30% of autoclaved yellow corn
9	Unautoclaved barley diet UBD	70% of reference diet	30% of unautoclaved barley
10	Autoclaved barley diet ABD	70% of reference diet	30% of autoclaved barley
11	Reference diet RD	100% of reference diet	-

for nine weeks using eleven treatments (diets) distributed over eleven ponds. A total of 55 fish (28.07±3.42 g) were used, 5 fish per tank. The fish were acclimatized for two weeks to the experimental diets. During the acclimation period and the experiment, the fish were fed one meal in the morning at a rate of 3% of their wet body weight. The tanks are cleaned with a siphon after the end of the meal serving period, which lasts for one hour, to remove feed residues and waste. During the cleaning process, almost most of the tank water is replaced. The waste collection process begins immediately after that. The feces collected during each three weeks were isolated separately as a replicate. Therefore, three different groups of feces were obtained at the end of the experiment to represent three replicates for statistical analysis.

Second experiment (diet digestibility, fish growth, and feed efficiency): The second experiment was conducted to study the effect of thermal treatment by autoclaving the feed ingredients individually for each and including them in the diet in the same proportions as the control diet. The reference diet used in the first experiment was the control diet in the second experiment, as the effect of treatments on the diet's digestibility, fish growth, and feed efficiency, were studied.

Diets: Eight diets were used in the second experiment. The first is the control diet. Seven other diets were prepared (Table 5). Five of these were prepared by replacing one of the five raw (unautoclaved) feed ingredients from which the control diet was formed with its cooked (autoclaved) counterpart in the same ratio. The sixth diet was prepared by replacing three raw feed ingredients in the control diet (wheat bran, yellow corn, and barley) with their cooked counterparts. The seventh diet was prepared by replacing all five raw feed ingredients used in the control diet with their cooked counterparts.

Experimental procedures: The second experiment was conducted to study the apparent digestibility of the diets, the growth rates of the fish, and the feed efficiency, which lasted for 60 days using eight treatments distributed over 16 tanks. In the experiment, 80 fish with an average weight of 15.25±1.30 g were used (5 fish per tank). The fish were acclimatized for two weeks to the experimental diets. During the acclimation period and the experiment, the fish were fed one meal in the morning at a rate of 3% of their wet body weight. The tanks are cleaned with a siphon after the end of the meal serving period, which lasts for one hour. The feces collection process begins immediately after that. The feces collected from each tank were isolated separately as a replicate. The fish were weighed at the beginning of the experiment, and three and six weeks after the start

Table 5. Diets used in the second experiment.

Ingredients	Diets							
	Control CD	Fishmeal FD	Soybean meal SD	Wheat bran WD	Yellow corn YD	Barley BD	Wheat bran, yellow corn, and barley WYBD	All five ingredients AD
Fishmeal	20	-	20	20	20	20	20	-
Soybean meal	28	28	-	28	28	28	28	-
Wheat bran	15	15	15	-	15	15	-	-
Yellow corn	15	15	15	15	-	15	-	-
Barley	15	15	15	15	15	-	-	-
Autoclaved fishmeal	-	20	-	-	-	-	-	20
Autoclaved soybean	-	-	28	-	-	-	-	28
Autoclaved wheat bran	-	-	-	15	-	-	15	15
Autoclaved yellow	-	-	-	-	15	-	15	15
Autoclaved Barley	-	-	-	-	-	15	15	15
Vit. Mix.	2	2	2	2	2	2	2	2
CMC	2	2	2	2	2	2	2	2
Sunflower oil	2	2	2	2	2	2	2	2
Cr_2O_3	1	1	1	1	1	1	1	1

of the experiment to monitor growth and correct the amount of food provided accordingly, and at the end of the experiment. At the end of the experiment, the weight gain (WG), relative growth rate (RGR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV) were calculated according to the following equations:

WG g = final weight g - initial weight g

RGR % = (weight gain g / initial weight g) × 100

SGR %/day = ((ln final weight g - ln initial weight g) / period days) × 100

FCR = feed intake g / weight gain g

PER = weight gain g / protein intake g

PPV % = (increase in body protein g / protein intake g) × 100

Digestibility: The indirect method was used to measure the apparent digestibility of the diets (Talbot, 1985). Chromic oxide (1%) was used as an indicator for this purpose. Feces were collected that were in the water for less than 20 minutes using the siphon. Then, the feces were filtered using a cloth, washed with distilled water, collected on filter paper, left to dry in the air at room temperature, then placed in plastic bottles and kept refrigerated. The percentage of chromic oxide in feces was estimated according to Olvera-Novoa et al. (1994) at a wavelength of 350 nm using spectrophotometry. The following equations were used to calculate the apparent digestibility coefficients of dry matter ADC_{dm} and nutrients ADC_n of the diets.

ADC_{dm} % = 100 - (100 × (% chromic oxide in feed / % chromic oxide in faeses))

ADC_n % = 100 - (100 × ((% chromic oxide in feed / % chromic oxide in faeses) × (% nutrient in faeses / % nutrient in feed))

The equations below were used to calculate the apparent digestibility coefficients of ingredients $ADC_{ingredients}$ according to NRC (2011).

$ADC_{ingredients}$ % = ADC of test diet + ((ADC of test diet – ADC of reference diet) × (0.7 × % of nutrient in reference diet / 0.3 × % of nutrient in feed)

Chemical composition: The chemical composition of feed ingredients, diets, and feces were measured according to AOAC (2005). The moisture was measured by drying in the oven at a temperature of 105°C. The protein was measured by the Semi-Micro-Kjeldahl method by calculating the total nitrogen content. The lipid by the soxhlet method using diethyl ether, and the ash by incineration of the sample in a muffle furnace at a temperature of 550°C. The carbohydrates, by subtracting the sum of the above components from 100. The gross energy was estimated based on the values reported by Zhang et al. (2009), which are 4.5, 8.5, and 3.49 kcal/g for protein, lipid, and carbohydrates, respectively.

Statistical analysis: The t-test was used to compare the treatments in the first experiment by comparing

Table 6. Apparent digestibility coefficients (%) of autoclaved and unautoclaved feed ingredients, fed to common carp fingerlings.

	Apparent digestibility coefficient ADC					
	Dry matter	Protein	Energy	Carbohydrate	Lipid	Ash
Unautoclaved fishmeal	81.58±3.01a	81.05±1.96a	81.53±2.90a	81.42±2.67a	87.15±14.25a	81.08±2.03a
Autoclaved fishmeal	82.38±2.32a	81.54±1.61a	82.29±2.24a	82.11±2.09a	91.06±10.04a	81.60±1.66a
Unautoclaved soybean meal	73.73±0.98b	75.23±0.89b	73.65±1.00b	58.23±2.30b	72.80±1.05b	72.68±1.06b
Autoclaved soybean meal	82.81±2.02a	82.20±1.69a	82.85±2.04a	89.19±5.56a	83.20±2.23a	83.24±2.25a
Unautoclaved wheat bran	52.54±4.94b	36.28±7.57b	52.17±5.00b	35.67±7.67b	58.22±4.02b	45.68±6.05b
Autoclaved wheat bran	73.89±3.29a	70.20±4.86a	73.81±3.33a	70.06±4.91a	75.18±2.75a	72.33±3.95a
Unautoclaved yellow corn	62.49±4.31b	37.47±9.42b	64.08±3.98b	58.37±5.15b	68.25±3.13b	39.15±9.07b
Autoclaved yellow corn	76.05±1.46a	70.21±3.50a	76.42±1.34a	75.09±1.78a	77.39±1.04a	70.61±3.36a
Unautoclaved barley	60.07±3.51b	32.66±7.48b	59.81±3.54b	25.86±8.47b	65.65±2.70b	40.86±6.29b
Autoclaved barley	73.23±2.85a	63.78±6.06a	73.14±2.88a	61.43±6.86a	75.16±2.21a	66.60±5.10a

Different letters in each column indicate significant differences ($P \leq 0.05$) between autoclaved and unautoclaved for each ingredient separately.

Table 7. Apparent digestibility coefficients (%) of diets containing autoclaved feed ingredients, fed to common carp fingerlings.

	Apparent digestibility coefficient ADC					
	Dry matter	Protein	Energy	Carbohydrate	Lipid	Ash
FD	72.92±1.58b	76.77±0.59b	74.61±1.24b	73.11±3.71b	94.73±2.06a	69.18±0.68a
SD	80.18±1.55a	81.50±0.02a	82.90±1.16a	83.33±2.93a	94.20±2.10a	54.57±1.17b
WD	73.46±1.60b	76.35±2.15b	76.73±0.67b	70.78±3.35b	90.97±1.96a	53.35±6.76b
YD	73.75±1.53b	76.63±2.27b	77.08±1.04b	70.26±4.03b	90.58±1.76a	53.79±0.78b
BD	71.79±0.85b	75.06±3.11b	74.86±0.32b	66.67±3.53b	90.22±3.38a	58.45±0.47b
WYBD	80.07±1.27a	75.30±0.19b	81.56±0.97a	85.97±2.62a	95.08±1.67a	64.56±0.36b
AD	82.18±1.22a	84.09±0.04a	84.16±1.19a	83.80±2.74a	93.40±1.23a	65.28±2.62b
CD	72.62±2.53b	76.09±1.10b	75.80±0.99b	72.20±3.46b	89.46±2.07a	49.43±15.09b

Different letters in each column indicate significant differences ($P \leq 0.05$) between each diet and the control diet.

every two treatments containing the same 30% feed ingredient in its raw (unautoclaved) and thermally-treated (autoclaved), each treatment contained three replicates. In the second experiment, a one-way analysis of variance was used to compare treatments, Dunnett's test was used to compare treatments with the control treatment, and each treatment contained two replicates. A significance level of less than or equal to 0.05 ($P \leq 0.05$) was used to judge the presence of significant differences between the treatments and the control.

Results

First experiment

Feed ingredients digestibility: Table 6 shows the ADCs of dry matter and nutrients of autoclaved and unautoclaved feed ingredients. The results of ADCs of dry matter and nutrients in autoclaved feed ingredients (soybean meal, wheat bran, yellow corn, and barley) indicated that the digestibility of autoclaved feed ingredients increased significantly ($P \leq 0.05$) compared to unautoclaved feed ingredients, except in the case of fishmeal, which recorded a slight and insignificant ($P > 0.05$) increase.

Second experiment:

Diets Digestibility: Table 7 shows the ADCs of dry matter and nutrients of diets that contain autoclaved and unautoclaved feed ingredients. The results show that the dry matter digestibility of diets including autoclaved soybean meal (SD) or wheat bran, yellow corn, and barley all together (WYBD), or all five feed ingredients (AD) significantly ($P \leq 0.05$) increased compared to the control diet. The barley diet (BD) had the lowest value of 71.79% for dry matter digestibility, whereas the AD diet had the greatest value of 82.18%.

The highest value of protein digestibility was recorded in the AD, as it reached 84.09%, significantly higher than the control diet (CD). In turn, the SD showed a value significantly higher than the CD (81.50%). The digestibility values were similar for the rest of the diets, ranging between 75.06% in the BD and 76.77% in the FD.

The energy digestibility values showed identical results with the dry matter digestibility, a significant

Table 8. Growth parameters of common carp fingerlings during the second experimental period.

	Initial weight	Final weight	Weight increment	RGR (%)	SGR
FD	15.13±0.34a	20.13±0.22a	5.00±0.12b	33.04±1.56b	0.48±0.02b
SD	15.58±2.88a	24.69±3.27a	9.10±0.38a	59.19±8.49a	0.77±0.09a
WD	16.25±0.64a	21.47±0.25a	5.22±0.39b	32.21±3.68b	0.47±0.05b
YD	16.06±1.76a	22.25±1.87a	6.19±0.11b	38.73±3.53b	0.55±0.04b
BD	14.43±0.10a	19.66±0.94a	5.23±0.84b	36.19±5.56b	0.51±0.07b
WYBD	13.45±0.06a	19.60±0.44a	6.14±0.38b	45.67±2.58b	0.63±0.03b
AD	15.44±1.30a	25.75±0.73a	10.31±0.57a	67.20±9.36a	0.86±0.09a
CD	15.64±0.23a	21.68±0.30a	6.04±0.53b	38.63±3.91b	0.54±0.05b

Different letters in each column indicate significant differences ($P \leq 0.05$) between each diet and the control diet.

Table 9. Feed efficiency parameters of common carp fingerlings during the second experimental period.

	FCR	PER	PPV%
FD	4.66±0.19b	0.71±0.03b	9.81±0.40b
SD	2.91±0.32a	1.13±0.13a	16.26±1.80a
WD	4.79±0.47b	0.69±0.07b	9.46±0.93b
YD	4.08±0.31b	0.81±0.06b	11.22±0.86b
BD	4.35±0.57b	0.76±0.10b	10.32±1.34b
WYBD	3.56±0.16b	0.92±0.04b	12.76±0.59b
AD	2.64±0.28a	1.25±0.13a	17.60±1.84a
CD	4.10±0.35b	0.80±0.07b	10.86±0.92b

Different letters in each column indicate significant differences ($P \leq 0.05$) between each diet and the control diet.

improvement in the values was observed in the SD (82.90%), WYBD (81.56%), or AD (84.16%) compared to the CD. The results of carbohydrate digestibility, in turn, were quite comparable to the results of dry matter and energy digestibility. Its levels ranged from 83.80% in the AD diet to 66.67% in the BD. The differences between the diets concerning the lipid digestibility decreased compared to the differences in the above nutrients, the values ranged between 89.46 to 95.08% in the CD and WYBD, respectively. The FD recorded the highest value (69.18%) for the digestibility of ash, with a significant superiority to the CD (49.43%).

Growth and feed efficiency: The impact of involving autoclaved feed ingredients in diets on the growth rates of common carp fingerlings is shown in Table 8. The weights of the fish at the beginning of the experiment were close among the treatments, ranging between 13.45 and 16.25 g, with no significant differences ($P > 0.05$) between the treatments and the control. Differences in the final weight between the treatments and the control became clear at the end of the experiment after 60 days. The results of weight gain in fish indicated a significant ($P \leq 0.05$) superiority for the AD (10.31 g/fish). The weight gain was 9.10 g/fish in fish fed the SD diet i.e. in second place. The weight gain in the fish was similar in the rest of the treatments, as it ranged between 5.00 g/fish in the FD and 6.14 g/fish in the WYBD, with no significant differences between the treatments and the control.

The relative growth rates and specific growth rates were largely identical except for a slight difference in the case of the FD. The highest values of RGR and SGR were recorded in the AD treatment (67.20% and 0.86%/day, respectively), followed by the SD (59.19% and 0.77%/day, respectively). Although the WYBD did not show a significant superiority compared to the CD, but it was in third place with values of 45.67% and 0.63%/day, respectively.

Table 9 shows the values of the feed efficiency parameters, namely the FCR, PER, and PPV. The best FCR (2.64) was recorded in the AD as significantly superior ($P \leq 0.05$) to the control one, followed by the WYBD (3.56), while the rest of the treatments recorded values ranging between 4.08 and 4.79. About the PER, the AD treatment recorded the significantly highest value (1.25), compared to the control, followed by the SD (1.13) significantly superior to the control one, while the rest of the treatments, whose values ranged between 0.69 and 0.81, showed no significant differences compared to the control group. Similar to the previous two cases, the treatment of AD recorded the highest value (17.60%) of PPV, and significantly compared to the control, followed by the values in the SD (16.26%) and the WYBD (12.76%) treatments. Other values ranged between 9.46 and 11.22%.

Discussions

Digestibility: Thermal treatment with autoclave did not improve or reduce the digestibility of fishmeal, or the nutrients contained in it, especially protein. Studies varied in their results on the effect of thermal treatment of fishmeal on its digestibility. Satoh (2005) reported the effect of thermal treatment on protein digestibility *in vitro* using the pepsin and trypsin enzymes extracted from yellowtail bluefin fish, *Seriola quinqueradiata* fed raw fish or fishmeal> In this work, the activity of the two enzymes extracted from fish fed raw fish had significantly higher than the two enzymes extracted from fish fed fishmeal, and these enzymes worked better on raw fish compared to fishmeal.

The negative effect can be attributed to the fact that thermal treatment can affect protein digestibility in several ways, including the occurrence of the protein denaturation process, as some researchers reported that some amino acids in fishmeal are not used efficiently or become unavailable (Mu et al., 2000, Chu et al., 2015). This was also confirmed by Orisasona (2021), who indicated the possibility of protein denaturation occurring as a result of thermal treatment, and thus it may lead to a reduction in digestibility. Opstvedt et al. (2003) pointed reason for the negative effect of thermal treatment on protein digestibility due to Maillard reactions. Feiner (2006) defines the Maillard reaction, sometimes called browning, which is a non-enzymatic reaction, a complex process resulting from a thermally-induced reaction between reducing sugars and proteins, which reduces the available lysine content and causes a reduction in the nutritional value of the protein. This was confirmed by Morken (2011), who indicated that the decrease in the nutritional value of thermally-treated protein is an indication of the decrease in the protein content of available amino acids and available lysine as a result of Maillard reactions occurring between reducing sugars and the amine group in lysine. In addition, thermal treatment may reduce the solubility of the protein, especially with increased exposure time, which reduces the amount of amino acids released into the digestive tract during the hydrolysis process by digestive enzymes (Arndt et al., 1999).

These negative effects may not occur unless the temperature of the thermal treatment exceeds 150°C, or if the thermal treatment continues for a long period (Takakuwa et al., 2022). Takakuwa et al. (2022) studied the effect of thermal treatment of fishmeal on the digestibility of white trevally fish (*Pseudocaranx dentex*) and noticed that the digestibility did not begin to decrease until the treatment temperature exceeded 150°C. This is also consistent with what was found by Morken *et al*. (2011, 2012) who showed that most of the major nutrients (including protein) in thermally-treated diets fed to *Oncorhynchus mykiss* and *Salmo salar*, respectively, had increased digestibility when the treatment temperature was raised from 110 to 150°C in the case of rainbow trout or from 110 to 141°C in the case of Atlantic salmon. The results of the digestibility of fishmeal in the first experiment were confirmed by the digestibility of diets containing it in the second experiment.

Ma et al. (2018) pointed out that anti-nutritional factors in soybean meal can reduce the digestibility of protein by forming complex compounds containing proteins and minerals and interfering with digestive enzymes such as protein-digesting and carbohydrate-digesting enzymes. In addition, soybeans contain fiber that can reduce its digestibility. The current study's results indicated a significant improvement in the apparent digestibility of dry matter and nutrients of autoclaved soybean meal and for the diet containing it. This improvement in digestibility can be attributed to the effect of thermal treatment. Caprita and Caprita (2010) stated that thermal treatment of soybean meal can reduce the activity of trypsin enzyme inhibitors and thus improve digestibility. Jannathulla et al. (2017) found that the concentrations of trypsin enzyme inhibitors had become imperceptible in autoclaved soybean meal for 20 minutes, in addition to an increase in the nitrogen-free extract NFE in the thermally-treated meal as a result of the hydrolysis of a portion of the complex saccharides (fibers) into dissolved ones, furthermore, phytic acid decreased to 24% of its level in the unautoclaved meal. Ljùkjel et al. (2000) also indicated that even the denaturation process of proteins present in soybean meal occurs when using moderate thermal treatment can lead to improving the digestibility of the protein. Morken et al. (2012) found that the digestibility of protein and many amino acids present in soybean meal thermally treated at temperatures of 110 and 150°C and fed to Atlantic salmon was improved, this was also attributed to the role of thermal treatment in reducing the levels of trypsin inhibitors.

The results of the current study revealed a significant improvement in the apparent digestibility of dry matter, protein, and energy of autoclaved wheat bran, yellow corn, and barley, in the first experiment. The positive effect of autoclave treatment on digestibility can be attributed to several reasons, including starch gelatinization, increased digestive enzymes activity, modification of the dietary fiber content and composition, and decreased levels of anti-nutrients. Vidal et al. (2017) indicated that thermal treatment (extrusion) can improve the nutritional value of wheat and its by-products for juvenile Nile tilapia. Fernandes et al. (2004) also stated that the digestibility in thermally-treated feed ingredients is usually higher due to the occurrence of the starch gelatinization process, which makes carbohydrates more susceptible to the activity of the amylase. Najim and Al-Tameemi (2023) mentioned that there is an improvement in carbohydrate digestibility due to the higher percentage of highly digestible gelatinized starch in bakery waste as a result of their being thermally processed (cooked) during the manufacture of bread. Gao et al. (2019) indicated that starch gelatinization also improves the activity of digestive enzymes. On the other hand, Amirkolaie et al. (2006) indicated that starch gelatinization may reduce feed evacuation, by increasing its viscosity, which gives digestive enzymes a longer time to work. Furthermore, thermal treatment can also change the fiber content and composition of the feed ingredients, as it can lead to a reduction in the total or indigestible fiber content, thus increasing the percentage of soluble fiber and increases the digestibility, thus increasing the amount of digestible energy (Cheng and Hardy, 2003). Takeuchi et al. (1994) pointed out that increasing the level of starch gelatinization in feed ingredients of plant origin can help increase the digestibility of starch and energy. Romano and Kumar (2018) also noted that increasing the level of gelatinization of starch leads to an increase in its digestibility because gelatinized starch contains more sites for attracting digestive enzymes. Also, Irungu et al. (2018) confirm that thermal treatment could enhance digestibility and bioavailability of nutrients.

The digestibility of dry matter and nutrients did not differ significantly in diets containing autoclaved wheat bran, yellow corn, or barley in the second experiment. This could be attributed to the low level of their involvement in the diets (only 15%) for each of them separately. While the positive effect was clear when they were involved together, they constituted 45% of the diet components, and more clearly when the all-autoclaved feed ingredients were involved in the diet.

Growth and feed efficiency: The results in the current study indicated a significant positive effect of thermal treatment in the case of the use of autoclaved soybean meal and all feed ingredients in the diets with regard to the SGR, FCR, PER, and PPV compared to the control group. This could be attributed to their higher digestibility, which was indicated in the second experiment, especially concerning the apparent digestibility of protein and energy. These results are consistent with the results of Hossain et al. (2001) with autoclaved Sesbania in the diets of mirror carp, Gao et al. (2019) with extruded diets in gibel carp, *Carassius gibelio*, Kanmani et al. (2018) with pre-gelatinized starch in the diets of red hybrid tilapia *Orecohromis* sp., Alqabili et al. (2022) with autoclaved soybean meal in the diets of Nile tilapia *O. niloticus*, and Naret (2019) with autoclaved Pea in the diets of Sea bass, *Dicentrarchus labrax*.

Conclusion

The current study concluded that there is a positive effect of autoclaving on the nutritional value of the studied feed ingredients, especially carbohydrate sources (wheat bran, yellow corn, and barley), in addition to its significant positive effect on soybean meal. The current study also did not record any negative effects on the nutritional value of fishmeal regarding the used parameters.

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