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EFFECT OF FEEDING RATE ON MEAT BIOCHEMICAL COMPOSITION OF *ACIPENSER STELLATUS* (PALLAS, 1771) REARED IN A RECIRCULATING AQUACULTURE SYSTEM

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Abstract

The meat nutritional value is an important factor that influence consumer preferences for certain fish species. Thus, the aim of this study is to identify the influence of feeding rate on stellate sturgeon meat biochemical composition, assessed by determining the percentage of dry matter, crude fat, crude protein and ash. Two feeding rates were tested (F1 - 1% BW, respectively F2 - 2% BW), in replicate. The biochemical evaluation of stellate sturgeon meat revealed better values in terms of crude protein content at F2 duplicate (17.86±0.14 %, respectively 17.70±0.17%), compared to F1 experimental variant duplicate (16.71±0.2%, respectively 16.78±0.18%). Also, F2 registered a higher crude fat (1.32±0.04%, respectively 1.36±0.03) and ash content (1.69±0.06%, respectively 1.77±0.09), compared to F1 (crude fat: 1.21±0.04%, respectively 1.27±0.05%; ash: 1.33±0.10%, respectively 1.39±0.11%). The water-to-protein ratio indicate a high nutritional value for meat of stellate sturgeon exemplars, reared by applying a feeding rate of 2% BW (4.31±0.15, respectively 4.35±0.17 at F2 duplicate, compared to 4.71±0.11, respectively 4.67±0.10 at F1). As a conclusion, the use of 2% BW feeding rate for rearing stellate sturgeons in a recirculating aquaculture system (RAS) assures a superior protein and fat retention and improves protein use efficiency (PUE).

Key words*: biochemical composition, crude protein, feeding rate, RAS, stellate sturgeon.*

INTRODUCTION

Aquaculture has a key contribution in maintaining the balance of global economy, considering the present worldwide status, which is characterized in Cao and Li (2013) study by rapid population expansion, urban development, improved quality of life in most parts of the world and continuous increase of demand for animal protein. Thus, intensive production fish farms may represent a solution for above mentioned challenges, only if proper technical and technological solutions are applied, in order to maximize their economic performance.

In order to be able to assure continuous intensive production over the entire year, aquaculture farmers must adopt recirculating aquaculture systems (RAS). Several studies, characterized RAS as high capital and operating costs, compared to other systems such as cage culture in natural waters and raceway and/or pond culture systems. Therefore, as Petrea et al. (2019) stated, most aquaculture facilities based on RAS are focusing on rearing high economic value fish species as sturgeons, turbot, salmon, rainbow trout or tilapia, in order to maintain the profitability (Cristea et al., 2002; Engle et al., 2010; Timmons et al., 2018).

The fish rearing technologies applied in RAS are based both on high stocking densities and feeding rates, in order to maximize the productivity and, therefore, the profitability of aquaculture facilities. However, beside assuring a high productivity, the main goal of fish rearing technologies is to maintain or even improve the nutritional value of fish meat. According to Dorojan et al. (2014), the biochemical composition of fish can be significantly influenced by the composition of administrated feed, fish development stage, or rearing

conditions. Antache et al. (2013) revealed that fish body composition is largely influenced by feed composition. An increase in other parameters such as feeding rate and fish size also results in enhanced adipose deposition and decreases water content in the fish body (El-Zaeem et al., 2012). Therefore, the aim of present study is to identify the influence of feeding rate on stellate sturgeon (201.72±32.72 g) meat proximate composition, reared in RAS.

MATERIALS AND METHODS

The description of RAS Pilot Station

The present study was conducted in RAS pilot station of Food Science, Food Engineering, Biotechnology and Aquaculture Department, Food Science and Engineering Faculty - „Dunărea de Jos'' University of Galați, during a 28 days experimental period.

The configuration of the pilot RAS was sized according to specific technology described by Cristea (2008). The RAS pilot system consists in a number of four octagonal rearing units, connected to water conditioning modules, as described in Figure 1.

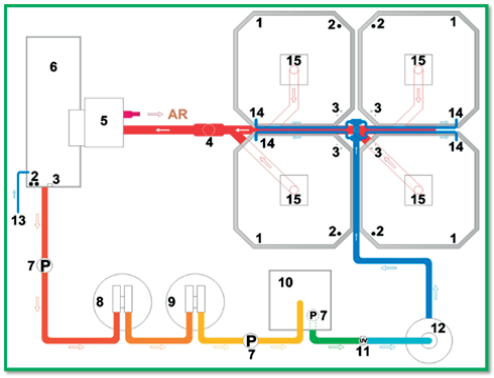

Figure 1. The design of RAS pilot station: rearing units - No.1, nitrogen compounds sensors - No.2; water level sensors – No.3; RAS outlet structure – No.4; mechanical drum filter – No.5; sump – No.6; pumps–No.7; sand filter – No.8; activated charcoal filter – No. 9; biological trickling filtration unit – No.10; sterilization UV filter – No.11; oxygenation unit–No.12; automatically fresh water inlet – No.13; rearing units water inlet/outlet structure–No.14, 15 (Petrea et al., 2019)

Biological material and experimental design

The fish biomass composed of 92 specimens of stellate sturgeon (201.72±32.72 g), which are the subject of the present study, was equally distributed within the four rearing units B1, B2, B3, B4 (4 rearing units x 23 specimens). Two feeding rates were tested (F1 - 1% biomass weight - BW, respectively F2 - 2% BW), in replicate (F1 in B1, B2, respectively F2 in B3, B4).

Feed biochemical composition

During the experimental period, Classic extra 1 P pellets were administrated by using automatic feeders. The feed biochemical composition is presented in Table 1.

Table 1. The biochemical composition of administrated feed

Crt.no.	Composition	Quantity
1	Crude Protein %	41.0
2	Crude fat%	12.0
3	Crude cellulose %	3.0
4	Crude ash %	6.5
5	Phosphorus %	0.9
6	Digestible energy (MJ/kg)	14.2
7	Vitamin A (UI)	10000
8	Vitamin D3 (UI)	1250
9	Vitamin E (mg)	150
10	Vitamin C (mg)	75
11	Cystine%	0.6
12	Lysine %	2.4
13	Methionine %	0.75
Note:	Fish meal. fish oil. haemoglobin. full fat soybean. soybean oil. wheat gluten. sunflower flour. wheat and wheat products. BHT.	

Water quality assessment

In order to determine the water quality parameters throughout the experimental period, temperature, dissolved oxygen (DO) and pH were measured daily. Also, the rest of water quality parameters, presented in Table 2, were determined twice per week. The water quality evaluation methods and equipment are presented in Table 2.

Table. 2. Water quality evaluation methods and equipment

Analysed Parameter	Method	Equipment
DO pH Temperature	Sensor method	HQ40d Portable Multi-Parameter (HACH)
NO_3 NO_2 NH_4 PO4 COD	Spectrophotometric method using Merk kits	Spectroquant photometer, Nova 400
Percentage removal of BOD_5	Winkler`s method	Velp IP54 analyzer
Turbidity	Spectrophotometric method	Turbidometer VELP, TB1.

Stellate sturgeon meat proximate composition assessment

The determination of stellate sturgeon meat proximate composition was made both in the initial and final stage of experimental period, from fresh meat tissue. During sampling process, ensuring the uniformity of the analysed exemplars was targeted, in order to eliminate the possible errors due to biomass differences. The proximate composition analyses were performed on homogenized muscle tissue by using the Association of Analytical Chemists (AOAC) methods (AOAC, 2000).

Chemical composition of meat crude protein was determined according Kjeldahl method (N x 6.25). Crude lipids were determined according to Soxhlet solvent extraction method (petroleum ether). Dry matter was determined by heating the muscle tissue samples at a temperature of 105±2ºC, using Sterilizer Esac, while ash was evaluated by calcification at a temperature of 550±20ºC in a Nabertherm furnace.

Stellate sturgeon meat quality indicators

The main stellate sturgeon meat quality indicators were determined as follows:

1. $PUE = 100 (W_f \times P_f - W_i \times P_i)/(F \times P_b)$ (%), where: PUE - Protein utilization efficiency; P_f - muscle tissue protein at the end of experimental period (%), P_i - muscle tissue protein at the initial stage of experimental period (%); W_f – final biomass (kg); W_i – initial biomass (kg); F – total feed quantity consumed (kg); P_b – administrated feed protein concentration. (%).
2. $PR = W_f \times P_f - W_i \times P_i$ (g/specimen), where: *PR* - retained protein
3. $LR = W_f \times L_f - Wi \times L_i$ (g/specimen), where: LR- retained lipids; L_f = muscle tissue lipids at the end of experimental period (%), L_i = muscle tissue lipids at the initial stage of experimental period (%)

Statistical methods

Statistical analysis was performed using the IBM SPSS Statistics 20 for Windows. Statistical differences between treatments were tested using T test ($\alpha = 0.05$) after a normality test (Kolmogorov-Smirnov). Comparisons between variants were assessed using post-hoc Duncan test for multiple comparisons (ANOVA).

RESULTS AND DISCUSSIONS

Water quality parameter

During the experimental period, the technological water quality parameters registered proper concentrations for rearing stellate sturgeon in the analysed development stage. Thus, the nitrogen compounds ($N\text{-}NH_4$; $N\text{-}NO_2$; $N\text{-}NO_3$), as well as P_2O_5, registered a higher average concentration for both duplicate trials (B3: 0.28±0.08 mg L^{-1} for $N\text{-}NH_4$; 0.13±0.04 mg L^{-1} for $N\text{-}NO_2$; 91.83±11.6 mg L^{-1} for $N\text{-}NO_3$; 25.11±6.9 mg L^{-1} for P_2O_5, respectively B4: 0.30±0.11 mg L^{-1} for $N\text{-}NH_4$; 0.11±0.05 mg L^{-1} for $N\text{-}NO_2$; 87.39±16.1 mg L^{-1} for $N\text{-}NO_3$; 23.94±5.1 mg L^{-1} for P_2O_5) which composed the F2 experimental variant, compared to B1 (0.21±0.09 mg L^{-1} for $N\text{-}NH_4$; 0.09±0.03 mg L^{-1} for $N\text{-}NO_2$; 79.26±18.6 mg L^{-1} for $N\text{-}NO_3$; 21.32±6.2 mg L^{-1} for P_2O_5) and B2 (0.22±0.07 mg L^{-1} for $N\text{-}NH_4$; 0.08±0.02 mg L^{-1} for $N\text{-}NO_2$; 74.74±14.9 mg L^{-1} for $N\text{-}NO_3$; 19.77±5.3 mg L^{-1} for P_2O_5) duplicates, part of F1 experimental variant (Table 3). This may be due to high feed input, collaborated to low recirculation flow of the RAS, respectively low hydraulic retention time (HRT) recorded at the level of each of the four rearing units.

Also, this hypothesis is confirmed by low pH values of technological water recorded in F2 experimental variant rearing units (6.18±0.54 upH at B3, respectively 6.15±0.51 upH at B4), compared to F1 rearing units (6.32±0.43 upH at B1, respectively 6.37±0.48 upH at B2) (Table 3).

As well, high values of turbidity (5.33±0.52 NTU at B3 respectively, 5.19±0.42 NTU at B4), percentage removal of BOD5 (64.95±16.85% at B3, respectively 67.68±15.86% at B4) and COD concentration (81.07±22.79 mg L^{-1} at B3, respectively 79.81±24.93 mg L^{-1} at B4), correlated to low DO concentrations (7.42±0.88 mg L^{-1} at B3, respectively 7.49±1.09 mg L^{-1} at B4), recorded at F2 experimental variant rearing units, compared to F1 rearing units (turbidity 4.88±0.38 NTU at B1 respectively, 4.68±0.49 NTU at B2; BOD5 56.96±14.78% at B1, respectively 53.73±12.84% at B2; COD 68.45±14.84 mg L^{-1} at B1, respectively 73.12±16.04 mg L^{-1} at B2; DO 7.76±0.62 mg L^{-1} at B1, respectively 7.63±0.56 mg L^{-1} at B2), indicates a possible superior organic matter

accumulation rate due to feed rate (2% feeding rate applied at F2 vs. 1% feeding rate applied at F1 experimental variants).

Not significant differences ($p>0.05$) were recorded between the experimental variants in terms of technological water temperature.

Table 3. Water quality parameters

Water quality parameter	B1	B2	B3	B4
N-NH_4 (mg L^{-1})	0.21±0.09	0.22±0.07	0.28±0.08	0.30±0.11
N-NO_2 (mg L^{-1})	0.09±0.03	0.08±0.02	0.13±0.04	0.11±0.05
N-NO_3 (mg L^{-1})	79.26±18.6	74.74±14.9	91.83±11.6	87.39±16.1
P_2O_5 (mg L^{-1})	21.32±6.2	19.77±5.3	25.11±6.9	23.94±5.1
pH	6.32±0.43	6.37±0.48	6.18±0.54	6.15±0.51
Turbidity (NTU)	4.88±0.38	4.68±0.49	5.33±0.52	5.19±0.42
Percentage removal of BOD5 (%)	56.96±14.78	53.73±12.84	64.95±16.85	67.68±15.86
DO (mg L^{-1})	7.76±0.62	7.63±0.56	7.42±0.88	7.49±1.09
Temperature (^oC)	22.82±0.44	22.83±0.46	22.90±0.39	23.87±0.41
COD (mg L^{-1})	68.45±14.84	73.12±16.04	81.07±22.79	79.81±24.93

Stellate sturgeon meat proximate composition assessment

The proximate composition of stellate sturgeon meat, reared in both experimental variants (F1 and F2), by applying different feeding rate conditions was determined both at the initial and final stage of the experimental period, in order to identify the ash, lipids, proteins and moisture content, as well as the moisture: protein (M/P) ratio (Table 4).

According to ANOVA test, not significant differences ($p>0.05$: $p=0.532$ for ash, $p = 0.278$ for lipid, $p=0.636$ for protein, $p=0.734$ for moisture and $p=0.324$ for M/P) were recorded between the exemplars reared in the rearing units corresponding to the same experimental variant. Thus, the duplicate experimental design is validated.

However, according to ANOVA test, statistically significant differences ($p<0.05$) were recorded between initial and final stage of experimental period, for both variants (F1 and F2) in terms of ash ($p=0.021$), lipids ($p=0.033$), proteins content ($p=0.048$), as well as for M/P ratio ($p=0.028$) (Table 4).

Also, by analysing the data, according to ANOVA test, it can be stated that statistically significant differences ($p<0.05$) were recorded, at the end of experimental period, between the two experimental variants (F1 and F2) in terms of moisture ($p=0.033$), protein ($p=0.045$), lipids ($p=0.042$) and ash content ($p=0.027$) (Table 4).

Thus, superior content of protein (17.86±0.14% - B3, respectively 17.70±0.17% - B4) and lipids (1.32±0.04% - B3, respectively 1.36±0.03 % - B4) are recorded at F2, were the highest feeding rate was applied, compared to F1 (protein: 16.71±0.2% - B1 and 16.78±0.18% - B2; lipid: 1.21±0.04% - B1 and 1.27±0.05% - B2) (Table 4).

However, the M/P ratio and moisture content indicates lower values for F2 variant (M/P: 4.31±0.15 at B3 and 4.35±0.17 at B4; moisture: 76.99±0.17% at B3 and 77.05±0.21% at B4), compared to F1 (M/P: 4.71±0.11 at B1 and 4.67±0.1 at B2; moisture: 78.72±0.33% at B1 and 78.38±0.27% at B4) (Table 4), situation which emphasizes a better condition status and meat nutritional quality for stellate sturgeon reared by using a 1% BW feeding ratio. The M/P ratio is considered an important indicator for the evaluation of meat quality, since it does not take into account the lipids content.

The PUE, PR and LR indicators were calculated in order to asses the nutrient retention efficiency of stellate sturgeon experimental biomass (Figure 2).

Therefore, results reveal a higher statistically significant ($p<0.05$) degree (ANOVA test) of both proteins ($p=0.008$; PR: 14.16 g/specimen at F1, respectively 30.17 g/specimen at F2) and lipids accumulation ($p=0,011$; LR: 0.76 g/specimen at F1, respectively 1.92 g/specimen at F2), for stellate sturgeons' specimens reared in F2, compared to F1 experimental variant (Figure 2).

Also, the evolution of PUE (Figure 2) emphasizes a better protein valorisation if stellate sturgeons' specimens are reared by

applying a 2 % BW feeding rate (51.18%), compared to 1 % BW (50.33%).

Thus, it can be stated that a higher feeding rate can led to an improvement of meat quality due to statistically significant ($p<0.05$, $p=0.041$) decrease of moisture (ANOVA test), collaborated with the statistically significant ($p<0.05$, $p=0,039$) increase of protein content (ANOVA test).

The findings recorded in present study confirm the statement of El-Zaeem et al. (2012) according to which the protein of the fish meat can range between 12.3 – 28%.

Also, according to Sikorski et al. (1994) classification, all the stellate sturgeons' specimens from present experiment can be included in second quality class (1st class - >20% protein content ; 2nd class – 15 – 20% protein content; 3rd class – 10 – 15% protein content; 4th class - <10% protein content), with meat protein content ranging between 16.71 – 17.86%.

According to Ackman et al. (1989) classification, all the stellate sturgeons' specimens from present experiment can be included in the first class (1st class - < 2% lipids content ; 2nd class – 2 – 4% lipids content; 3rd class – 4 – 8% lipids content; 4th class - >8% lipids content), with a lipid content ranging between 1.21 – 1.36%.

According to Ionescu et al. (2006), the percentage of lipids content in fish meat varies within 0.1 – 28 %. However, multiple studies which analysed lipids content variation in sturgeon's meat (Dorojan et al., 2014; Dicu et al., 2013; Paltenea et al., 2007; Vasilean et al., 2010) have reported values less than 4%, therefore characterizing sturgeons' species as low-fat fish.

The results recorded in present study, related to stellate sturgeon meat proximate composition assessment are have been compared to those reported by other authors, for stellate sturgeons' specimens reared in RAS condition during similar development stage (Table 5).

Thus, in terms of ash content, the registered results are higher compared to those reported by Dorojan et al. (2014) (1.14-1.17%), respectively Dorojan et al. (2015) (0.99 – 1.20%), (Table 5).

However, compared to present study, Dorojan et al (2015) recorded superior lipids content (2.58 – 5.50%), while Dorojan et al. (2015) and Dorojan et al. (2014) reported lower protein content (14.45 – 15.69%, respectively 12.48 – 14.80%), (Table 5).

The results in terms of moisture content and M/P ratio were similar to those reported by Dorojan et al. (2015) (moisture: 70.91 – 76.54%; M/P ratio: 4.55 – 5.30), (Table 5).

Table 4. Stellate sturgeon meat proximate composition assessment

Parameters	Experimental variant		Ash (%)	Lipid (%)	Protein (%)	Moisture (%)	M/P
Initial	*F1; F2*	*B1; B2; B3; B4*	1.22±0.03	1.09±0.02	16.10±0.12	79.02±0.45	4.91±0.03
Final	*F1*	*B1*	1.33±0.1	1.21±0.04	16.71±0.2	78.72±0.33	4.71±0.11
		B2	1.39±0.11	1.27±0.05	16.78±0.18	78.38±0.27	4.67±0.1
	F2	*B3*	1.69±0.06	1.32±0.04	17.86±0.14	76.99±0.17	4.31±0.15
		B4	1.77±0.09	1.36±0.03	17.70±0.17	77.05±0.21	4.35±0.17

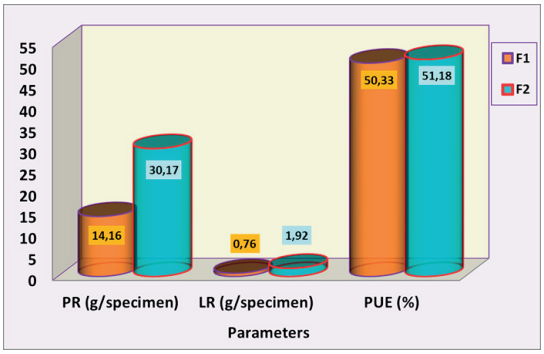

Figure 2. Protein utilization efficiency (PUE), retained protein (PR) and retained lipids (LR) for stellate sturgeon specimens reared in both experimental variants (F1 and F2)

Table 5. The meat proximate composition of stellate sturgeons reared in RAS, during similar development stage, reported by different authors

Reference	Stellate sturgeon average biomass (g)	Ash (%)	Lipid (%)	Protein (%)	Moisture (%)	M/P
Dorojan et al., 2015	188.33- 201.03	0.99 – 1.20	2.58 – 5.50	14.45 – 15.60	70.91 – 76.54	4.55 – 5.30
Dorojan et al., 2014	140 - 166	1.14 – 1.17	0.30 – 0.43	12.48 – 14.80	80.55 - 83.44	6.45 – 5.64
Dicu et al., 2013	204 ± 8	-	0.41 – 0.76	-	-	6.51 – 7.12

CONCLUSIONS

The results recorded in present study concludes that the use of 2% BW feeding rate for rearing stellate sturgeons, in RAS conditions, assures a superior protein and fat retention and improves protein use efficiency.

However, if analysing M/P ratio and moisture content, it can be concluded that a 1% BW feeding rate assures a better condition status for the biological material and superior stellate sturgeon meat quality.

However, the findings are influenced by the RAS capacity to maintain proper conditions for stellate sturgeon growth throughout the experimental period. For future studies, it is recommended to extend the experimental period in order to have a better view related to the influence of feeding rate on stellate sturgeon meat proximate composition.

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