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Effects of intelligent feeding method on the growth, immunity and stress of juvenile *Micropterus salmoides*

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A B S T R A C T

The feeding method is of great significance for aquaculture production and cost. In recent years, research on intelligent feeding, a method based on fish appetite, has been trending. Few studies, however, have focused on fish welfare issues that can result from intelligent feeding. In this study, an adaptive feeder based on a practical intelligent feeding method was designed to evaluate whether this intelligent feeding method would impair fish welfare compared to traditional automatic feeding. The results indicated that the amount of residual feed and size inhomogeneity in the traditional group was significantly higher than that in the intelligent group. The results of the growth indicator showed that the weight gain rate (WGR) in the intelligent feeding group was significantly higher (30.17%) than in the traditional feeding group. Although no significant differences were observed in the survival rate (SR), the condition factor (CF) and the hepatosomatic index (HSI), the specific growth rate (SGR) in the intelligent feeding group was significantly increased by 8.33% while the feed conversion rate (FCR) was reduced by a remarkable 17.07% compared to the traditional feeding group. Moreover, intelligent feeding significantly improved the pepsin activity of the bass. In terms of immunity and antioxidant capacity, however, the fish in the intelligent feeding group had a significantly lower lysozyme (LZM) level than those fed with the traditional method. For superoxide dismutase (SOD), the intelligent group also displayed a lower activity value, although it was not significant. The intelligent feeding group had 0.14 nmol/mg protein higher malondialdehyde (MDA) activity than the traditional group. Regarding stress, although no statistical significance was observed, the cortisol level in the intelligent group was 1.9 ng/ml higher than in the traditional group. Together, these data suggested that the intelligent feeding method can significantly improve fish growth but may also result in stress and suppress innate immunity.

1. Introduction

Feed management is a non-trivial aspect of fish aquaculture. The feeding method, to a great extent, determines fish production (Føre et al., 2011; López-Olmeda et al., 2012; Zhao et al., 2019). Feeding according to fish requirements is effective and as such is becoming increasingly accepted in aquaculture (Zhou et al., 2018b). Until now, many studies have attempted to establish the endpoint of feeding based on fish appetite (Zhou et al., 2018b; Li et al., 2020). For example, underwater acoustic technology has long been applied to determine the feeding endpoint of salmon in sea cages by detecting the residual feeds (Juell, 1991; Juell et al., 1993). Similarly, Atoum et al. (2015) also established models to determine the feeding endpoint by detecting the residual feeds using different cameras. Residual feed detection-based feeding depends heavily on high-quality detection instruments and the breeding environment. In view of this, novel intelligent feeding methods that characterize the variation of dissolved oxygen (DO) and temperature in the water during feeding have been developed (Wu et al., 2015; Zhao et al., 2019). Although this kind of feeding revealed better performance compared to traditional feeding, it does not apply to increasingly industrialized aquaculture (such as recirculating aquaculture systems (RAS)) to a great extent as the DO and temperature are stably maintained in this setting.

In contrast to the methods above, fish appetite quantification in real production using the characteristics of fish feeding behavior is more feasible. With the rapid development of artificial intelligence and technology, feeding according to fish feeding behavior has been a trending area of research in aquaculture (Sun et al., 2016; Zhou et al., 2017). Chang et al. (2005) constructed a feedback control system, which could

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determine the feeding endpoint, by quantifying the aggregation behavior of eels during feeding. Zhou et al. (2017, 2018a) measured the degree of fish feeding activity by analyzing the aggregation and dispersion of fish schools. Liu et al. (2014) quantified the feeding intensity of Atlantic salmon in RAS using inter-frame differential characteristics caused by fish feeding behaviors. Similarly, Ye et al. (2016) developed a novel method to assess the feeding activity of tilapia in RAS based on the Lucas-Kanade optical flow algorithm and entropy. Zhou et al. (2019) also graded the feeding activity of tilapia in RAS using a customized convolutional neural network (CNN). The above methods depend heavily on the water turbidity and light environment, where the clearer the foreground (location of the fish school), the better the performance, however, these conditions are difficult to achieve in practical production. Zhao et al. (2017) established a model based on the motion characteristics of the reflective areas caused by fish feeding behaviors to assess appetite. This method, to a great level, can avoid the above limitations.

The intelligent feeding methods above could improve production and reduce costs to a large extent (Zhou et al., 2018a), however, whether these intelligent methods are beneficial to fish welfare in aquaculture remains little unknown. Therefore, to understand the effects of the intelligent feeding method on fish growth more comprehensively, an adaptive feeder based on a practical and typical intelligent feeding method (Zhao et al., 2017) was designed. Fish welfare was evaluated by measuring the corresponding growth and biochemical and immune indexes throughout a 47-day production experiment.

In this study, *Micropterus salmoides*, one of the five domestic fish in China, was used as the experimental objects as its indispensable status in the Chinese freshwater aquaculture system. Besides, in view of the trend of industrial RAS in aquaculture, our study was implemented in the environment of the industrial RAS.

2. Materials and methods

2.1. Fish samples

All *Micropterus salmoides* were purchased from the fish hatchery of Suzhou (Jiangsu, China). Before the experiment, the fish were acclimated in a laboratory RAS and fed a commercial diet (protein: 42%, lipid: 8%, ash: 13%) daily for 14 days (Zhao et al., 2017). During acclimation, the fish were fed 3 times a day (08:30, 16:00, 19:30), and the total amount of feed did not exceed 5% of the bodyweight per day. After that, 384 fish (32 ± 5 g) of a similar size were chosen and randomly and evenly distributed to six cylindrical culture ponds (two groups, each in triplicate).

2.2. Experimental system

2.2.1. RAS structure

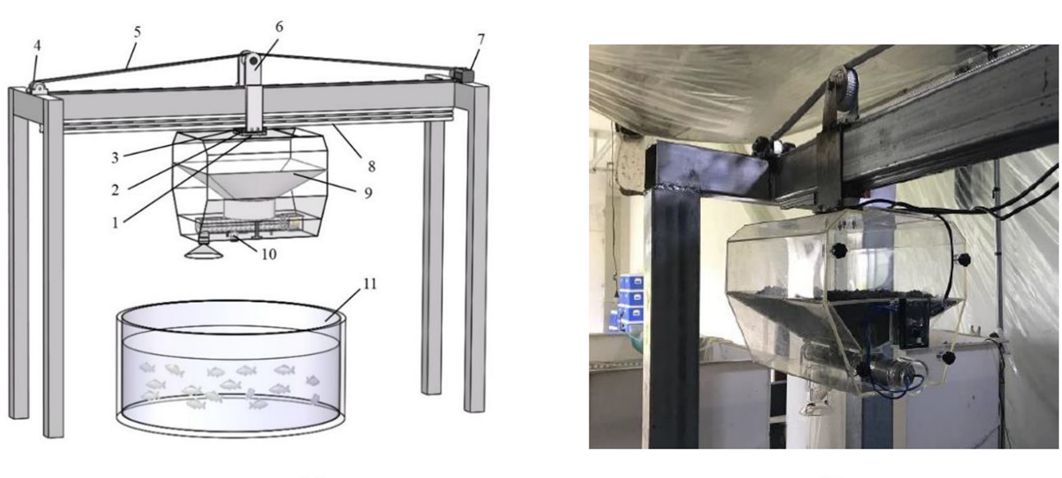
The experimental system consisted of two independent RAS, where each RAS contained three culture ponds (diameter: 1 m, water depth: 0.5 m) coupled with a biofilter, solid removal, feeder, oxygen supply, light emitting diode (LED), and ultraviolet lamp (UV). The water used in this experiment was ammonia removal tap water with aeration for >24 h and UV sterilization. The circulating water volume of each pond was 300 L/h, and the daily water exchange volume was 15% of the total. The full spectrum energy-saving lighting lamp was used to simulate the light period (08:00–20:00 light, 20:00–08:00 dark) during the experiment. The temperature was maintained at 25 ± 0.5 °C, dissolved oxygen ≥5.5 mg/L, pH at 7.2 ± 0.5, nitrate ≤0.5 mg/L, and ammonia nitrogen ≤0.8 mg/L for the entirety of the acclimation period and experiment.

2.2.2. Feeder

Fig. 1 (a) shows the feeder, which includes the walking system, weight sensor and feeder. The camera used for monitoring the fish activity was installed at the bottom of the feeder, facing down the water surface (1.3 m above the water surface). For the intelligent group, feeding was performed using the adaptive feeder, which was controlled by the adaptive feeding algorithm (see adaptive feeding algorithm below for details). For the traditional group, the fish were fed quantitively (see experimental design and sampling for details). The total feeding amount (TFA) at each time point was divided into 3 parts on average, and then the fish were fed at intervals of approximately 30 s.

2.3. Adaptive feeding algorithm

The adaptive feeding system is outlined in Fig. 2. The feeding system proposed in this paper can adjust the feeding amount according to fish appetite. The appetite of juvenile *Micropterus salmoides* was assessed using a modified kinetic energy model (Zhao et al., 2017). During the feeding (at intervals of approximately 30 s), the appetite assessment (E_k) at each feeding point was compared with a kinetic energy threshold T_h (set to 140 in this study), which was determined by K-means clustering. The feeding continued until E_k was smaller than T_h, and the feeding



(a) (b)

Fig. 1. Adaptive feeder. (a) Diagram of the feeder structure: 1 = weight sensor, 2 = installation and positioning platform, 3 = slider, 4 = synchronous wheel, 5 = synchronous belt, 6 = drive connecting plate, 7 = stepper motor, 8 = track, 9 = feeder, 10 = camera, and 11 = pond. (b) Adaptive feeder prototype.

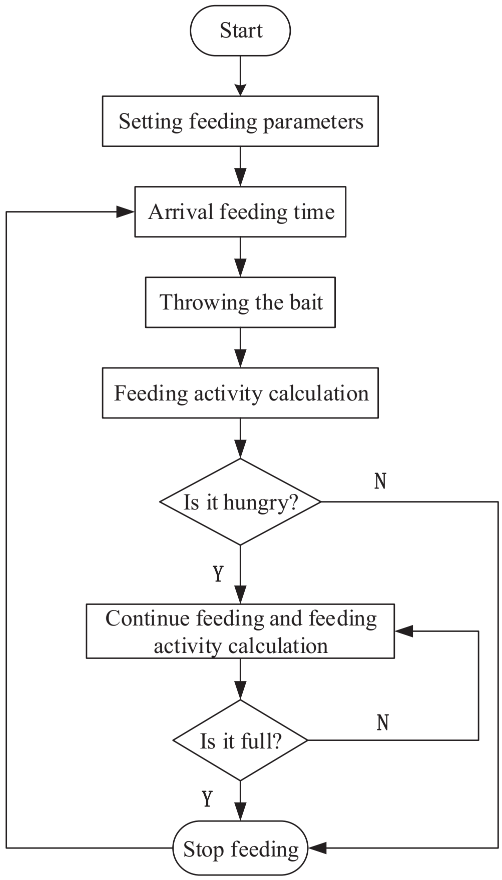


Fig. 2. The flowchart of the adaptive feeding method.

amount at each feeding point (except the first two feedings) was obtained by Eq. (1):

$$X(i+1)=\frac{E_k(i)+100}{E_{k(i-1)}+100}X(i), i\geq 2 \tag{1}$$

where $X(\mathrm{i})$ and $E_k(\mathrm{i})$ are the i-th feeding quantity and i-th appetite assessment result, respectively. Note that the first two feeding amounts were set to 10% TFA. The feeding amount decreased over the feeding time. When $X(\mathrm{i})$ decreased to a certain extent (set to 3% TFA), the minimum feed amount (i.e., 3% TFA) was implemented.

2.4. Experimental design and sampling

The two treatment groups in this experiment were the same except for the feeding method. The fish were fed 3 times a day (08:30, 16:00, 19:30) with the same commercial feed during the experiment and weighed every 15 days. The feeding amount in the traditional group was adjusted according to the weight change of the fish. The uneaten feed remaining on the water surface was collected and weighted 15 min after feeding of each group separately to prevent the residual feed produced in the experiment from contaminating the water.

At the end of the experiment, all fish were deprived of food for 24 h. The fish were then anesthetized (in the whole pond) deeply with MS-222 (120 mg/L) followed by weighing, grading, and sampling. The fish were divided into 3 levels according to the thickness of the back using grading sieves with a gap width of 1.2 cm and 1.7 cm, and the number of fish in each level was counted. The five fish from each level were randomly sampled and stored at −20 °C for whole-body proximate composition analysis (all fish at this level would be sampled if the fish less than 5). The length and weight were measured from an additional five fish randomly selected from each level. In addition, the livers were also removed and individually weighed to determine the hepatosomatic index. Then, a blood sample was immediately collected for centrifugation to separate the serum, and serum samples were frozen and stored at −40 °C for subsequent analysis. In addition to the blood samples, livers were also sampled. After the rinse in normal saline solution, the sampled livers were then stored at −40 °C for the analysis of antioxidant enzyme activities and immune-related parameters (analyzed by Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Growth performance evaluation

The long-term growth performance of the feeding methods was assessed using the fish survival rate (SR), the weight gain rate (WGR), the fish specific growth rate (SGR), the feed conversion rate (FCR), the condition factor (CF), and the hepatosomatic index (HSI). These parameters are defined in Eqs. (2)–(7), respectively.

$$SR=\frac{N_2}{N_1}\times 100\% \tag{2}$$

$$WGR=\frac{W_2-W_1}{W_1}\times 100\% \tag{3}$$

$$SGR=\frac{ln\,W_2-ln\,W_1}{t}\times 100\% \tag{4}$$

$$FCR=\frac{F}{W_2-W_1+W_0} \tag{5}$$

$$CF=\frac{W_B}{L^3}\times 100\% \tag{6}$$

$$HSI=\frac{W_L}{W_B}\times 100\% \tag{7}$$

W_1, W_2 and W_0 are the total weight (g) of the fish at the beginning of the trial, the end of the trial and the dead individuals during the test, respectively. F is the amount of feed intake in each pond, N_1 is the initial total number of fish, and N_2 is the number of fish at the end of the trial. W_B is the fish weight (g), L is the fish length (cm), W_L is the fresh liver weight (g), and t is the breeding time.

2.6. Proximate composition analysis

The proximate composition of the fish diets was determined by standard methods of the Association of Official Analytical Chemists (AOAC, 2000). The amounts of crude protein and crude lipid were measured by Coomassie blue staining and the Soxhlet extraction method, respectively. Moisture was measured by drying at 100 °C to a constant weight, and ash was determined by heating at 550 ± 25 °C for 4 h in a muffle furnace.

2.7. Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics 20 for Windows, and all the data are presented as the mean ± standard error (SE). Following the test of normality and heteroscedasticity, independent sample t-tests were used to analyze the test data with p set at 0.05 significance threshold.

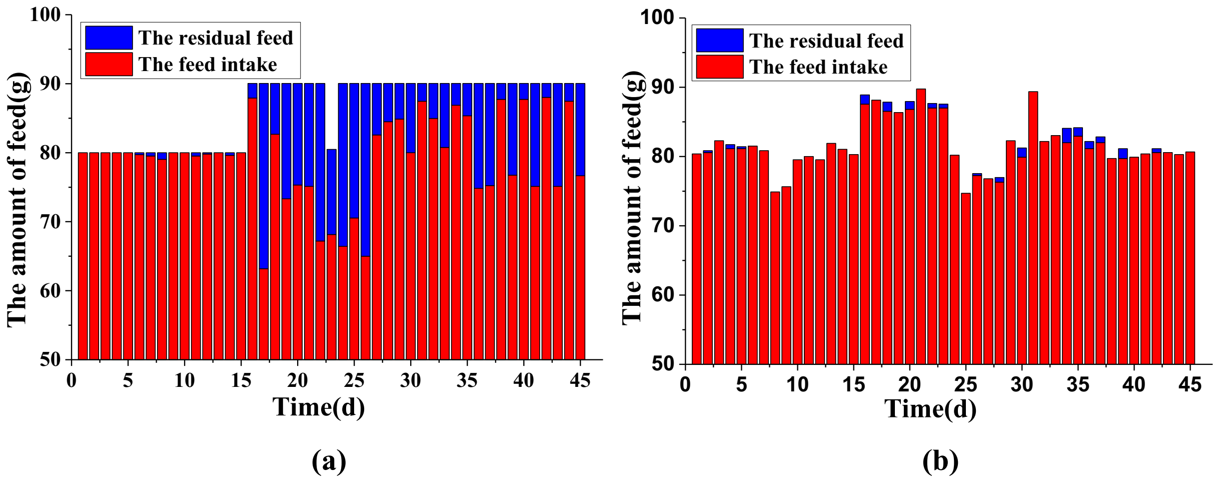


Fig. 3. Daily feed intake—residual map of the traditional group (a) and the intelligent group (b).

3. Results and discussion

3.1. Growth performance

3.1.1. Daily feed intake and size homogeneity

In order to explore the relationship between feed intake and residues in different feeding methods, the daily feed intakes, and residues of the *Micropterus salmoides* were recorded and shown in Fig. 3. During the experiment, the total feeding amount in the intelligent group was 11,077 g, and the feed residue accounted for 0.39%. Whereas in the traditional group, the total feeding amount and the feed residue proportion were 11,700 g, and 8.84%, respectively. Although no significant difference was observed in the total feed amount of the two groups, the amount of residual feed in the traditional group was significantly higher than that in the intelligent group, which indicated that the intelligent feeding method could better meet the food requirement of juvenile *Micropterus salmoides* and might be more conducive to fish growth.

In real production, the homogeneity of fish size is being emphasized due to its indispensable role in quality and yield to aquatic products. In order to study the influence of different feeding methods on size homogeneity, the fish in the fish in the two groups were graded into three levels (level 1: smaller than 1.2 cm; level 2: between 1.2 cm and 1.7 cm; level 3: bigger than 1.7 cm) according to their body thickness at the end of the experiment. Fig. 4 shows the grading result of the fish, where the horizontal axis is the value of the grading level, and the vertical axis denotes the number of fish. It can be seen from Fig. 4 (a) and (b) that fish in the intelligent group have a better size homogeneity compared to the traditional group. In other words, the intelligent feeding method can weaken the size differentiation of fish in aquaculture to some extent.

3.1.2. Growth indicator

To evaluate the performance of the intelligent feeding and traditional feeding methods, the fish growth parameters (SR, WGR, FCR, HIS, CF, and SGR) were measured and illustrated in Table 1. Generally, the feeding method had no significant influence ($p > 0.05$) on SR, HIS, and CF, however, significant differences ($p < 0.05$) were observed in

Table 1
Effect of different feeding methods on growth indicators.

Item	Intelligent group	Traditional group
SR (%)	98.40 ± 0.00^a	98.93 ± 0.92^a
WGR (%)	308.74 ± 10.35^a	278.57 ± 10.36^b
FCR	1.02 ± 0.06^a	1.23 ± 0.09^b
HIS (%)	0.03 ± 0.01^a	0.03 ± 0.00^a
CF (%)	0.02 ± 0.00^a	0.02 ± 0.00^a
SGR (%)	2.86 ± 0.71^a	2.64 ± 0.60^b

Note: Significant differences ($p < 0.05$) are indicated by different superscript letters within rows.

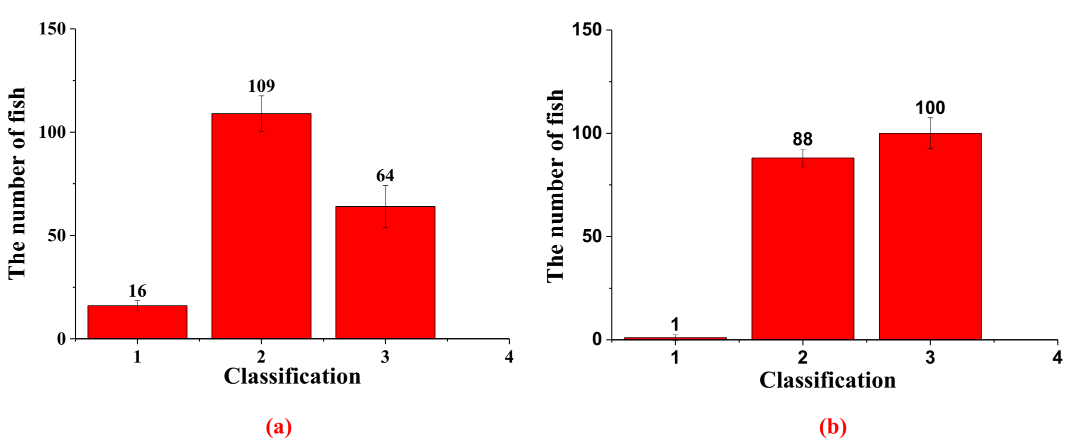


Fig. 4. Fish grading map of the traditional group (a) and the intelligent group (b).

WGR, SGR, and FCR. Specifically, WGR in the intelligent group was 30.17% higher than in the traditional group. In addition, the intelligent feeding group displayed an FCR decrease of 17.07% compared to the traditional group.

As shown above, fish fed with the adaptive feeder showed a higher WGR and SGR. The higher FCR in the traditional group may be due to insufficient feeding, which would decrease the proportion of food energy used for growth. In addition, food wastage during a lower appetite period could also increase FCR (Juell et al., 1993). In other words, the lower FCR of the intelligent group indicates that the intelligent feeding method can effectively reduce the cost of feed and improve feed efficiency. Therefore, the intelligent feeding method showed benefits, such that all fish growth parameters (especially the FCR) in the intelligent group were improved to a certain extent compared to the traditional group.

3.1.3. Digestive enzyme activity

Fish digestive enzyme activity is closely related to fish growth (Guerrera et al., 2015; Nofouzi et al., 2019; Thongprajukaew et al., 2011). No significant difference in amylase and lipase activity was observed between the two feeding methods (Table 2). Pepsin activity in the intelligent feeding group was significantly higher than the traditional group. These results indicate that fish fed with the intelligent feeding method had better protein digestion. This feature is critical to the digestion and absorption of protein in the feed and promotes the rapid growth of fish.

3.2. Biochemical and immunological indicators

For a comprehensive evaluation of feeding methods in aquaculture, fish welfare must also be assessed (Santurtun et al., 2018). Previous studies have demonstrated biochemical parameters and immunological indicators are closely related to the health and welfare of fish (Sankian et al., 2018; Shi et al., 2019; Yildiz and Altunay, 2011). To evaluate the effects of different feeding methods on fish welfare, both the biochemical and immunological indicators were measured here (Table 3). Although no significant differences in the cortisol level or superoxide dismutase (SOD) and malondialdehyde (MDA) activities were detected between the two groups ($p > 0.05$), the cortisol level and MDA activity in the intelligent group increased by 9.45% and 19.44%, respectively, and the SOD activity declined by 6.25%. The traditional group had a significantly higher level of lysozyme (LZM) compared to the intelligent group ($p < 0.05$).

Cortisol, extracted from the adrenal cortex, is an adrenocortical hormone that plays a key role in carbohydrate metabolism and is usually used to assess the fish stress response (Brijs et al., 2018; Ellis et al., 2012; Fevolden et al., 1994; Hoseini et al., 2020; Santurtun et al., 2018). A short-term increase in the cortisol level can promote the breakdown of body proteins while accelerating sugar synthesis and fat oxidation and enhancing non-specific immunity (Demers and Bayne, 1997), however, if the cortisol level remains high, it will result in reduced fat synthesis, bodyweight loss, growth stagnation and innate immunosuppression (Demers and Bayne, 1997; Gregory and Wood, 1999). Previous studies suggested that frequent feeding stimulation would cause continuous excitement and alertness of the fish, which would increase the cortisol level and affect normal growth and fish welfare (Huntingford et al., 2012). In the current aquaculture, most breeding objects, especially the *Micropterus salmoides*, tend to eat non-stationary feed. Thus, many feeding methods, including intelligent feeding, are based on the feeding strategy of repeated feeding in an event. This feeding strategy can not only make feed loss easy to be observed and determine when to stop feeding, but also leave enough time for the intelligent feeding algorithm to calculate. Few studies, however, have focused on fish welfare that can result from intelligent feeding. In this study, the intelligent feeding method stimulates the fish many times during feeding, which may increase the cortisol level. Furthermore, the feeding strategy (continuous feeding of a low amount per time) in the intelligent group may lead to individuals with a higher social hierarchy occupying the feeding area and prevent others from approaching this area. The fish in a lower social hierarchy may then be continually excited and alert, which would increase the cortisol level in the intelligent group. Although the cortisol level may gradually return to normal when the feeding is finished, the cortisol in the intelligent group would eventually accumulate and be higher than compared to the traditional group. In this study, the lack of a significant difference in the cortisol level may be due to the relatively short breeding time. Nonetheless, the cortisol level is still an effective parameter that reflects fish welfare.

Table 2
Effects of different feeding methods on digestive enzyme activity ($n = 15$).

Item	Intelligent group	Traditional group
Liver amylase (U/mg protein)	0.15 ± 0.03^a	0.17 ± 0.06^a
Intestinal amylase (U/mg protein)	0.37 ± 0.12^a	0.48 ± 0.13^a
Liver lipase (U/mg protein)	2.01 ± 0.66^a	2.18 ± 0.54^a
Intestinal lipase (U/mg protein)	3.12 ± 0.88^a	3.19 ± 0.84^a
Liver protease (U/mg protein)	132.53 ± 34.27^a	152.84 ± 37.48^a
Intestinal trypsin (U/mg protein)	80.03 ± 17.48^a	72.61 ± 13.65^a
Pepsin (U/mg protein)	6.83 ± 1.64^a	5.19 ± 1.12^b

Note: Significant differences ($p < 0.05$) are indicated by different superscript letters within rows.

Table 3
Effects of different feeding methods on biochemical and immunological indicators ($n = 15$).

Item	Intelligent group	Traditional group
Cortisol (ng/ml)	22.00 ± 2.07^a	20.10 ± 2.24^a
SOD (U/mg protein)	255.45 ± 20.15^a	272.47 ± 29.20^a
MDA (nmol/mg protein)	0.86 ± 0.25^a	0.72 ± 0.22^a
LZM (U/mg protein)	21.95 ± 4.75^a	26.68 ± 3.43^b

Note: Significant differences ($p < 0.05$) are indicated by different superscript letters within rows.

Lysozyme, an alkaline enzyme that can hydrolyze mucopolysaccharides of pathogenic bacteria, has anti-inflammatory, antibacterial and antiviral effects on the body (Rauta et al., 2012). In the intelligent group, multiple feeding stimulations resulted in depressed lysozyme levels, however, previous studies have shown that cortisol plays a mediating role in the process of environmental impact on immune organs. Demers and Bayne (1997) also emphasized that lysozyme may be a rather small component of innate resistance, whereas cortisol may have rather widespread negative effects on resistance mechanisms. For the intelligent group, the phenomenon of higher cortisol but lower lysozyme levels indicates that a higher susceptibility to pathogens can lead to a negative effect on the health and welfare of the fish.

The antioxidant capacity of the immune system is also closely related to fish health (Dawood et al., 2020; Liu et al., 2020). The SOD and MDA activities can indirectly reflect the fish antioxidant capacity (Viarengo et al., 1995). In this study, the SOD activity in the intelligent group was lower than the traditional group, but the MDA activity in the intelligent group was higher than in the traditional group. The intelligent group, which was subjected to multiple stimulations during the feeding process, may have an increased consumption of metabolism and energy. This phenomenon, to a certain extent, would promote the generation of oxygen free radicals and increase the MDA activity in the intelligent group. Although no significant differences in the SOD and MDA activities were observed, the generation of oxygen free radicals slightly increased the MDA activity in the intelligent group compared to the traditional group. The intelligent group, however, demonstrated lower SOD activity.

How to realize intelligent feeding for the cultured fish in the RAS, is not only the challenge in production management but the key scientific problem in welfare farming. Different from the traditional feeding

Table 4
Effects of different feeding methods on biochemical components ($n = 15$).

Item	Intelligent group	Traditional group
Moisture (%)	79.18 ± 0.86^a	78.73 ± 2.00^a
Ash (%)	2.92 ± 0.71^a	2.83 ± 0.83^a
Crude protein (%)	12.68 ± 0.42^a	16.67 ± 2.74^b
Crude lipid (%)	4.03 ± 0.88^a	4.77 ± 0.99^a

Note: Significant differences ($p < 0.05$) are indicated by different superscript letters within rows.

method, intelligent feeding can greatly weaken the size differentiation of fish school and improve fish growth but may also result in stress and suppress innate immunity. At present, although intelligent feeding has been an active area in aquaculture research, few studies have focused on how to combine the appropriate feeding strategy with an intelligent feeding algorithm. This deficiency may underlie the stress and innate immune suppression observed during intelligent feeding. Moreover, fish feeding and appetite vary from species to species and are susceptible to a variety of external factors, including the environment and management practices. Therefore, intelligent feeding in the future should target specific breeds, not only combining feeding strategies but also adapting to changes in the environment.

3.3. Proximate composition

Energy intake plays an important role in determining the primary proximate composition in growing fish (Cardinal et al., 2011). To study the effects of the two feeding methods on the proximate composition of fish flesh, the moisture, ash, crude protein, and crude lipid levels were measured as shown in Table 4. No significant differences in moisture, ash, or crude lipid ($p > 0.05$) were observed between the two groups, however, the traditional group had a significantly higher level of crude protein (3.99%) compared to the intelligent group ($p < 0.05$).

In theory, the fish in the intelligent group can acquire sufficient energy intake, which would promote a more preferable body proximate composition versus the traditional group, but the opposite result was obtained. In this study, the fish in the intelligent feeding group were stimulated many times during a feeding, resulting in relatively accelerated metabolism and consequently higher energy consumption. Hence, juvenile *Micropterus salmoides* may consume body protein first, which would result in significantly lower crude protein levels observed in the intelligent group.

4. Conclusions

To explore whether the intelligent feeding methods that have been proposed so far would impair fish welfare in real production, an adaptive feeder based on a practical intelligent feeding method was designed. In contrast to traditional feeding, the intelligent feeding method was more conducive to improving fish growth performance and reducing feed cost in aquaculture, however, intelligent feeding would eventually result in a higher cortisol level and lower lysozyme level, which is harmful to the fish immune system and general welfare. In the future, with the development of multi-functional, high-precision sensors, the improved or potential methods will be proposed to provide more and more intelligent solutions to address as many feeding problems as possible, and these methods could potentially improve breeding efficiency and fish welfare.

Declaration of competing interest

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agreed to submission to the journal of "**Artificial Intelligence in Agriculture**". No conflict of interest exists in the submission of this manuscript.

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