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Research article

Buspirone administration: Influence on growth, spawning, immune response, and stress in female goldfish (*Carassius auratus*)

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ABSTRACT

The current study evaluated the impact of buspirone supplementation on the growth, physiology, stress response, spawning, and immunity in female goldfish (*Carassius auratus*). For this purpose, buspirone was dissolved in absolute methanol and sprayed onto the feed to create four experimental groups: B0 (control), B25 (25 mg kg^{-1}), B50 (50 mg kg^{-1}), and B100 (100 mg kg^{-1}). Fish were fed their respective diets for 56 days and subjected to stress using the air exposure method at the end of the experiment. Growth performance analysis revealed that fish in the B100 group exhibited significantly higher final weight, weight gain, specific growth rate, and average daily gain than the other groups ($P < 0.05$). Plasma stress response indicated that cortisol levels were significantly lower in the B100 group after stress exposure, accompanied by a simultaneous decrease in glucose levels. The mucus stress response also showed lower cortisol and glucose levels in the B100 group compared to the other groups. Immunological analysis revealed significant increases in total protein, albumin, complement C3 and C4, and immunoglobulin M concentrations in both plasma and mucus of the B100 group ($P < 0.05$). Reproductive performance showed a notable enhancement in the number of eggs, fertilization rate, hatching rate, and survival rate in the B100 group compared to other groups ($P < 0.05$). Buspirone at higher concentrations, positively impacted various physiological aspects of goldfish, including growth, stress, immune activity, and reproductive performance. The significant improvements observed in growth parameters, cortisol levels, immunological markers, and reproductive outcomes suggest the potential of buspirone supplementation as a beneficial strategy in aquaculture practices.

1. Introduction

In aquaculture, fish are frequently exposed to natural and artificial stressors, including changes in water quality (temperature, oxygen, pH, salinity), rearing conditions (stocking density, water flow), and routine handling (grading, netting, transportation). These factors can adversely affect fish physiology [1,2].

It is well established that stress can activate the immune system, reducing pathogen resistance and fish survival rates [3–5]. The relationship between the endocrine and immune systems under stress has been extensively documented, particularly in lower vertebrates like fish, where cytokines and neuropeptides influence both systems [6,7], data on the interactions between these systems at

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the mucosal level in stressed fish remain limited [8,9]. Stress affect immune function through alterations in the hematopoietic system, leukocyte profile, and biochemical stress markers [8,10]. The mucosal immune system in vertebrates comprises a wide range of innate and adaptive immune cells and molecules that defend against pathogens [11,12]. The mucus layer also acts as a reservoir for numerous immune factors [13,14].

Research on steroid levels, such as cortisol, in skin mucus and their role in fish stress responses, is limited, though skin mucus may be a useful non-invasive stress biomarker [15,16]. Acute stressors can enhance immune responses by increasing the activity of dendritic cells, neutrophils, macrophages, and lymphocytes [17–19]. However, prolonged stress may lead to immune system suppression or dysregulation due to insufficient energy to sustain the stress response [17,20]. Anti-stress drugs are commonly used to manage stress, with selective serotonin reuptake inhibitors (SSRIs) being the main class of antidepressants in recent years. Buspirone, an antianxiety medication, is prescribed for certain anxiety and stress disorders and for short-term relief of anxiety symptoms [21,22]. While buspirone has shown antidepressant-like effects [23–25], its therapeutic impact is attributed to adaptive changes from prolonged use rather than immediate effects on the 5-HT system [26]. Although buspirone has been used to study anxiolytic behavior in zebrafish (*Danio rerio*) [27,28], the mechanisms and physiological effects of azapirone-class drugs, like buspirone, are not well understood compared to other pharmaceuticals like Diazepam and Citalopram [21]. Additionally, recent studies have extensively explored probiotics, prebiotics, synbiotics, fruit extracts, and medicinal herbs, along with their byproducts, as natural substances that can serve as growth promoters, enhance immune responses, improve disease resistance, and support stress responses, either as alternatives or supplements to antibiotics [4,29–35].

These studies focus on identifying, quantifying, and characterizing tissues to evaluate various therapeutic modalities and understand the mechanisms of stress response [8,31,36,37]. In this study, goldfish (*Carassius auratus*) were selected due to their ease of handling, genetic similarity to humans, and cost-effectiveness as a high-throughput model [38–41]. They are well-suited for stress research and can advance neuropharmacological understanding and drug discovery [42,43]. A comprehensive review of published and unpublished studies indicates that the effects of buspirone on fish growth or stress response have received little attention [21,44,45]. Therefore, the current investigation aims to analyse the impact of Buspirone on the growth, stress response, immune activity, and reproduction of female goldfish.

2. Materials and methods

2.1. Ethical information

All animal procedures were conducted in compliance with the ethical standards of the institution and adhered to the guidelines outlined in Directive 2010/63/EU.

2.2. Fish maintenance

The present investigation was conducted in aquaculture facilities at the Faculty of Natural Resources, University of Guilan (Sowmeh Sara, Iran). Four hundred goldfish (Average weight 11.2 ± 0.2 g) were sourced from a private aquaculture operation in Rasht, Guilan, Iran. Before beginning the study, the fish were acclimated to experimental conditions for three weeks and fed with commercial pellets (Thai Luxe Enterprises PCL, Phetchaburi, Thailand; comprising protein: 41 %, fat: 6 %, fibre: 5 %, and moisture: 12 %). The temperature and dissolved oxygen levels were consistently maintained at 22.8 ± 0.5 °C and 8.0 ± 0.5 mg L^{-1}, respectively, with continuous aeration. The photoperiod was set to 16 h of light and 8 h of darkness. To ensure optimal water quality, 40 % of the water in each tank was replenished daily [46]. Acclimated female broodstock goldfish with an average weight of 14.1 ± 0.5 g were randomly allocated into four groups (n = 50) across eight fiberglass tanks with a 500 L capacity.

2.2.1. Experimental design

Buspirone hydrochloride was procured from Sigma-Aldrich (CAS number: 33386-08-2; USA) and dissolved in absolute methanol sourced from Merck (Darmstadt, Germany). To create the experimental diet, buspirone was incorporated by spraying it onto the feed pellets [47,48]. The study established four experimental groups based on dosage levels adapted from the studies by Maximino et al. and Abozaid and Gerlai [49,50],: B0: Control group, which received feed without buspirone, B25: Group receiving feed supplemented with 25 mg kg^{-1} of buspirone, B50: Group receiving feed supplemented with 50 mg kg^{-1} of buspirone, B100: Group receiving feed supplemented with 100 mg kg^{-1} of buspirone, then pellets were left overnight at room temperature and let to evaporate the ethanol, and finally pellets were coated with 1 % gelatin solution (Merck, Darmstadt, Germany). Each group of fish was fed their respective diets to apparent satiation three times a day (9:00, 13:00, and 17:00) for 56 days [2]. After the 56-day feeding period, stress was induced across all experimental groups to evaluate the effects of b,uspirone under stress conditions. This was achieved by exposing the fish to air for 2 min using a dip net, a method designed to simulate a common stressor in aquaculture and assess the fish's stress response [2].

2.3. Growth performance

Prior to any handling or sampling, all fish were fasted for 24 h. All fish from each group were sampled to measure total length and weight at the end of the experiment. Concerning total length and weight, weight gain (WG), specific growth rate (SGR), average daily growth (ADG), condition factor (CF), and feed conversion ratio (FCR) were recorded. The calculations for these metrics were performed using the following formulas [51]:

WG (g) = final weight – initial weight

SGR (% day^{-1}) = 100 × (Ln final weight-Ln initial weight)/days

ADG (g day^{-1}) = (final weight – initial weight)/days

CF = weight/(total $length^3$)

FCR = (feed consumed)/(WG)

2.3.1. Sample analysis

For blood sampling, 2 mL of blood was collected using heparinized syringes (10,000 U mL^{-1}, Caspian Tamin, Tehran, Iran) from the caudal vein. Additionally, mucus was collected directly from the skin following the procedure described by Vervarcke et al. [52]. Briefly, mucus was gently collected by scraping the body with a sterile spatula. Subsequently, blood plasma and mucus supernatant were separated using a centrifuge (Hettich, Kirchlengern, Germany) at 1600 g for 8 min, and then stored in a refrigerator at −20 °C for further analysis. Cortisol measurement was performed using the enzyme-linked immunosorbent assay (Monobind, Lake Forest, California, USA) following the protocol by Abdollahpour et al. [53]. The intra-assay and inter-assay coefficients of variation were 5.2 % and 6.7 %, respectively. Biochemical analyses of blood plasma and mucus, including glucose, cholesterol, triglycerides, total protein, and albumin, were performed using a commercial diagnostic kit (Pars Azmun, Karaj, Iran). Glucose levels were determined via the Glucose Oxidase- Phenol Aminoantipyrine Peroxidase method, while cholesterol and triglyceride levels were measured using the Cholesterol Oxidase-Phenol Aminoantipyrine Peroxidase method and Glycerol Phosphate Oxidase- Phenol Aminoantipyrine Peroxidase methods, respectively. Total protein and albumin were analysed using the biuret and bromocresol green binding methods, respectively. Based on the total protein and albumin levels, globulin levels were calculated and expressed as g dL^{-1}. Blood plasma and mucus complement 3 (C3), complement 4 (C4), and immunoglobulin M (IgM) were assessed using the immunoturbidometric method with a commercial kit (Biorexfars, Shiraz, Fars, Iran).

2.3.2. Reproductive performance

Broodstock were induced to spawn using a semi-artificial technique. To facilitate reproduction, fish were moved to aquaria (dimensions: 80 cm length × 60 cm width × 40 cm height; 180 L volume), housing 1 female and 2 males. The fish were administered OvaprimTM (Tianjin Pharmaceutical Holdings Co, Shanghai, China) via a single injection at the base of their pectoral fins. The hormone dosages were 0.1 mg L^{-1} for males and 0.2 mg L^{-1} for females [8]. Plastic ropes were placed in each aquarium to provide a substrate for the adhesive eggs, which naturally attach to submerged materials. Post-spawning, the number of eggs, fertilization rate, hatching rate, and survival rate of larvae at 3 days post-hatch (dph) were recorded using the following methods:

Hatching rate (%) = 100 × ((number of fertilized eggs)/(numbers of total eggs))

Survival rate at 3 dph (%) = 100 × (number of live larvae)/(number of total larvae)

2.4. Statistical analysis

All data related to growth, cortisol levels, biochemical and immunological markers, and reproductive performance were statistically analysed using the SPSS software (Chicago, IL, USA, version 28). The normality of the data and homogeneity of variance for each parameter were assessed using the Kolmogorov-Smirnov and Levene's tests, respectively. Data were analysed using a One-way ANOVA followed by Tukey's post hoc test, with a significance threshold set at 0.05. The experimental outcomes were presented as mean ± SE.

3. Results

3.1. Growth performance

The growth performance of goldfish reared under different diets is summarized in Table 1. The administration of buspirone significantly enhanced the growth performance of female goldfish. Fish in the B100 group showed significantly higher final weight (24.03 ± 0.60 g) compared to other groups ($P < 0.05$). A significant increase in WG was observed in the B100 group (9.96 ± 0.60 g) compared to those in other groups ($P < 0.05$). Clear differences in SGR (0.95 ± 0.04 % day^{-1}) and ADG (0.17 ± 0.01 g day^{-1}) were observed, with fish in the B100 group showing higher values ($P < 0.05$). Fish in the B0 group showed the lowest values for all growth parameters, including final weight (15.62 ± 0.36 g), WG (1.55 ± 0.36 g), SGR (0.18 ± 0.04 % day^{-1}), and ADG (0.02 ± 0.00 g day^{-1}) ($P < 0.05$). The feeding experiment also led to a significant decrease in the FCR, with a clear decreasing trend observed as the buspirone concentration increased. Fish in the B0 and B100 groups had the highest (3.44 ± 0.09) and lowest (0.91 ± 0.15) FCR levels, respectively ($P < 0.05$).

Table 1
Effects of buspirone administration on growth performance of female goldfish (*Carassius auratus*) after 56 days.

	B0	B25	B50	B100
Initial weight (g)	14.12 ± 0.23	14.87 ± 0.14	14.19 ± 0.52	14.34 ± 0.34
Final weight (g)	15.62 ± 0.36^{c}	17.71 ± 0.54^{bc}	19.93 ± 0.70^{b}	24.03 ± 0.60^{a}
WG (g)	1.55 ± 0.36^{c}	3.64 ± 0.44^{bc}	5.86 ± 0.07^{b}	9.96 ± 0.60^{a}
SGR (%day^{-1})	0.18 ± 0.04^{c}	0.41 ± 0.05^{bc}	0.62 ± 0.00^{b}	0.95 ± 0.04^{a}
ADG (g day^{-1})	0.02 ± 0.00^{c}	0.06 ± 0.00^{bc}	0.10 ± 0.00^{b}	0.17 ± 0.01^{a}
FCR	3.44 ± 0.09^{a}	2.00 ± 0.18^{b}	1.53 ± 0.18^{bc}	0.91 ± 0.15^{c}

B0: control, without buspirone administration; B25: 25 mg kg^{-1} buspirone administration; B50: 50 mg kg^{-1} buspirone administration; B100: 100 mg kg^{-1} buspirone administration.
WG: weight gain; SGR: specific growth rate; ADG: average daily growth; FCR: feed convention ration.
Data are presented as mean ± SE. Groups values statistically different from each other have different small letters ($P < 0.05$).

3.2. Stress responses

Fig. 1a and b displays the plasma stress response of different experimental groups in goldfish. The cortisol levels were significantly lower in fish from the B100 group (26.79 ± 2.89 ng ml^{-1}) compared to other groups ($P < 0.05$), while fish in the B0 group indicated the highest cortisol levels (62.88 ± 1.79 ng ml^{-1}) after stress ($P < 0.05$). Under the B100 diet, fish in B100 group also showed a decrease in glucose levels (3.59 ± 0.11 mg dL^{-1}) compared to other groups ($p < 0.05$), whereas fish in the B50 group had the highest glucose levels (15.73 ± 3.32 mg dL^{-1}). Fig. 2a and b shows the mucus stress response of different experimental groups in goldfish. The experimental diets affected the mucus stress response, with cortisol levels being significantly lower in the B100 group (19.04 ± 1.06 ngml^{-1}) compared to other groups ($p < 0.05$). Fish in the B0 group had the highest mucus cortisol levels (51.67 ± 3.61 ng ml^{-1}). Similarly, fish in the B100 group had lower mucus glucose concentrations (0.68 ± 0.05 mg dL^{-1}) compared to other groups ($P < 0.05$). There was no significant difference in mucus glucose levels among groups B0, B25, and B50 ($P > 0.05$).

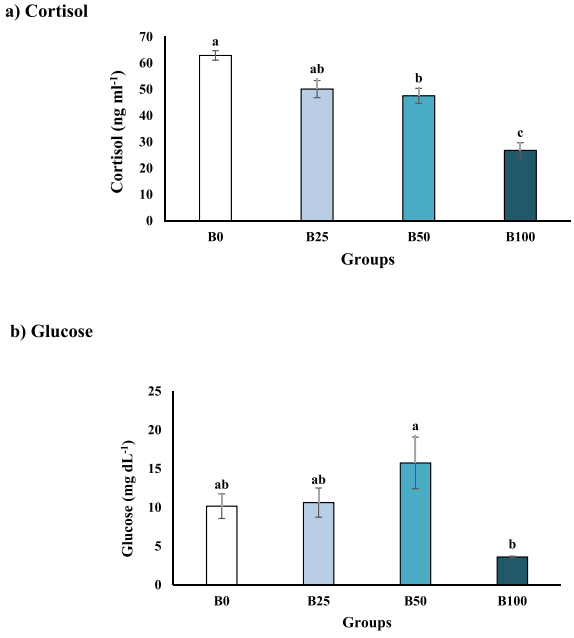

Fig. 1. Effects of buspirone administration on plasma stress response of female goldfish (*Carassius auratus*) after 56 days. B0: control, without buspirone administration; B25: 25 mg kg^{-1} buspirone administration; B50: 50 mg kg^{-1} buspirone administration; B100: 100 mg kg^{-1} buspirone administration. Data are presented as mean ± SE. Groups values statistically different from each other have different small letters ($P < 0.05$). a) Cortisol, b) Glucose.

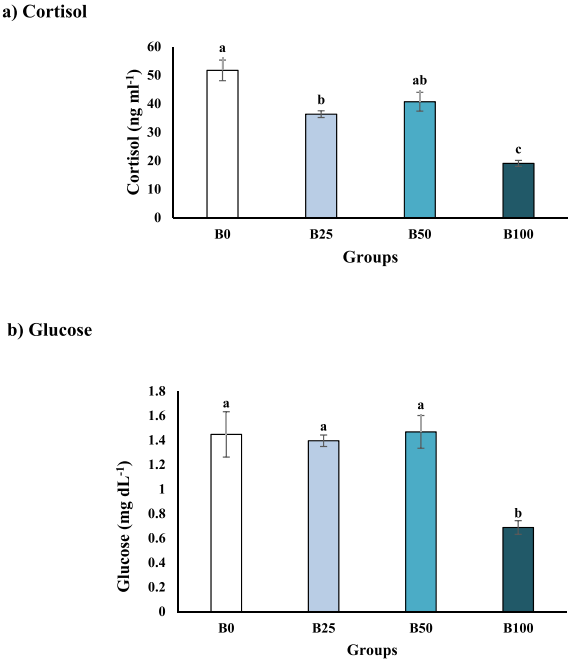

Fig. 2. Effects of buspirone administration on mucus stress response of female goldfish (*Carassius auratus*) after 56 days. B0: control, without buspirone administration; B25: 25 mg kg^{-1} buspirone administration; B50: 50 mg kg^{-1} buspirone administration; B100: 100 mg kg^{-1} buspirone administration. Data are presented as mean ± SE. Groups values statistically different from each other have different small letters ($P < 0.05$). a) Cortisol, b) Glucose.

3.3. Immunological parameters

The findings from the plasma immunological assay in goldfish are summarized in Table 2. Notably, group B100 exhibited a remarkable increase in total protein levels (1.56 ± 0.14 mg dL^{-1}), significantly surpassing other groups ($P < 0.05$). Moreover, buspirone administration influenced albumin levels and B100 group displayed the highest concentration (1.33 ± 0.18 mg dL^{-1}) compared to other groups ($P < 0.05$). Significant differences were observed in plasma C3 and C4 levels across the experimental groups ($P < 0.05$), with the B100 group exhibiting notably elevated concentrations of C3 (1.82 ± 0.36 mg dL^{-1}) and C4 (2.92 ± 0.36 mg dL^{-1}). Similarly, IgM concentration showed a similar trend, with the B100 group exhibiting the highest levels (4.57 ± 0.42 mg dL^{-1}), showing a significant difference compared to the other groups ($P < 0.05$). Additionally, the mucus immunological assay results shown in Table 2 revealed significant differences in total protein concentration. The B100 group had notably higher levels (1.60 ± 0.14 mg dL^{-1}), while the B25 and B50 groups did not show a significant impact compared to the B0 group ($P > 0.05$). Furthermore, albumin levels displayed a significant increase ($P < 0.05$) in the B100 group (0.67 ± 0.13 mg dL^{-1}). C3 concentration exhibited significant differences among experimental groups, with group B100 displaying the highest levels (4.09 ± 0.36 mg dL^{-1}) compared to others ($P < 0.05$). Similarly,

Table 2
Effects of buspirone administration on immunological parameters of female goldfish (*Carassius auratus*) after 56 days.

	B0	B25	B50	B100
Plasma				
Total protein (mg dL^{-1})	0.56 ± 0.19^{b}	0.74 ± 0.19^{b}	0.62 ± 0.08^{b}	1.56 ± 0.14^{a}
Albumin (mg dL^{-1})	0.14 ± 0.03^{b}	0.19 ± 0.04^{b}	0.3 ± 0.11^{b}	1.33 ± 0.18^{a}
C3 (mg dL^{-1})	0.68 ± 0.11^{b}	0.74 ± 0.17^{b}	0.91 ± 0.11^{ab}	1.82 ± 0.36^{a}
C4 (mg dL^{-1})	1.46 ± 0.05^{b}	1.76 ± 0.29^{b}	1.83 ± 0.09^{ab}	2.92 ± 0.35^{a}
IgM (mg dL^{-1})	1.52 ± 0.21^{b}	2.67 ± 0.27^{b}	2.21 ± 0.41^{b}	4.57 ± 0.42^{a}
Mucus				
Total protein (mg dL^{-1})	0.29 ± 0.01^{b}	0.39 ± 0.16^{b}	0.31 ± 0.16^{b}	1.60 ± 0.14^{a}
Albumin (mg dL^{-1})	0.22 ± 0.04^{b}	0.22 ± 0.05^{b}	0.22 ± 0.51^{b}	0.67 ± 0.13^{a}
C3 (mg dL^{-1})	1.44 ± 0.15^{b}	1.39 ± 0.12^{b}	1.81 ± 0.21^{b}	4.09 ± 0.36^{a}
C4 (mg dL^{-1})	0.96 ± 0.18^{b}	1.01 ± 0.41^{b}	1.02 ± 0.11^{b}	2.37 ± 0.12^{a}
IgM (mg dL^{-1})	1.45 ± 0.24^{b}	1.85 ± 0.41^{b}	2.21 ± 0.19^{b}	3.61 ± 0.28^{a}

B0: control, without buspirone administration; B25: 25 mg kg^{-1} buspirone administration; B50: 50 mg kg^{-1} buspirone administration; B100: 100 mg kg^{-1} buspirone administration.
Data are presented as mean ± SE. Groups values statistically different from each other have different small letters ($P < 0.05$).

Table 3
Effects of buspirone administration on reproductive performance of female goldfish (*Carassius auratus*) after 56 days.

	B0	B25	B50	B100
Number of eggs	74.00 ± 2.59^{c}	111.00 ± 7.72^{c}	228.33 ± 14.63^{b}	425.67 ± 1.49^{a}
Fertilization rate (%)	46.67 ± 1.49^{c}	51.67 ± 3.95^{bc}	59.33 ± 0.61^{b}	75.00 ± 2.59^{a}
Hatching rate (%)	40.00 ± 2.59^{b}	44.00 ± 4.42^{b}	55.00 ± 2.59^{b}	73.33 ± 2.99^{a}
Survival at 3 dph (%)	50.00 ± 2.59^{bc}	56.67 ± 1.49^{bc}	63.33 ± 1.49^{b}	80.00 ± 2.59^{a}

B0: control, without buspirone administration; B25: 25 mg kg^{-1} buspirone administration; B50: 50 mg kg^{-1} buspirone administration; B100: 100 mg kg^{-1} buspirone administration.
Data are presented as mean ± SE. Groups values statistically different from each other have different small letters ($P < 0.05$).

significant variations were noted in C4 levels among different groups ($P < 0.05$), with the B100 group demonstrating the highest concentration (2.37 ± 0.12 mg dL^{-1}). The experimental diets had a significant effect on plasma IgM concentrations ($P < 0.05$), with the B100 group showing higher IgM levels (3.61 ± 0.28 mg dL^{-1}). In contrast, the other experimental groups did not show a significant difference compared to the B0 group ($P > 0.05$).

3.4. Reproductive performance

Table 3 presents the variations in reproductive performance among the experimental groups. Notably, there was a clear improvement in reproductive performance as the dosage of buspirone increased. As shown in Table 3, buspirone administration had a significant impact on the number of eggs compared the different groups. B100 group exhibited the highest number of eggs (425.67 ± 1.49), while group B0 displayed the lowest (74.00 ± 2.59), showcasing significant differences ($P < 0.05$). Similarly, the fertilization rate significantly increased with buspirone supplementation in the diet, with the B100 group achieving the highest average fertilization rate (75.00 ± 2.59 %) compared to the other groups ($P < 0.05$). Moreover, the utilization of buspirone significantly increased both the hatching rate and survival by the experiment's conclusion, with group B100 achieving the highest levels of hatching rate (73.33 ± 2.99 %) and survival (80.00 ± 2.59), presenting statistically significant disparities ($P < 0.05$). Conversely, Group B0 recorded the lowest hatching rate (40.00 ± 2.59 %) and survival rate (50.00 ± 2.59 %) among the experimental groups.

4. Discussion

In this study, our objective was to evaluate the potential impact of administering buspirone on the growth, spawning, immune response, and stress activity in female goldfish, a commonly used vertebrate model for physiological research. The effects of stress response induced by air exposure were examined, which typically result in elevated cortisol and glucose levels, as well as reduced growth, spawning performance, and immune function in female goldfish broodstock. The findings are consistent with previous studies on various fish species [2,54,55].

Based on the present results, a higher dosage of buspirone (B100) led to greater WG, SGR, and ADG, while achieving lower FCR compared to other treatment groups. Fish growth is regulated through various physiological pathways that influence energy acquisition and balance [56,57]. The drive to feed is modulated by neuropeptides (as reviewed by Volkoff, [58]), with the somatotropic axis playing a crucial role in regulating the growth and metabolism of fish [54]. Administration of buspirone did not adversely affect growth and spawning performance at the tested concentrations. Previous studies have highlighted the impact of antidepressants on fish development and physiology across different species [59]. Exposure to anti-anxiety medications can also influence fish development and growth. For example, Duarte et al. reported a significant decline in the weight of juvenile meagre (*Argyrosomus regius*) exposed to 0.3 or 3 μg L^{-1} fluoxetine for 15 days [60]. In contrast, Chen et al. observed no significant changes in body growth and hatch rate in zebrafish embryos exposed to 1, 10, or 100 mg L^{-1} carbamazepine for 6 days [61]. A crucial aspect of growth involves the endocrine regulation of feeding and metabolism [56,57].

Growth hormone (GH) and insulin-like growth factors (IGFs) are major components of the somatotropic axis, which regulates growth and metabolism in fish [62,63]. The functioning of the somatotropic axis is modulated by monoaminergic stimulation in fish species [64,65]. For example, serotonin stimulation enhances GH release [66], while catecholamines, including norepinephrine, decrease GH production [67]. Dopamine also plays a role in controlling GH production in fish (reviewed by Peng and Peter, [68]). In vertebrates, many researchers have advocated using diazepam in animal reproduction, fish fry transportation, and veterinary management [69,70]. Extensive research, including both published and unpublished studies, has shown that the effects of diazepam on fish growth and stress responses have received minimal attention [44,45,71]. Khan et al. observed that the significant increase in body mass, protein, and lipid accumulation at the 12 mg dosage can be attributed to diazepam's well-documented role as a potent appetite stimulant [72]. This effect is mediated by Gamma-aminobutyric acid and central inhibition at the serotoninergic receptors that control the satiety center in the hypothalamus. The studies by Belal and Assem on diazepam, along with the current investigation on buspirone administration in fish, offer valuable insights into the role of psychoactive drugs in aquaculture [73]. Both studies highlight the potential benefits of using these drugs to enhance fish growth, although the mechanisms and effects may differ. Belal and Assem's research on Nile tilapia (*Oreochromis niloticus*) indicates that diazepam can significantly enhance growth metrics without compromising food safety, as the drug is effectively cleared from the fish's system over time [73]. This finding is crucial for aquaculture, as it ensures that growth enhancements do not compromise consumer health. Belal and Assem's research show that diazepam can increase

growth in Nile tilapia safely, also, our study introduces buspirone, which influences growth in goldfish. These results highlight the need to consider species-specific responses and physiological stages when using such drugs. Future research should compare drugs like diazepam and buspirone across different species and stages to optimize dosages and improve aquaculture practices, while also investigating their long-term health effects and ecological impacts. Maintaining female broodstock health is essential for high-quality progeny and population recruitment [74,75]. The observations highlight the need to evaluate the impact of stress on goldfish spawning and consider buspirone as a potential enhancer of spawning performance. The results revealed higher egg production, fertilization rates, hatching percentages, and survival rates in the B100 group compared to the lower rates observed in the B0 (control) group. Previous studies have reported that fluoxetine administration for a week increased estrogen levels but decreased testosterone levels and sperm quantity in male goldfish [76]. Similarly, venlafaxine in female fathead minnows resulted in increased total egg production at a high dosage of 88 μg L^{-1}, while a low dosage of 0.88 μg L^{-1} led to a reduction in ovipositor length [77]. It appears that the high dosage of buspirone in current study increased estrogen release, leading to higher reproductive output. However, the mechanisms by which antidepressants affect the reproductive axis need further investigation. Future studies should explore how buspirone administration influences estrogen levels. Buspirone, a serotonin receptor agonist, may affect reproductive functions and growth in fish by influencing serotonin pathways, similar to other serotonergic drugs. Serotonin impacts fish reproduction through both central (preoptic-hypothalamic region and pituitary) and peripheral (gonads) mechanisms [78]. In the hypothalamus, GnRH neurons are crucial for regulating vertebrate reproduction. Serotonin stimulates GnRH release in seabream and goldfish [79,80], and in zebrafish, serotonin receptors are present in brain regions with GnRH neurons, indicating possible co-expression of serotonin receptors and GnRH neurons, as seen in mammals [81,82]. Additionally, serotonin, along with GnRH, stimulates LH secretion in Atlantic croaker [83] and influences growth hormone (GH) release in goldfish [83,84]. The varied effects of SSRIs on fish reproduction may result from differences in dosage, administration methods, treatment durations, physiological and reproductive status, sex, and species [85]. SSRIs are thought to interact with and inhibit certain P450 isozymes involved in steroid metabolism [86], potentially affecting reproductive neuroendocrine control. Future research should focus on species-specific responses, dose effects, long-term impacts, and molecular mechanisms of buspirone in fish, as well as its interactions with neuroendocrine systems, life stages, and environmental factors. It should also consider human health implications and alternative treatments to ensure the safe use of buspirone in aquaculture.

The current study specifically investigates how buspirone administration affects both stress responses (e.g., cortisol and glucose levels) and endocrine functions (e.g., hormone regulation and blood biochemical changes) in female goldfish broodstock. The endocrine feedback of stress is highly conserved in vertebrates and plays a crucial role in enabling animals to cope with stress responses [2,87,88]. The results indicated that the highest cortisol levels in blood and mucus were found in the B0 group, while the lowest levels were observed in the B100 group. In fish, cortisol is the main corticosteroid released in response to stress, and its secretion is regulated by the activation of the hypothalamus-pituitary-interrenal axis [89,90]. Once released into circulation, cortisol acts on target tissues through the activation of either the glucocorticoid receptor (GR) and/or the mineralocorticoid receptor [91,92]. The findings suggest that buspirone administration affects the function of the stress axis, as fish exposed to the drug exhibited lower cortisol levels than control fish during an acute stress test. Both benzodiazepines and SSRIs possess anxiolytic properties [69,93], and it is reasonable to suggest that these effects on the stress axis are associated with the central action of these drugs. The mechanism by which buspirone reduces the stress response may be directly linked to the hypothalamus and/or pituitary gland, potentially excluding the involvement of interrenal tissue. The serotonergic system in the brain plays a vital role in the autonomic, neuroendocrine, and behavioural integration of the stress response in fish and mammals [94]. Benzodiazepines have been shown to influence the hypothalamus-pituitary-adrenal axis in humans by decreasing basal adrenocorticotropic hormone (ACTH) and cortisol levels following acute administration [95–97]. Our results demonstrated decreased cortisol levels after stress induction in all buspirone concentrations. While the effects of diazepam and fluoxetine on cortisol levels have been previously reported in other species, the ability of buspirone to decrease stress-induced hormone release has not been definitively demonstrated [98]. Previously, protective effects of 1 mg kg^{-1} of buspirone on T cell immune responses were reported in stressed mice, with a corresponding reduction in ACTH levels [99]. As an increase in plasma corticosterone levels, due to ACTH release, can have detrimental effects on specific cells and tissues essential for optimal immune defense [100], the buspirone-induced decrease in ACTH concentration could partially explain the immunoprotective role of this drug. Interestingly, Levy and Van de Kar found that in rats, a dose of 0.5 mg kg^{-1} of buspirone inhibited the stress-induced increase in corticosterone release [98]. However, previous fish investigation on the impact of buspirone administration has been accomplished only following a single dose [49,50,101]. Moreover, the gender of the fish is an important factor that may influence the results of the antidepressant effect [102–104], and should be considered in future studies on the effects of buspirone in male goldfish. Consistent with our results, Wang et al. reported diazepam exposure could significantly reduce swimming activity (800 ng L^{-1}) and anxiety (800 and 8000 ng L^{-1}), indicating a sedative effect on medaka (*Oryzias latipes*) [105]. In comparison to buspirone, a multitude of investigations have examined the utilization of natural substances to alleviate stress responses and enhance health indicators [106,107]. For instance, Yousefi et al. performed a study on rainbow trout (*Oncorhynchus mykiss*) with an initial mass of 35.48 ± 0.34 g to evaluate the influence of dietary supplementation with (*Lactobacillus delbrueckii* subsp. *bulgaricus* (LDB)) and (*Asparagus officinalis* L. (AR)) root, either separately or in combination as synbiotics, on growth metrics, digestive enzyme activity, and multiple biomarkers in response to crowding stress. Their results indicated that these dietary supplements effectively reduced stress-induced effects on serum immune responses and significantly diminished the adverse impacts of crowding stress on immune function, hematological profiles, antioxidant status, and serum biochemical markers. Consequently, they advocate for the dietary inclusion of AR5 + LDB7 (1 × 10ˆ7 CFU/g CG + 5 g/kg CG) in high-stocking density rainbow trout rearing systems and aquaculture practices such as fish transportation [4]. Several studies have examined the impact of medicinal plant essential oils on aquatic species, consistently demonstrating reduced cortisol and glucose levels [8,108,109]. For instance, Shahbazi Naserabad et al. found that administering 1 % and 1.5 % (*Allium jesdianum*) essential oil (AJEO) significantly lowered cortisol and glucose levels, along with reductions in alkaline phosphatase, lactate

dehydrogenase, aspartate aminotransferase, and alanine aminotransferase activities. Additionally, AJEO showed potential in mitigating the toxic effects of cypermethrin in rainbow trout [110]. Similarly, Abdul Rahim et al. reported that in zebrafish, treatment with (*Polygonum minus*) leaf extract (at concentrations of 1 mg L^{-1} and 100 mg L^{-1}) led to a decrease in cortisol levels [111]. These findings on medicinal plant extracts underscore the importance of exploring natural alternatives to conventional pharmaceuticals like Buspirone in managing stress and improving health parameters in aquatic animals.

Glucose is a vital nutrient in blood serum, modulated by both external and internal factors. In the current investigation, the rapid increase in plasma and mucus glucose levels following air exposure stress is likely due to the mobilization of glucose from muscle and liver sources. When fish are subjected to stress, glucose is quickly mobilized from muscle and hepatic stores, resulting in a significant rise in plasma glucose to provide the necessary energy to counteract stress. Stressful stimuli trigger the rapid release of glucocorticoids and catecholamines from the adrenal tissue of fish species, both of which induce quick hyperglycemia. The occurrence of increased metabolism during stress activity has been documented in numerous studies, linking hyperglycemia in stressful conditions to a positive correlation between plasma glucose concentrations and metabolic rate [112–115]. Plasma glucose, an organic constituent of blood, is often used as an index of stress in fish subjected to environmental changes [116]. Several studies have shown that long-term stress exposure, such as crowding stress, thermal stress, and low salinity, can increase serum glucose levels [115,116]. The results of the current study indicated that Buspirone decreased glucose levels, which may be related to an increase in insulin levels in the blood [117]. This decrease in blood glucose can be explained by recent studies showing that buspirone reduces blood glucose through serotonergic receptors, independent of insulin. It has been determined that stimulating factors of the serotonin system, manifested as direct stimulation of 5-HT receptors, increase insulin secretion and reduce blood glucose levels. Buspirone, as a serotonin system stimulant, likely plays a role in reducing blood glucose [118,119]. In summary, the findings from this study align with previous research indicating that Buspirone can modulate glucose levels in stressed fish by influencing serotonergic receptors and potentially increasing insulin secretion, thereby reducing blood glucose levels. Future research should explore the dose-response relationships of buspirone across various fish species, developmental stages, and exposure durations. Comparative endocrinology could provide broader insights into buspirone's effects across vertebrates. Longitudinal studies are needed to evaluate its long-term impacts on immune function, behavior, and physiology. Additionally, environmental research should assess buspirone's bioaccumulation, non-target effects, and persistence. Understanding these factors will help develop safe usage guidelines and minimize environmental risks. In summary, future studies should thoroughly investigate buspirone's mechanisms, optimize dosages, and assess ecological impacts to guide informed aquaculture practices.

This study highlights that buspirone significantly affects the immune parameters of female goldfish under stress, with higher levels of key immune markers (albumin, IgM, C3, and C4) in the B100 group compared to the lower levels in the B0 group. These parameters are essential indicators of the overall health and immune status of the fish [8,120], with immunoglobulins playing a crucial role as the first line of immunity system during stress. Enhanced IgM levels, for instance, reflect improved immune responses, as seen in other species such as Nile tilapia under salt stress [121]. Complement C3 also plays a vital role in pathogen recognition and elimination [122]. Under stress, fish activate homeostatic mechanisms to modulate their immune responses [123]. The observed increase in IgM and complement C3 activities suggests a heightened immune response in Buspirone-treated fish. Similar enhancements in immune parameters have been reported in other species under stress, such as yellowhead catfish and pacu [123,124]. Stress typically reduces immune capacity, leading to increased susceptibility to infections, which further activates immune responses [125,126]. Our findings show that stress significantly suppresses immune responses, reducing levels of total protein, albumin, C3, C4, and IgM in both blood and mucus. These results are consistent with previous studies [8,127,128]. The administration of buspirone in mice has been shown to modulate immune responses to stress, with protective effects on NK cell and phagocytic activities at certain doses [129]. Buspirone, a 5-HT1A agonist, and its metabolite 1-PP, which has antagonist activity, may impact the immune system either directly or indirectly through neuroendocrine pathways [21,98,130,131]. In comparison to buspirone, several studies have highlighted the benefits of natural substances and medicinal plants in improving growth and immune parameters. For example, Ahmadifar et al. reported that supplementing cornelian cherry (*Cornus mas* L.) fruit extract at 0.5–1% enhanced growth performance and mitigated the effects of (*Aeromonas hydrophila*) infection in common carp (*Cyprinus carpio*) fingerlings after 8 weeks of feeding trials [30]. Similarly, Yousefi et al. found that incorporating 200 mg kg^{-1} of marjoram (*Origanum majorana*) extract in carp feed stimulated growth, antioxidant activity, and immune responses, reducing mortality during (*Aeromonas septicemia*) [32]. Additionally, oral administration of p-coumaric acid at 1–1.5 g kg^{-1} improved growth performance, digestive enzyme activity, and immune responses in common carp [33]. Furthermore, a study by Hoseinifar et al. demonstrated that zebrafish fed a sodium propionate (SP) supplemented diet exhibited elevated mucosal immune parameters, indicating the beneficial effects of dietary SP on mucosal immune response, growth, and appetite-related gene expression [35].

5. Conclusion

This study demonstrates that buspirone supplementation significantly enhances growth, stress response, immune activity, and reproductive performance in goldfish, with the highest dose (100 mg kg^{-1}) yielding the most notable improvements. Specifically, buspirone at 100 mg kg^{-1} reduced cortisol levels, indicating reduced stress, and elevated key immune parameters such as total protein, albumin, complement C3 and C4, and immunoglobulin M in both plasma and mucus. Reproductive performance, as measured by egg production, fertilization, hatching, and survival rates, also improved with higher buspirone doses. While these findings highlight buspirone's positive effects, the precise mechanisms by which it modulates immune responses remain unclear, suggesting a complex network of signaling pathways. Further research is needed to clarify these mechanisms, determine optimal dosages, assess long-term effects, and explore the environmental impact. Future studies should also compare buspirone with other stress-reducing agents to

expand aquaculture management strategies and ensure sustainability.

CRediT authorship contribution statement

Hamed Abdollahpour: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Naghmeh Jafari Pastaki:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Milad Karimzadeh:** Visualization, Validation, Methodology, Data curation, Conceptualization. **Hosseinali Zamani:** Validation, Methodology, Data curation.

Data availability statement

The data supporting the findings of this study are available from the corresponding author (H.A.) upon reasonable request.

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Declaration of competing interest

The authors declare that they have no conflicts of interest in this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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