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Research Article

Effects of fermented poultry waste with *Saccharomyces cerevisiae* on growth performance, survival, carcass composition, and selected immune parameters in juvenile Siberian sturgeon (*Acipenser baerii*) as a fishmeal replacement

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Abstract

In this study, 300 juvenile Siberian sturgeon with an average weight of 100.25±3.17 g were distributed in 12 fiberglass tanks (300 liters each) with a density of 25 fish per tank. Over 56 days, they were fed diets containing fermented poultry waste derived from *Saccharomyces cerevisiae* at 0% (control), 20% (T1), 30% (T2), and 40% (T3) as a replacement for fishmeal. The growth results showed that the final weight, weight gain, and specific growth rate increased significantly in T2 treatment compared to other treatments, especially the control group ($p<0.05$). The lowest and highest feed conversion ratio was observed in T2 treatment (1.2±0.11) and the control (1.5±0.23) respectively ($p<0.05$). The fish fed with T2 treatment showed the highest crude protein content (18.4±1.26) and the lowest crude fat content (1.35±0.29). However, there was no significant difference in the moisture and fat content between the different experimental treatments. The results indicate a significant difference in the levels of immunoglobulin IgM, respiratory activity (production of oxygen radicals), and serum lysozyme activity in the fish fed diet containing 30% treatment compared to other treatments, especially the control ($p<0.05$). Overall, the addition of 30% fermented poultry waste from *S. cerevisiae* instead of fishmeal is recommended to maximize growth performance and improve the immune system.

Introduction

The Siberian sturgeon (*Acipenser baerii*) is a commercially valuable species, prized both for its high-quality meat and its caviar. In Iran, this species is also farmed; however, specific production statistics for *A. baerii* are not reported separately. In 2019, the total sturgeon production in the country was recorded at 2,839 tons (Morshedi *et al*., 2013). Fishmeal remains a cornerstone of aquaculture nutrition due to its high-quality protein, essential amino acids, phospholipids, and superior digestibility. Nevertheless, the increasing global demand for fishmeal, coupled with declining wild fish stocks, has led to a steady rise in price and limited availability (Tacon, 2004). This volatility in fishmeal supply has prompted extensive global efforts to identify alternative protein sources that are both sustainable and cost-effective. Replacement strategies have focused on both plant- and animal-based materials, with key evaluation criteria including availability, cost, nutritional composition, and digestibility (Xue *et al*., 2012). Enhancing the nutritional value of such alternative ingredients through biotechnological interventions has emerged as a promising approach to maintaining growth performance and health in aquaculture species while contributing to global food security.

The poultry processing industry generates considerable volumes of organic waste—including offal, feathers, fat, bones, and blood—which can be converted into valuable protein sources via enzymatic hydrolysis, biosilage, or microbial fermentation. Notably, approximately 16.5% of a chicken's body weight consists of non-edible by-products, and large-scale poultry slaughtering operations produce thousands of tons of such waste daily. Utilizing modern biotechnological methods allows for the transformation of this waste into high-value feed ingredients with minimal environmental impact. Fermented products, such as silage, are produced through autolytic, chemical, or microbial processes and offer a versatile application as biofertilizers in agriculture or as alternative protein sources in aquafeeds. Yeasts such as Saccharomyces cerevisiae are particularly advantageous for single-cell protein production due to their high growth rates, efficiency, ease of protein extraction, and low contamination risks (Dawood *et al*., 2019).

Among animal-derived feed ingredients, poultry by-product meal (PBM) is of particular interest. Defined by the Association of American Feed Control Officials (AAFCO) as a rendered product derived from poultry slaughterhouse waste—comprising heads, feet, viscera, beaks, and minimal feather content—PBM is rich in protein (55–67%) and offers a favorable amino acid profile for fish nutrition (Meeker, 2009; Gaylord and Rawles, 2005). Studies have demonstrated that PBM can replace fishmeal by up to 75% in the diet of gilthead seabream (*Sparus aurata*) (Nengas *et al*., 1999) and up to 100% in red seabream (*Pagrus major*) (Takagi *et al*., 2000) without negative impacts on growth or health.

Given the economic significance of the Siberian sturgeon and its potential for caviar production, as well as the promising outcomes of using S. *cerevisiae*-fermented poultry waste in aquafeeds, this study aims to investigate the effects of such feed ingredients on growth performance and innate immunity in *A. baerii*. The findings are expected to contribute to the development of sustainable and cost-effective feeding strategies in sturgeon aquaculture.

Materials and methods

Experimental design and fish rearing conditions

A total of 300 clinically healthy Siberian sturgeon (*Acipenser baerii*), with an average initial weight of 100.25±3.17 g, were procured from a certified hatchery authorized by the Veterinary Organization and transferred to the rearing facility at the Caspian Sea Ecology Research Center. To acclimate the fish to experimental conditions, all individuals were fed a basal control diet for a two-week preconditioning period. Upon completion of the adaptation phase, fish were randomly assigned to 12 fiberglass tanks (300 L capacity each), with 25 fish per tank, under identical environmental conditions. Water quality parameters—dissolved oxygen, pH, and temperature—were monitored daily using a portable multi-parameter water quality meter (WTW model 320, Germany). The average measured values during the trial were: temperature 21±0.5°C, dissolved oxygen 6.8±0.2 mg/L, and pH 7.6±0.3. The experiment was conducted under natural photoperiod conditions.

Diet preparation and feeding regimen

The study was conducted following a completely randomized design, consisting of four dietary treatments with three replicates each. The experimental diets were formulated by substituting fishmeal with *S. cerevisiae*-fermented poultry by-product meal at 0% (control), 20% (T1), 30% (T2), and 40% (T3) replacement levels (Table 1) (Dawood *et al*., 2020a).

Table 1: Composition of the experimental diets for juvenile Siberian sturgeon in different experimental treatments.

Composition (%)	Control	20%	30%	40%
Kilka fishmeal [۱]	57	37	27	17
Wheat flour	18	18	18	18
Fermented poultry wastes	0	20	30	40
Meat meal	6	6	6	6
Soybean meal	11.5	11.5	11.5	11.5
Fish oil	2	2	2	2
Plant oil	2	2	2	2
Gelatin	1	1	1	1
Vitamin premix [2]	1	1	1	1
Mineral premix [2]	1	1	1	1
Binder [3]	0.5	0.5	0.5	0.5

1: Clupeonella cultriventis

2: Premix detailed by Kongnum and Hongpattarakere (2012).

3: Amet binder (MehrTaban,Yazd, Iran).

In the control group, saline solution and vegetable oil were used to standardize moisture and fat content across diets. All diets were vacuum-sealed in polyethylene bags and stored at 4±1°C until use. Fish were hand-fed to apparent satiation three times daily (08:00, 12:00, and 18:00) for a period of eight weeks. To maintain optimal water quality, 30% of the tank water was exchanged daily, and fecal matter was removed via siphoning.

Growth performance and biometric analysis

Fish were anesthetized using clove powder (100 mg/L) before biometric sampling, which occurred every 15 days. Feed was withheld 24 hours prior to sampling to minimize stress. The following growth and performance indices were calculated (Adel *et al*., 2021):

Weight gain (WG) = final weight – initial weight
Daily growth rate (DGR) = (final weight – initial weight) / number of feeding days
Feed conversion ratio (FCR) = total feed intake / weight gain
Body Weight Gain Percentage (BWG%) = [(Final weight – Initial weight) / Initial weight] × 100
Specific growth rate (SGR) = [(ln final weight – ln initial weight) / duration in days] × 100
Survival rate (%) = (final fish count / initial fish count) × 100

Blood sampling and hematological analysis

At the conclusion of the 56-day feeding trial, blood samples were collected from five randomly selected fish per tank via caudal venipuncture using heparinized syringes. Samples were centrifuged at 5,000 rpm for 5 minutes to separate serum, which was stored at −4°C until further biochemical and immunological assays (Adel *et al*., 2021).

Serum indices

Immunoglobulin M (IgM) levels were quantified using the microprotein precipitation method (C-690; Sigma), involving precipitation with 12% polyethylene glycol and measurement of the differential protein content before and after precipitation. Lysozyme activity was measured as described by Ellis (1990), with modifications. Fifty microliters of serum were added to 2 mL of *Micrococcus lysodeikticus* suspension (0.2 mg/mL in 0.05 M sodium phosphate buffer, pH 6.2), and absorbance was recorded at 450 nm after 0.5 and 3 minutes using a spectrophotometer (Biophotometer, Eppendorf). The respiratory burst activity of leukocytes was assessed via chemiluminescence, following the method of Binaii *et al*. (2014), using a LUMI Skan Ascent T392 reader (Finland).

Proximate composition

At the end of the period, 5 pieces of fish were selected completely at random from each tank. After removing the intestines and viscera, the fish muscle was minced 3 times and kept at freezing temperature. To determine the moisture, an oven with a temperature of 105°C was used until a constant weight was reached. An electric furnace with a temperature of 550°C was also used to determine the ash. The Kjeldahl method was used to measure the protein content, while the Soxhlet method was used to evaluate the amount of fat (AOAC, 1995).

Statistical analysis

Data analysis was done using SPSS 22 software. First, the variance homogeneity of the data was checked using the Leven test and the normality using the Kalmogorov-Smiranov test. In order to evaluate significant changes between the groups, a one-way analysis of variance was used and for intra-group comparison, Duncan's multiple-range test was used at the 5% probability level ($p<0.05$).

Results

Growth performance and survival rates

The effects of dietary inclusion of *S. cerevisiae*-fermented poultry by-product meal at varying replacement levels for fishmeal on the growth performance and survival of Siberian sturgeon are summarized in Table 2. The results demonstrate that fish fed the diet with 30% replacement exhibited significantly higher final body weight, weight gain, and specific growth rate (SGR) compared to other treatment groups, particularly the control group ($p<0.05$). All treatment groups receiving fermented poultry waste showed significantly higher daily growth rates compared to the control ($p<0.05$). The lowest feed conversion ratio (FCR) was recorded in the 30% replacement group (1.21±0.11), while the control group exhibited the highest FCR (1.53±0.23), indicating more efficient feed utilization in the experimental diets containing fermented by-products. Survival rate analysis showed no statistically significant differences among treatment groups compared to the control, suggesting that dietary modifications had no adverse effect on fish survivability.

Table 2: Average growth and survival indices in Siberian sturgeon fed with diets containing fermented poultry wastes by *Saccharomyces cerevisiae*

Index	Control	T1	T2	T3
PW	99±1.2^{a}	100±2.2^{a}	99±2.1^{a}	99±2.4^{a}
FW	310±4.5^{c}	328±3.7^{b}	345±2.9^{a}	325±2.9^{b}
WG	211±3.3^{c}	228±1.5^{b}	246±0.8^{a}	226±0.5^{b}
DGR	3.76±0.45^{b}	4.07±0.23^{a}	4.39±0.41^{a}	4.03±0.23^{a}
FCR	1.5±0.23^{a}	1.3±0.17^{a}	1.21±0.11^{b}	1.31±0.17^{a}
SGR	2.03±0.11^{a}	2.12±0.13^{a}	2.20±0.14^{a}	2.12±0.10^{a}
(%)SR	99±0.91^{a}	100±0.00 ^{a}	100±0.00^{a}	100±0.00^{a}

PW: primary weight; FW: Final Weight: WG: weight gain (g); DGR: Daily growth rate; SGR: specific growth rate; FCR: fed conversion ratio; %SR: percentage survival rate. Statistical difference between treatment groups is indicated by different letter notations.

Serum immunological parameters

Table 3 illustrates the serum immunological responses of Siberian sturgeon subjected to different dietary levels of *S. cerevisiae*-fermented poultry waste. A significant elevation in total serum immunoglobulin (IgM) levels was observed in fish fed the 30% replacement diet compared to the other groups (*p*<0.05), with the lowest IgM concentration recorded in the control group (27.1±1.1 mg/dL) and the highest in the 30% group (41.3±3.3 mg/dL). Furthermore, lysozyme activity—a key component of innate immune defense—was significantly higher in the 30% group (*p*<0.05), while the control group exhibited the lowest activity (23.6±1.23 µg/mL/min). A similar pattern was observed for respiratory burst activity, as measured by reactive oxygen species (ROS) production. The highest ROS levels were recorded in the 30% replacement group, with significant differences observed relative to other treatments, particularly the control group (*p*<0.05).

Table 3: Average serum immunity indices in Siberian sturgeon fed diets containing fermented poultry wastes by *Saccharomyces cerevisiae*.

Index	**Control**	**T1**	**T2**	**T3**
IgM (mg.d L $^{-1}$)	27.1±1.1^{c}	32.1±4.25^{b}	41.1±3.31^{a}	31.1±6.17^{b}
Lysozyme (µg. mL.min)	23.1±6.23^{c}	35.1±2.45^{b}	37.1±1.20^{a}	34.1±1.1^{b}
Respiratory burst activity (RLU. S $^{-1}$)	1520.25±2.1^{d}	1860.56±2.3^{c}	2100.32±2.2^{a}	1910.25±2.4^{b}

Statistical difference between treatment groups is indicated by different letter notations.

Proximate composition of fish muscle

Table 4 presents the results of the proximate analysis of muscle tissue in Siberian sturgeon at the conclusion of the feeding trial. Fish receiving the diet containing 30% fermented poultry by-product meal demonstrated the highest crude protein content (18.4±1.26%) and the lowest crude fat content (1.35±0.29%). No statistically significant differences were detected in moisture or ash content among the treatment groups, indicating that the inclusion of fermented poultry waste did not adversely affect the general tissue composition of the fish.

Table 4: The carcass composition of Siberian sturgeon fed diets containing fermented poultry waste from *Saccharomyces cerevisiae*.

Index (%)	**Control**	**T1**	**T2**	**T3**
Protein	16.1±1.05^{c}	17.1±3.2^{b}	18.1±4.26^{a}	17.1±2.17^{b}
Fat	9.0±45.9^{a}	9.0±3.75^{a}	9.0±2.63^{a}	9.0±3.51^{a}
Moisture	73.2±1.35^{a}	73.2±5.17^{a}	73.2±6.46^{a}	73.2±5.21^{a}
Ash	1.0±39.23^{a}	1.0±45.21^{a}	1.0±35.29^{a}	1.0±53.33^{a}

Statistical difference between treatment groups is indicated by different letter notations.

Discussion

Optimizing nutritional and immunological parameters plays a pivotal role in enhancing the ecological adaptability, growth performance, and survival of aquaculture species (Adel *et al*., 2021). In the present study, the inclusion of *Saccharomyces cerevisiae*-fermented poultry waste at a 30% substitution level significantly improved growth performance metrics—namely final weight, weight gain, and specific growth rate (SGR)—in Siberian sturgeon, compared to other treatments, particularly the control. All treatment groups receiving fermented poultry waste also exhibited significantly higher daily growth rates than the control group, highlighting the positive impact of dietary fermentation. These findings are supported by previous studies. Sayed Hassani *et al*. (2014) demonstrated that up to 100% replacement of fishmeal with poultry by-product protein in catfish diets did not adversely affect growth performance, which was attributed to the nutritional quality of poultry by-products and the adaptive physiology of fish such as catfish and sturgeon (Dabrowski and Guderley, 2002). Similarly, Dawood *et al*. (2020a, 2020b) observed improved growth indices in Nile tilapia and common carp when fermented poultry by-products were incorporated into the diet at levels ranging from 11–25% and 15–20%, respectively. Hao and Yu (2003) also reported no adverse effects on the growth of *Silurus glanis* catfish when 80% of fishmeal was replaced with poultry and meat-bone meal, citing the importance of enhanced processing techniques. Davis and Arnold (2000) found similar outcomes in white shrimp (*Litopenaeus setiferus*) diets. In the current study, the lowest feed conversion ratio (FCR) was recorded in the 30% replacement group (1.11±0.21), while the control group had the highest FCR (1.23±0.53). These improvements likely stem from the high protein content, favorable amino acid profile, and palatability of the fermented poultry by-product meal, which collectively enhance feed efficiency and growth performance. Comparable outcomes were reported in seabass (*Dicentrarchus labrax*) and red sea bream (*Pagrus major*) where high levels of poultry waste substitution did not compromise growth (Nengas *et al*., 1999; Takagi *et al*., 2000). The observed enhancement in growth metrics can be attributed to fermentation-mediated breakdown of anti-nutritional factors and macromolecules, improving digestibility and nutrient bioavailability (Oliva-Teles and Gonçalves, 2001; Dawood *et al*., 2020b). Additionally, *S. cerevisiae* fermentation generates bioactive compounds such as B-complex vitamins, peptides, and organic acids, which stimulate feed intake and growth (Ringø *et al*., 2010). Fermented products may also positively modulate gut microbiota, thereby enhancing intestinal health and nutrient assimilation (Dimitroglou *et al*., 2009).

From an immunological perspective, serum immunoglobulin production involves interactions between antigen-presenting cells, T-helper cells, and interleukins activating B lymphocytes, with immunoglobulins playing a central role in humoral immunity (Magnadottir, 2010). Lysozyme, an essential component of innate immunity, contributes to bacterial lysis, complement activation, and phagocytosis enhancement. Elevated lysozyme activity reflects an improved innate immune response and has been linked to the use of immunostimulants, vaccines, and prebiotics in fish diets.

In this study, fish fed the 30% fermented poultry waste diet showed significantly higher serum IgM levels and lysozyme activity compared to other groups, especially the control. These outcomes align with Dawood *et al*. (2020b), who observed decreased immune responses when 40% fermented poultry waste replaced fishmeal, possibly due to increased oxidative stress and inferior protein quality. Moreover, respiratory burst activity—an indicator of phagocytic response—was significantly elevated in the 30% group, confirming improved innate immunity. Supporting evidence from Dawood *et al*. (2020a) and Siddik *et al*. (2020) demonstrated enhanced lysozyme, phagocytosis, and bactericidal activity in Nile tilapia and freshwater crayfish, respectively, fed diets enriched with fermented poultry waste and yeast derivatives such as β-glucans and mannan oligosaccharides (MOS). These compounds modulate immune responses by stimulating macrophages and cytokine activity (Paulsen *et al*., 2001), in addition to improving gut microbiota balance and overall health (Ringø *et al*., 2010).

In terms of body composition, fish fed diets containing 30% fermented poultry waste exhibited the highest crude protein (18.4±1.26%) and lowest crude fat (1.35±0.29%). These results are consistent with Nengas *et al*. (1999), who reported reduced fat deposition in seabass with high poultry by-product inclusion. Xue *et al*. (2012) also demonstrated that poultry meal could replace fishmeal in sturgeon diets without adversely affecting carcass composition, due to its similar amino acid profile. Conversely, Zhu *et al*. (2011) reported no significant impact on body protein in sturgeon fed a mixed animal protein diet, indicating potential variability depending on ingredient source and proportion. The improved protein retention and reduced fat accumulation observed in this study could result from enhanced nutrient digestibility, better amino acid availability, and the emulsifying effects of fermentation byproducts. These benefits may translate into improved fillet yield, muscle deposition, and reduced visceral fat—traits desirable for marketability. Fermentation breaks down complex proteins into peptides and amino acids, increasing their absorption and utilization in muscle growth (Oliva-Teles and Gonçalves, 2001; Dawood *et al*., 2020b). Additionally, yeast-derived nucleotides and organic acids can enhance flesh quality, firmness, and color by supporting metabolic efficiency and antioxidant defense (Zhou *et al*., 2010). The reduction in abdominal fat deposition may be linked to more efficient nutrient partitioning and suppressed lipogenesis, potentially mediated by improved gut health (Ringø *et al*., 2010).

Given the importance of Siberian sturgeon as a cultivated species, the inclusion of 30% *S. cerevisiae*-fermented poultry waste in the diet appears to be optimal for promoting growth performance, enhancing immune responses, and improving body composition. However, further investigations are warranted to determine the safety and efficacy of inclusion levels beyond 30%, as well as potential long-term physiological impact.

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