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# INFLUENCE OF PROBIOTIC FEED ADDITION ON CARP MEAT CHEMICAL COMPOSITION

**Irina Manevska[1*], Ivica Panchevski[2], Natalija Atanasova Panchevska[3], Julijana Arsovska[3], Vasil Kostov[1]**

**[1]Fishery Department, Institute of Animal Sciences, Ss. Cyril and Methodius University, Boulevard Ilinden 92a, 1000 Skopje, Macedonia**
**[2]JPUZPP Jasen, 11 Oktomvri, 1000 Skopje, Macedonia**
**[3]Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University Arhimedova 5, 1000 Skopje, Macedonia**

**[*]e-mail: irinamanevska@yahoo.com**

## Abstract

For the first time in our country, an attempt for intensive carp production reared in an intensive cage culture farm was made, without the use of antibiotics and chemical treatments i.e. probiotic food supplementation in carp production. This experiment aimed to assess the effect of the probiotic *Paenibacillus alvei*, added to carp food on meat chemical composition.

The experiment was set in reservoir Kozjak, Macedonia on a registered aquaculture production subject for 180 days. *Paenibacillus alvei* DZ-3 was incubated in NB (nutrient broth) at 37$^0$C/24h/180 rpm. The biomass was collected at 4000 rpm/15 min. and washed with 5 mL of PBS (phosphate buffer) (pH = 7.2) twice consecutively. After that, it was diluted in PBS to 1.5 x $10^8$ CFU/mL (= Mcfarland 0.5). There were three experimental groups (A, B, and C). Probiotic concentration for groups A and B was 1 mL/kg food and 2 mL/kg, respectively. Group C was a control group and fish were fed without probiotic addition. The handling procedure of commercial food and probiotic addition was carried out by spraying, mixing, and drying. The percentage representation of fat, protein, moisture, and ash in the meat of common carp (*Cyprinus carpio*) was determined according to standard methods. These research results were analyzed through the ANOVA variance test, (Principal component analysis-PCA), Tucky test (post-hoc analysis) descriptive statistical presentation through the measures of dispersion: (min-max), standard deviation (sd), and coefficient of variance (CV).

The protein percentage of fish from group B, fed with a higher concentration of probiotics, presented higher values compared to control group C. Fat percentage in meat of group A fed with lower probiotic concentration presented higher values, compared to the control group. The moisture results showed a statistically significant difference ($p < 0.05$) between groups A and B, but not with group C. Ash percentage did not present statistical significance between all three groups.

The highest percentage of proteins was presented in group B. It was concluded that a higher concentration of probiotics added to fish food affects the higher percentage representation of proteins in carp meat.

***Key words***: *Probiotic, Food, Carp, Cage system, Meat chemical composition.*

## 1. Introduction

Following the increased trend line of fish meat market demand, worldwide aquaculture producers are focused on intensive cage fish rearing. It is a modern method of intensive cultivation of freshwater fish species and is popular due to its many advantages, in contrast to conventional methods of cultivation of freshwater fish species (De Silva *et al.*, 1991) [1]. The carp *(Cyprinus carpio)* is the most suitable and adequate species, characterized by relatively fast growth and development, good taste and quality of the meat, and high demand in the Balkan countries market.

The intensive cultivation of carp, in addition to all the advantages, also brings an increased incidence of diseases, and thus a significantly increased need for the application of medicines, especially antibiotics, as well as regular use of disinfectants (Chen *et al.*, 2014) [2]. Experience over the years has shown that the application of antibiotics, causes many negative, unwanted consequences, such as nephrotoxicity (Hentschel *et al.*, 2005) [3], immunosuppression, and immunomodulation (Nayak *et al.*, 2007) [4], and their residues accumulation in fish tissues are causing carcinogenic effects in many teleosts (Gatesoupe, 2007) [5], as well as environmental hazards (Allameh *et al.*, 2015) [6].

Since antibiotics application is usually added through food, fish do not metabolize them efficiently and 70 - 80% of applied antibiotics are released through urine and feces, as well as unused food with antibiotics into the water, which is why it is difficult to imagine their harmfulness to the aquatic ecosystem (Burridge *et al.*, 2010) [7].

As a result of the current knowledge about the negative effects of the use of antibiotics in aquaculture, in recent years there has been a pronounced trend and effort among fish producers to reduce their use in fish rearing. The science is intensively committed to establishing a new alternative approach, with the main goal of increasing the resistance of fish to diseases, i.e. to improve the immune system without the use of antibiotics. In that relation, "antibiotic-free" fish meat results in healthy fish meat. One of the alternative approaches in aquaculture development is the addition of probiotics to the fish diet.

According to the definition of the World Health Organization (WHO), probiotics are living microorganisms, or rather "living microbial supplements" that, when taken in an adequate amount, contribute to the health of the recipient organism, Merriefield *et al.*, (2010) [8], defined them as "microbial cells provided through the diet or the water and have a benefit on the fish, the breeder or the consumer", ensuring the improvement of fish appetite, growth, utilization of food, improvement of meat quality and its composition and reduction of malformations.

An effective probiotic should contain the following properties: resistance to variations in pH and acids; non-pathogenicity; feasibility; stability during field storage; possibility of survival and potential colonization in the intestinal system; wide variety cultivation; adhesion ability of the intestinal tract epithelium and beneficial effect on the fish organism (De *et al.*, 2009) [9].

Probiotics most commonly used in aquaculture are those belonging to the genus Bacillus spp. *(B. subtilis*, *B. licheniformis*, *and B. circulans*), then *Bifidobacterium* spp. (*B. bifidum*, *B. lactis*, and *B. thermophilum*), lactic acid bacteria (*Lactobacillus* spp., and *Carnobacterium* spp.), and the yeast *Saccharomyces cerevisiae* (Lee *et al.*, 1999) [10], and (Sanders *et al.*, 2001) [11].

According to literature data, regarding the protein content, it is concluded that the total protein fractions in fish are quite stable concerning age (Suzuki, 1981) [12]. During the ontogeny of the carp, there is an initial increase in the protein content of the whole body and the musculature, until reaching values of 16 - 19% (Takeuchi *et al.*, 1979) [13], and (Hossain *et al.*, 1989) [14].

The addition of probiotics to fish feed affects the increase of fat and protein content (Allameh *et al.*, 2017) [6] in fish meat. The increase in the percentage of protein in the body of fish is most likely the result of the activity of probiotics that improve digestion and absorption of nutrients by increasing enzyme activity in the digestive system of fish (Ali *et al.*, 2018) [15]. According to the authors, probiotics can synthesize extracellular enzymes such as amylases, proteases, and lipases that improve the breakdown of food, and they can also synthesize growth factors such as vitamins, amino acids, and fatty acids that affect the efficiency of food absorption. As a result, there is a deposition of amino acids and peptides that affect the increase in the percentage of total proteins in the meat of the carp. Also, the secretion of proteins by probiotics in the digestive system of fish, and the efficient conversion of absorbed food into structural protein parts affect an increased percentage of proteins in fish muscles (Opiyo *et al.*, 2019) [16].

The previous research and the results obtained by previously mentioned authors led us to set up an experiment whose aim was in addition to the production characteristics, we wanted to determine the chemical composition of the carp meat reared in an intensive cage system in reservoir Kozjak.

## 2. Materials and Methods

The experimental carp individuals were fed with probiotics added to feed. The fish weren't treated with chemicals, antibiotics and disinfectants.

The experiment was performed on a registered production facility cage farm, located in the "Kozjak" reservoir. To carry out the research, we separated three partitions/cages with dimensions 5 x 5 x 5 m, that is, 3 x 125 $m^3$ volume (A, B, and C). Before starting the experiment, a preparatory phase was conducted.

At the beginning of the experiment, in the chemical laboratory of the UKIM Institute of Animal Science and Fishery, the food was controlled and its chemical composition was examined.

As a basis in the diet of the carp in the experiment commercial, pelleted food from a renowned manufacturer "Aqua" from Austria, with a pellet size of 4 - 6 mm, was used. The chemical composition of the commercial feed had the following declared values: protein 30%, fat 10%, crude fiber 4.5%, crude ash 6.5%, calcium (Ca) 0.90%, sodium (Na) 0.25%, and phosphorus (P) 1.10%.

According to standard chemical methods following parameters were analyzed:
- Moisture by drying in an oven at a temperature of 105 $^0$C to constant weight;
- Raw fiber;
- Crude proteins according to the Kjeldahl method (N x 6.25);
- Crude fats through extraction with diethyl ether according to the Soxhlet method;
- Crude ash through combustion in a furnace for 8 hours at a temperature of 600 $^0$C.

Bacterial culture - probiotic *Paenibacillus alvei* DZ-3, in strictly defined quantities, was added to the basic food in the form of pellets, with a special procedure.

The production and preparation of probiotics was carried out in the laboratory at the Department of Microbiology and Microbial Biotechnology at the Faculty of Natural Science and Mathematics in Skopje.

The preparation of the probiotic was carried out through the following procedure: *Paenibacillus alvei* was used, i.e. its 24-hour culture, at 37 $^0$C. Furthermore, culture multiplication was performed in NB (nutrient medium) at 37 $^0$C/24h/180 rpm. The biomass is collected at 4,000 rpm/15 min. and then washed with 5 mL of phosphate buffer (PBS) (pH = 7.2) twice consecutively. It is then diluted in PBS to 1.5 x $10^8$ CFU/mL (= Mcfarland 0.5). The probiotic is applied in two concentrations: 1 mL/kg and 2 mL/kg of food.

The preparation of the food with added probiotic took place in the following way: the probiotic in a liquid form was sprayed onto the pelleted food in a mixer for 3 minutes; and then mixed in a mixer for 5 minutes. The mixed food with the added probiotic was applied evenly distributed in a layer of 2 cm, in a dry, ventilated place to dry for 2 hours.

Food with probiotics for experimental groups A and B was prepared every two weeks, and daily rations were determined according to the table prescribed by the food manufacturer, depending on the water temperature and body weight of the fish.

The setup of the experiment started on 16.04.2018 and lasted until 30.09.2018 (one growing technological season). Before the start of the experiment, the sorting and selection of the biological material (1,000 carp individuals), necessary for the formation of the three experimental groups, was carried out. The sorted and separated individuals had an average body mass of 170 g. The same number of carp individuals (323), were stocked in each cage. After stocking the fish into separate groups, the total ichthyomas in each group were determined, which represented the initial weight of the groups.

In the food for the first experimental group A, probiotic was added at 1 mL/kg of food with a concentration of 1.5 x $10^8$ CFU/mL (colony forming units/mL).

In the food for the second experimental group B, a probiotic of 2 mL/kg of food was added, with a concentration of 1.5 x $10^8$ CFU/mL (colony-forming units/mL).

The third group C was the control group and the fish were fed with commercial food without the use of probiotics.

The fish were fed with automatic feeders for 24 hours.

The obtained results were statistically analyzed through the ANOVA variance test, Tuckey test (post-hoc analysis) descriptive statistical presentation through the measures of dispersion: (min-max), standard deviation (SD), and coefficient of variance (CV).

## 3. Results and Discussion

One of the goals of this experiment was to determine the probiotic influence on the chemical composition of carp meat. After standard chemical analyses, results of the chemical composition of fish meat from experimental groups A and B were compared with control group C. As mentioned, the percentage representation of the basic chemical parameters, moisture (v), ash (p ), protein (b), and fat (m), on 10 randomly selected individuals from each group (A, B, and C) extracted from three body regions: back (B), abdomen (A) and tail region (T). Meat tissues extracted for fish back, abdomen and tail sections were processed by standard chemical analysis. Data obtained was analyzed through descriptive statistics, in which the central tendency and dispersion values for each chemical parameter presented as moisture (M), ash (A), crude protein (P), and fat (F) are presented in Table 1.

**Table 1. Central tendency and dispersion values of analyzed chemical parameters of meat tissue samples**

| Group | Meat region | Chemical parameter % | Minimum | Maximum | $\bar{x}$ | Variance (n-1) | (SD) (n-1) | CV |
|---|---|---|---|---|---|---|---|---|
| A | B | M | 75.7900 | 78.1000 | 77.1730 | 0.5341 | 0.7308 | 0.0090 |
| | | A | 0.9500 | 1.0800 | 1.0170 | 0.0026 | 0.0508 | 0.0474 |
| | | P | 16.9500 | 18.6300 | 17.6610 | 0.2976 | 0.5456 | 0.0293 |
| | | F | 1.1900 | 5.5600 | 3.2790 | 2.5089 | 1.5839 | 0.4583 |
| | A | M | 72.0400 | 78.5000 | 76.0940 | 4.4984 | 2.1209 | 0.0264 |
| | | A | 0.8700 | 1.0200 | 0.9470 | 0.0021 | 0.0457 | 0.0458 |
| | | P | 17.1500 | 18.3300 | 17.6590 | 0.1821 | 0.4268 | 0.0229 |
| | | F | 2.8300 | 6.0000 | 4.5150 | 1.1954 | 1.0933 | 0.2297 |
| | T | M | 75.2700 | 78.3800 | 76.6490 | 0.7148 | 0.8455 | 0.0105 |
| | | A | 0.9200 | 1.1100 | 0.9920 | 0.0025 | 0.0496 | 0.0475 |
| | | P | 17.0100 | 18.9800 | 17.8100 | 0.4556 | 0.6749 | 0.0360 |
| | | F | 3.3200 | 6.0400 | 4.3800 | 0.7334 | 0.8564 | 0.1855 |
| B | B | M | 76.3400 | 78.1900 | 77.4890 | 0.3931 | 0.6270 | 0.0077 |
| | | A | 0.7300 | 1.2000 | 1.0200 | 0.0180 | 0.1342 | 0.1248 |
| | | P | 18.8700 | 21.1200 | 19.8940 | 0.5889 | 0.7674 | 0.0366 |
| | | F | 1.3400 | 2.6300 | 1.9140 | 0.1277 | 0.3573 | 0.1771 |
| | A | M | 76.5800 | 78.9000 | 77.6940 | 0.5660 | 0.7523 | 0.0092 |
| | | A | 0.8900 | 1.1000 | 0.9850 | 0.0034 | 0.0584 | 0.0562 |
| | | P | 18.6500 | 21.0600 | 19.6850 | 0.5313 | 0.7289 | 0.0351 |
| | | F | 2.4000 | 3.3000 | 2.8140 | 0.0998 | 0.3160 | 0.1065 |
| | T | M | 76.3300 | 78.6200 | 77.3340 | 0.4737 | 0.6883 | 0.0084 |
| | | A | 0.8900 | 1.0300 | 0.9570 | 0.0030 | 0.0550 | 0.0545 |
| | | P | 17.9300 | 19.9600 | 19.4250 | 0.4181 | 0.6466 | 0.0316 |
| | | F | 2.0600 | 3.7800 | 2.8580 | 0.2241 | 0.4734 | 0.1571 |
| C | B | M | 75.0000 | 78.2200 | 77.2900 | 0.9488 | 0.9741 | 0.0120 |
| | | A | 0.9300 | 1.0500 | 0.9840 | 0.0016 | 0.0406 | 0.0391 |
| | | P | 16.5700 | 17.7800 | 17.1430 | 0.1610 | 0.4013 | 0.0222 |
| | | F | 1.8700 | 2.9300 | 2.4270 | 0.2161 | 0.4649 | 0.1817 |
| | A | M | 75.7700 | 78.5100 | 77.2820 | 0.8066 | 0.8981 | 0.0110 |
| | | A | 0.9500 | 1.0400 | 0.9770 | 0.0008 | 0.0291 | 0.0282 |
| | | P | 15.9900 | 17.8300 | 16.8310 | 0.4069 | 0.6379 | 0.0360 |
| | | F | 1.4800 | 4.4500 | 2.7800 | 0.6569 | 0.8105 | 0.2766 |
| | T | M | 74.3300 | 78.8700 | 76.6100 | 1.8276 | 1.3519 | 0.0167 |
| | | A | 0.9500 | 1.0700 | 1.0050 | 0.0015 | 0.0387 | 0.0365 |
| | | P | 15.9100 | 17.9600 | 16.9200 | 0.2763 | 0.5257 | 0.0295 |
| | | F | 2.0200 | 5.2600 | 3.1430 | 1.0290 | 1.0144 | 0.3062 |

Legend: *G-back; S-abdomen; O-tail tissue. M-moisture; A-ash; P-proteins; V-fats.

Presented values of the standard deviation from Table 1, it can be noted that fats are characterized by significantly variable values, as well as a high standard deviation, concerning the other chemical parameters in the tissue regions of all the studied groups.

Univariate ANOVA, on the logarithmic chemical parameters, showed that proteins accounted for most of the overall variance between experimental groups Table 2, (F-ratio). The determination of the statistically significant difference ($p < 0.05$) between the studied groups was performed with a post-hoc Tucky test table ANOVA. The analysis of variance showed that the percentage representation of proteins differs between all three experimental groups, with a statistically significant level of $p < 0.05$. The fat percentage of group A was statistically significantly different from the fat of group B and the control group C. Analysis of the percentage of moisture showed that there was a statistically significant difference between groups A and B, but not with control group C. The percentage representation of ash, with this analysis, did not show a statistically significant difference between all three groups.

**Table 2. Analysis of variance in experimental groups (F-ratio)**

| Chemical parameter | F - ratio |
|---|---|
| Protein | 160.0637 |
| Fat | 14.9726 |
| Moisture | 4.5978 |
| Ash | 0.0470 |

Regarding the chemical composition of the meat, from the fish treated with a higher concentration of probiotics, the percentage of proteins was with higher values in group B 19.66%, and compared to the control group it differs by 13.74% where they were represented by 16.96%. Regarding fats, the probiotic with the lower concentration singles out group A with a higher percentage of 4.05%, compared to the control group it differs by 31.36% where they are represented by 2.78%. The analysis of moisture showed that between groups A and B there is a statistically significant difference ($p < 0.05$), but not with group C. The representation of ash is not a carrier of statistical significance between all three groups. Regarding the chemical parameters from the analyses of the separate tissue regions, statistical significance ($p < 0.05$) was shown by the values of proteins from (BBp), representation of fats from (AAf) and (ATm), and moisture from the region (A) of the groups A and B.

What can be concluded is that fats are distinguished by the greatest variability, proteins by less variability, but as a parameter contribute the most to a difference with statistical significance among all experimental groups A, B, and C.

Similarities in the protein representation in the meat can be observed in comparison with the research of Izci, (2010) [17], who found that the crucian carp contains 17.99 ± 0.38% protein, while Ozyilmaz *et al.*, (2016) [18], in their research, noted the representation of protein in the meat was 19.43 ± 0.21%. A higher protein percentage in carp meat (20.23 ± 0.31%) was also found by (Mahdi *et al.*, 2006) [19]. The results of the protein analysis from this research also correspond to the research by Ljubojević *et al.*, (2013) [20], who ascertained the protein values of 17.30 ± 0.39%.

As for the representation of fats, it is noted that they are characterized by a high standard deviation. Regarding the tissue representation of the chemical parameters within each experimental group, there is no difference, except in the tissue dorsal region (BBm), where fats are represented with a lower value with a statistically significant difference ($p < 0.05$).

Table 3 presents the values of the percentage representation of proteins from all tissue regions within the experimental groups.

**Table 3. Protein percentage content in carp meat from tissue sections in groups A, B, and C**

| Group | Proteins in meat sections % | | |
|---|---|---|---|
| | Back section | Abdomen section | Tail section |
| A | 17.66 | 17.66 | 17.81 |
| B | 19.89 | 19.69 | 19.43 |
| C | 17.14 | 16.83 | 16.92 |

The results showed that experimental group B stands out with a higher percentage of proteins in all three tissue regions (B, A, T) and that is 19.89%; 19.69%, and 19.43% respectively. Experimental group A is represented by a lower percentage representation of proteins in the tissue regions compared to group B, namely in the back and abdomen region are 17.66%, while the percentage of the same in the abdominal region is 17.81%. Group C is represented by the lowest representation of proteins, 17.14%, 16.83%, and 16.92% for the dorsal, abdominal, and tail regions, respectively.

The fat percentage representation in the meat tissue sections is presented in Table 4.

**Table 4. Fat percentage content in carp meat from tissue sections in groups A, B, and C**

| Group | Fats in meat sections % | | |
|---|---|---|---|
| | Back section | Abdomen section | Tail section |
| A | 3.28 | 4.52 | 4.38 |
| B | 1.91 | 2.81 | 2.86 |
| C | 2.43 | 2.78 | 3.14 |

Regarding this parameter the results presented a higher fat percentage in fish from group A, fed with 1ml/kg probiotic enriched food. In the back-section fats were represented by 3.28%, while the stomach and tail sections were represented by a higher fat percentage, 4.52%, and 4.38% respectively. Fish fed with commercial food without probiotic addition (control group C) presented the lowest fat percentage in the back, abdomen, and tail sections with values of 2.43, 2.78, and 3.14 respectively.

Total protein and fat content (%) in carp meat groups A, B, and C are presented in Table 5.

**Table 5. Total protein and fat content (%) in carp meat in experimental groups A, B, and C**

| Group | Total protein content% | Total fat content% |
|---|---|---|
| A | 17.71 | 4.05 |
| B | 19.66 | 2.52 |
| C | 16.96 | 2.78 |

The highest percentage of fat in the meat of the individuals from the experimental group A is 4.05%. The values for groups B and C are shown with a lower percentage of fat in the meat, 2.52% and 2.78% respectively (Table 5).

Factors that indirectly stimulate the increase in the nutritional regime, and thus the representation of fat, are temperature, space of movement of fish, and steroid supplementation (Lone and Matty, 1984) [21], (Sathynarayana Rao *et al.*, 1988) [22], (Basavaraja *et al.*, 1989) [23], and (Viola *et al.*, 1992) [24]. The lowest percentage of fat is found in fish grown in open waters (Kurćubić *et al.*, 2017) [25].

Ali *et al.*, (2018) [15], indicated that probiotics introduced into the body of fish through the diet can affect the increase in fat estimation, due to their ability to synthesize extracellular enzymes such as lipases. Together with other growth stimulants (vitamins, amino acids, fatty acids), they improve food breakdown and nutrient absorption.

Variations of the protein component are smaller and range from 14 - 18% (Vladau *et al.*, 2008) [26], (Trbović *et al.*, 2009) [27], and (Ćirković *et al.*, 2011) [28]. The percentage of protein in the meat of different fish species ranges from 12% to 24% (Huss, 1995) [29]. The total percentage representation of proteins in the meat from all three experimental groups is presented in Table 6. The values of this chemical component for groups A, B, and C are 17.71%; 19.66%, and 16.96 respectively. The results present that experimental group B stands out with a higher percentage of protein in meat compared to group A, while control group C is represented by the lowest percentage of protein in meat.

In our experiment, the highest percentage of moisture was determined in the samples taken from group B and was 77.50%, while the lowest percentage of moisture was recorded in group A, with a value of 76.63%. The content of moisture in the meat of carp from the experimental groups and the control group is within approximate limits with the results of Ljubojević *et al.*, (2013) [20], who ascertained a percentage of moisture in the meat of carp grown in a semi-intensive farming system, with a value of 78 .1% ± 1.09%. The same authors determined a moisture content of 70.32% ± 1.00% in the meat of carp grown in a cage system in Vrbas, Serbia.

The percentage representation of ash in our experiment showed no differences between the experimental groups, and the mean value for all three experimental groups was 0.98%.

From the research of Ljubojević *et al.*, (2013) [20], regarding the value of percentage of ash in fish meat, the highest value of 1.96% was determined, while the lowest value of ash content was determined in carp meat 0, 82%. According to Kurćubić *et al.*, (2017) [25], the percentage of ash in carp meat is 1.02%, i.e. 0.82%, and it is noted that there are no significant differences from those values from the Ečka pond (1.04 ± 0.02 %) and the cage system of the Vrbas pond (0.88 ± 1.00%). According to Opiyo *et al.*, (2019) [16], probiotics do not affect the change in the percentage of ash and moisture in fish meat.

In general, the chemical composition of carp meat is highly variable. These differences result in different fish categories, rearing methods, diet and types of food, stocking densities, and production systems.

## 4. Conclusions

- Obtained experiment results led to the conclusion that probiotic addition in carp food with a higher concentration (2ml/kg food) has fulfilled our set goal towards determination of its influence on protein and fat content. Regarding protein percentage probiotic food addition influenced increased values. Fat percentage was influenced by probiotics concerning low-fat carp meat.
- The highest value of total protein percentage in the carp meat of 19.66% was recorded in experimental group B (carp individuals fed with higher probiotic concentration).
- The lowest value of fats percentage in the carp meat of 2.52%, was recorded in experimental group B.
- Experimental groups A and B regarding percentage representation of moisture, present statistically significant differences between these two groups, but not concerning the control group C.
- The percentage representation of ash in our experiment presented no differences between all experimental groups, with the mean value for all three experimental groups being 0.98%.

**Table 6. Mean values of percentage content of total chemical parameters of experimental groups**

| Parameters | Moisture | Ash | Proteins | Fats |
|---|---|---|---|---|
| **Group A** | 76.63 (1.40)$^{b}$ | 0.98 (0.05)$^{a}$ | 17.71 (0.54)$^{b}$ | 4.05 (1.30)$^{a}$ |
| **Group B** | 77.50 (0.68)$^{a}$ | 0.98 (0.09)$^{a}$ | 19.66 (0.71)$^{a}$ | 2.52 (0.57)$^{b}$ |
| **Group** | 77.06 (1.10)$^{ab}$ | 0.98 (0.03)$^{a}$ | 16.96 (0.52)$^{c}$ | 2.78 (0.82)$^{b}$ |

Legend: *Small superscript characters present statistical difference ($p < 0.05$).

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