COMPARATIVE STUDY REGARDING THE INFLUENCE OF PHYTOBIOTCS AS FEED ADDITIVES ON BIOCHEMICAL COMPOSITION OF *OREOCHROMIS NILOTICUS* MEAT

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Abstract

The aim of this research was to evaluate the biochemical composition of the Nile tilapia meat in conditions of five phytobiotics administrated in feed. This experiment was performed in duplicate. The experimental variants were: V1 – control variant, V2 – 1% chilli pepper (Capsicum annuum) / kg feed, V3 – 1% black pepper (Pipper nigrum) / kg feed, V4 – 1% onion (Allium cepa) / kg feed, V5 – 1% goji fruits (Lycium barbarum) / kg feed and V6 – 1% basil (Ocimum basilicum) / kg feed. For biochemical analysis from muscle tissue, the sampling was performed at the beginning and at the end of the experiment from fresh meat. The results showed a significant differences ($p<0.05$), between variants in which were administered phytobiotics compared with the control variant, in case of the protein content (%), moisture content (%) and dry matter (%). Also, in this paper are presented the dynamics of the nutrient retention efficiency (PER-g/g, PUE-%, RP-g/fish and RL-g/fish) in the muscle tissue of the Nile tilapia. The best results were obtained in the case of the V3 variant regarding to retained protein (RP), PER and PUE and in V5 variant regarding to retained lipid (RL). In conclusion, the use of these phytobiotics in a concentration of 1% / kg feed in the Nile tilapia diet, led to significant changes ($p <0.05$) in the positive sense of the percentage moisture, protein and dry matter from muscle tissue and has contributed to the increasing the nutritional value of fish.

Key words: aquaculture system, basil, biochemical composition of meat, black pepper, chilli pepper, onion, goji, *Oreochromis niloticus*, phytobiotics

INTRODUCTION

Fish are an important animal protein and have been widely accepted as a good source of protein and other elements for the maintenance of health of the human body [19].

In recent years the body composition of fish has received more attention in studies on nutrition, genetics, and health [18] due to an increased interest on the quality and safety of fish products [7]. For this reason the biochemical composition of the fish represents an important aspect of the nutritional quality that can affect the nutritional value and implicitly the quality of the fish meat.

Fish fillet consists of several components, such as moisture, protein, essential amino acids, lipids – fatty acids such as omega, vitamins and minerals, all of which contribute to the overall meat composition [19]. Fish body composition is affected by both exogenous and endogenous factors [1]. Exogenous factors that affect fish body composition include the diet of the fish (composition, frequency) and the environment in which it is reared.

The main exogenous factor affecting proximate composition is diet. Differences in nutritional components of the fish could be as a result of the rate at which these components are available in the particular water body

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[22]. They could also be due to the capacity of the fish to absorb and assimilate the essential nutrients from the harvest water where they habitat or the available diet [10].

Nile tilapia is an omnivorous species and is the second most important group of farmed fish after carps, and the most widely grown of any farmed fish [23].

Due to high-quality proteins, omega-3 fatty acids and amino acids from Nile tilapia meat [3, 9, 20], the consumption of this fish has many benefits for human health in different ways by preventing the heart diseases. Tilapia is actually low in fat, and just contains around 128 calories in each 3.5-ounce bit. Tilapia also helps people to manage the blood sugar-levels [16].

Phytobiotics represent a wide range of bioactive compounds that can be extracted from various plant sources. In recent years, some interesting and novel applications of phytobiotics in the animal production appeared [21].

The aim of this research was to investigate the influence of *Capsicum annuum*, *Pipper nigrum*, *Allium cepa*, *Lycium barbarum* and *Ocimum basilicum* on biochemical composition of *Oreochromis niloticus* muscle tissue, reared in a recirculating aquaculture system conditions.

MATERIAL AND METHODS

The experiment was conducted during a four weeks in the pilot recirculating system of Food Science, Food Engineering, Biotechnology and Aquaculture Department from "Dunarea de Jos" University, Galati.

The recirculating system design includes 12 rearing units, with a volume of 0.15 m^3 each, and a series of water quality conditioning units. The recirculating system was describded in the paper of Petrea et al., 2013 [17]. A total number of 240 of Nile tilapia specimens, with an initial average weight of 100.26±12.19 g/fish, were randomly distributed in 12 rearing units.

Fish were fed with CLASSIC EXTRA 1P pelleted feed, with 41% crude protein and 12% crude lipid. The feed biochemical composition is presented in table 1.

Table 1. Feed biochemical composition CLASSIC EXTRA 1P

Composition	U.M.	Quantity
Protein	%	41
Fat	%	12
Cellulose	%	3
Ash	%	6.5
Total P	%	0.9
Digestible energy	MJ/kg	14.2
Vitamin A	UI/kg	10000
Vitamin D3	UI/kg	1250
Vitamin E	mg/kg	150
Vitamin C	mg/kg	75
Lysine	%	2.4
Methionine	%	0.75
Cysteine	%	0.6
Ingredients: *fish meal, fish oil, hemoglobin, soybean full fat, soybean oil, wheat gluten, sunflower meal, wheat and wheat products, BHT.*		

The feeding frequency was consisted in four meals per day (09:00, 12:00, 15:00, 18:00) with a daily ration of 3% from fish body weight. During the experiment, the feed was supplemented with five phytobiotics (chilli pepper - *Capsicum annuum*, black pepper - *Pipper nigrum*, onion - *Allium cepa*, goji fruits - *Lycium barbarum* and basil - *Ocimum basilicum*), in concentration to 1%. Thus, the experimental variants were organized as follows: V1–control, V2- 1% chilli pepper/kg feed, V3-1% black pepper/kg feed, V4-1% onion/kg feed, V5-1% goji fruits/kg feed and V6-1% basil/kg feed. The introduction of phytobiotics in feed was performed using an aqueous solution of gelatin with 2% concentration.

The sampling for biochemical analysis of muscle tissue was performed at the beginning and at the end of the experiment. When the samples were collected, the uniformity of exemplars was taken into account to eliminate the errors, which consist in weight differences between exemplars. The biochemical determinations were made from fresh muscle tissue samples. To ensure homogeneous samples for the analysis the samples were weighed and minced in a tissue grinder.

The proximate composition of meat was carried out using the Association of Analytical Chemists methods (AOAC).

For the analysis of *Oreochromis niloticus* meat biochemical composition was performed to determine the body percentage of protein content, fat content, ash, dry matter and moisture.

Proteins were determined with Gerhardt equipment by using Kjeldahl method, fats were determined by Soxhlet solvent extraction method (petroleum ether) with Raypa extraction equipment, dry matter was determined by heating at temperature of 105±2ºC using Sterilizer Esac and ash was evaluated by calcification at temperatures of 550±20°C, in a Nabertherm furnace [4].

The technological indicators of fish rearing which involves the biochemical composition of fish muscle tissue are: protein efficiency ratio (PER), protein utilization efficiency (PUE), retained protein (RP) and retained lipids (RL). These indicators were calculated based on following formulas:

Protein efficiency ratio (PER):

[PER] = (Bf-Bi)/(FxPb) (g/g),

where: Bi–initial body weight (g); Bf–final body weight (g); F–quantity of feed administrated (g); Pb–protein quantity from feed (%).

Protein utilisation efficiency:

[PUE] = 100(Bf x Pf – Bi x Pi)/(F x Pb) (%),

where: Pf–final body protein (%); Pi–initial body protein (%).

Retained protein (RP):

[PR] = Bf x Pf – Bi x Pi (g/fish).

Retained lipids (RL):

[RL] = Bf x Lf –Bi x Li (g/fish),

where: Lf–final body lipids (%); Li–initial body lipids (%).

The data were statistically analyzed using descriptive statistics and ANOVA One Way test. Programs used were Microsoft Excell (Office 2010) and SPSS Statistics 20.0 for Windows. The results are presented as mean±standard error.

RESULTS AND DISCUSSIONS

During the experiment, the physico-chemical parameters of technological water were situated into normal range for optimal growth (DO – 8,54±0,91 mg/L; T – 26,22±0,60°C; nitrite nitrogen ($N\text{-}NO_2$) – 0,16±0,01 mg/L; nitrate nitrogen ($N\text{-}NO_3$) – 65,48±1,16 mg/L; ammonium nitrogen ($N\text{-}NH_4$) – 0,16±0,03 mg/L).

The average individual weight, at the end of the experiment registered the following values: V1 – 192.55 g/fish, V2 – 198.25 g/fish, V3 – 188.58 g/fish, V4 – 191.25 g/fish, V5 – 192.00 g/fish and V6 - 180.58 g/fish.

Fish body composition appears to be largely influenced by feed composition. An increase in other parameters, such as feeding rate and fish size, results also in a enhanced adipose deposition and a moisture content decrease of fish body [8].

The main biochemical parameters (moisture, protein content, fat content and ash) of Nile tilapia meat, reared in a recirculating aquaculture system are presented in Fig. 1.

The results showed a significant increase ($p<0.05$) of the protein content (%) in the case of variants in which were administered phytobiotics compared with the control variant (V1), but insignificant compared to the initial moment (Vi). Compared with variant V1 was registered an increase of proteins content (%) with 2.69% in V6 variant, 3.68% in V2 variant, 6.65% in V4 variant, 7.71% in V5 variant, respectively with 12.10% in V3 variant. Protein content (%) ranged from 13.52% to 21.08%, values within the optimal range for Nile tilapia [12].

Concerning to fat content (%), there was no significant difference between experimental variants ($p>0.05$). However, compared with control variant was registered a decrease with 9.94% and 14.91% in V2, respectively in V3 variant, and an increase with 6.21%, 16.77% and 17.39% in V5, V6, respectively in V4 variant.

The results of ash content (%) from muscle tissue indicates an insignificant decrease ($p>0.05$) compared with the initial moment (Vi). Even if at the end of the experimental period was observed a decrease with 3.17% in V5 variant, 1.59% in V2 variant, respectively with 0.79% in V3 variant compared to the control variant (V1) the changes were insignificant ($p>0.05$). This aspect shows that the use of the five phytobiotics did not affect the mineral content of muscle tissue. The recorded values

(1.17 - 1.34% ash) fall within the reference range described in different researches for our species (1.17 - 1.79% ash content)[12].

Regarding the moisture content (%) a significant decrease ($p<0.05$, $p=0.008$) was registered in the case of the V5 variant and V4 variant with 2.26%, respectively with 2.12% in V3 variant, compared with control. In these conditions, we can say that the supplementation of the Nile tilapia feed with these phytobiotics led to improvement of the quality of the fish meat. In Table 2 are presented the values of the main biochemical parameters, of Nile tilapia meat, reported by different authors.

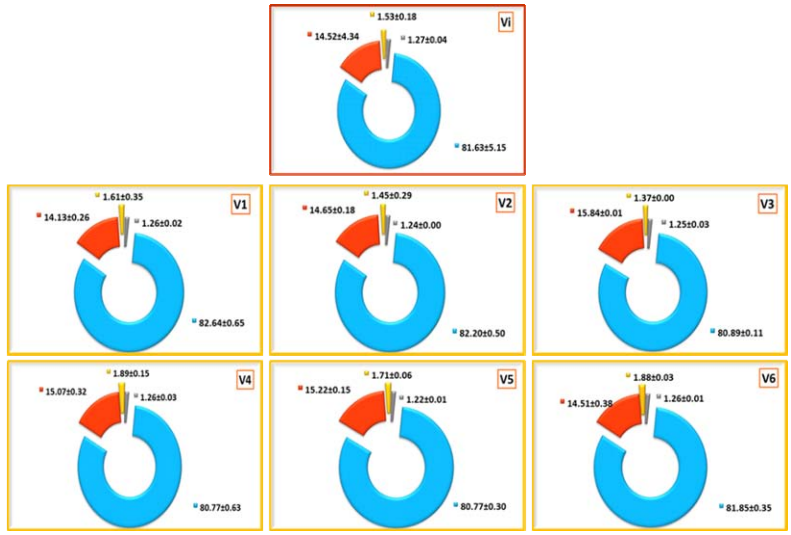

Fig. 1 The biochemical composition of *Oreochromis niloticus* meat, fed with five phytobiotics

Legend: ■ - protein (%); ■ - fat (%); ■ - ash (%); ■ - moisture (%).

Table 2 The biochemical composition of Nile tilapia meat

Reference	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Gaber, 2000 [11]	78.9±0.5	16.6±0.5	2.75±0.16	2.6±0.2
Agbo, 2008 [2]	73.69 ± 0.41	15.18 ± 0.21	6.43 ± 0.14	4.16 ± 0.05
Bag et al., 2012 [5]	75.50±1.21	13.36±0.20	4.60±0.05	5.10±0.06
Bozaoglu and Bilguven, 2012 [6]	80.06±0.11	13.62±0.21	2.47±0.27	2.06±0.12
Mabroke et al., 2013 [14]	74.49±0.3	16.37±1.0	1.16±0.1	7.92±1.1
Jim et al., 2017 [13]	75.3–80.82	13.86–17.12	1.73–3.17	1.76–3.36
Mahmoud et al., 2018 [15]	70.69±0.74	15.71±0.41	5.12±0.31	3.67±0.14

Concerning to the dry matter a significant increase ($p<0.05$) was registered in the variants in which there were administered black pepper (V3 variant) with 5.31% and onion (V4 variant) with 2.78%; respectively a significant decrease ($p<0.05$) in case of goji fruits (V5 variant) with 0.77%, basil (V6 variant) with 3.34% and chilli pepper (V2 variant) with 5.70% compared with the control (V1 variant) (Fig. 2).

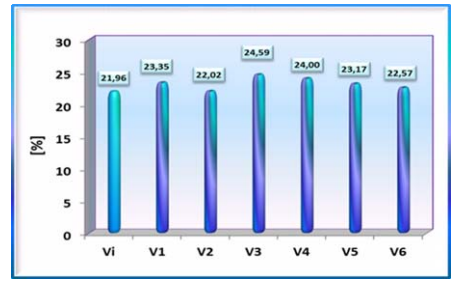

Fig. 2 Dynamics of dry matter content in Nile tilapia meat

Therefore, the muscular tissue of the Nile tilapia from variants V3 and V4 presented a much better nutritional value.

In order to evaluate the efficiency of nutrient retention in the muscle tissue of Nile tilapia, were calculated the following indicators: retained proteins (RP-g/fish), retained lipids (RL-g/fish), protein utilization efficiency (PUE-%) and protein efficiency ratio (PER-g/g). The best results were obtained in case of V3 variant (black pepper) – retained protein (RP), PER and PUE, respectively in V5 variant (goji fruits) regarding to retained lipid (RL) (Fig. 3 and 4).

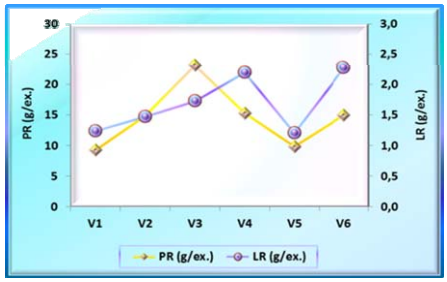

Fig. 3 Retained lipids (RL) and retained proteins (RP) at the end of the experiment

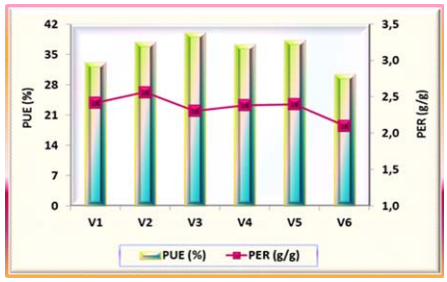

Fig. 4 The protein utilization efficiency (PUE) and the protein efficiency ratio (PER)

In Fig. 4 it is found that the highest efficiency of protein utilization (PUE) was recorded in the variant in which the *Piper nigrum* was used (V3 - 39.99%). Comparing the values obtained with the value registered in the control variant (V1 - 33.15%), there is an increase with 20.63% in variant V3, 15.63% in V5 variant, 14.33% in V2 variant, 12.64% in V4 variant and a decrease with 8.14% in V6 variant.

However, given the fact that the PER indicator has the best value in the variant in which the *Capsicum annuum* was administered, we can say that a good efficiency of nutrient use was also achieved in V2 variant.

CONCLUSION

In conclusion, based on the obtained results, we can see that the use of these phytobiotics in a concentration of 1% / kg feed in the Nile tilapia diet, led to significant changes ($p < 0.05$) in the positive sense of the percentage of moisture, protein and dry matter from muscle tissue.

At the same time, administration of these phytobiotics has contributed to the growth of retained proteins in the muscle tissue. Thus, an improvement in the quality of the meat was achieved by increasing the nutritional value.

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