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Research Article

Therapeutic Effect of Dietary Turmeric (*Curcuma longa*) on Growth Performance, Hematology Profile and Immunity Parameters of Common Carp (*Cyprinus carpio*)

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Abstract

Medicinal plants are rich in diverse nutrients and antioxidants that promote growth and have immunostimulating activities for fish. In aquaculture phytomedicines have gained importance worldwide because they are easily available, non-toxic to aquatic life. Turmeric (*Curcuma longa*) is among the most suitable natural ingredient due to its biological effects. Therefore, a study has been conducted to determine the effect of turmeric on the growth performance, hematology and immunity of common carp *Cyprinus carpio*. For this purpose, 180 fingerlings of common carp with average initial weight (30.23±0.04 g) were divided into six groups containing five treatment groups. Each tank was stocked with 10 fingerlings. Treatments were maintained in triplicates. Fish were fed with basal diet supplemented with graded level of turmeric at different concentrations i.e. TE0 (control), TE1 (1.0%), TE2 (2.0%), TE3 (3.0%), TE4 (4.0%) and TE5 (5.0%). Fish were fed twice a day at 3% body weight for 12-week. Results showed that fish growth was higher in all treatments as compared to the control group. The feed efficiency ratio (FER), feed conversion ratio (FCR), specific growth rate (SGR) and condition factor (K), of fish fed with turmeric enriched diet were found to be better than the control group. TE4 diet exposed to 4% Turmeric, showed maximum growth in terms of growth performance and feed utilization. Moreover, haematology profile (MCH, MCHC, MCV, PCV) and immunity parameters (WBCs) were also improved by turmeric supplementation. The result of the present study clearly shows that turmeric enhances the growth, improved haematological indices and boost up the immunity of common carp. Therefore, turmeric can be effectively used as natural dietary supplement in fish feed to improve overall fish health.

Keywords: *Cyprinus carpio*, turmeric, growth, haematology profile, immunity parameters.

1. Introduction

Aquaculture industry facing the challenge to provide advance formulated diets to enhance the growth and improve fish health. Feed additives are widely used to enhance growth performance, survival rate and feed efficiency in aquaculture [1]. In aquaculture, although a large number of fishes are produced through fish farming to overcome the difference between need and production of fish, but the major concern of the field is epidemic that reduces its profit ratio [2]. In aquaculture, disease prevention is very necessary and can be done by two methods. One method is by enhancing the immune response against the attack of microorganisms, and the other one is the use of chemical additives and veterinary medicines [3]. Use of drugs for controlling bacterial diseases are restricted because of their high prize and antibiotic resistant strains, pollution, and

deposition of chemical substances in fish meat are very dangerous for human health [4]. Presence of great variety of pathogens in water also reduces the vaccines application [5]. In other circumstances, useful vaccines are not formed for all fish pathogens and are also expensive to use [6]. The excessive use of antibiotics, disinfectants, and pesticides for controlling diseases has increased antibiotic resistant bacteria that may lead towards adverse side effects and are responsible for environmental pollution. This situation has accelerated the need to find the supplements that are safe to use, and additives that heightened the fish activity, health and immunity of fish [7]. World Health Organization suggests instead of using chemicals, we should widely use herbal medicines as an alternative and back to nature. For this reason, uses of medicinal herbs as immune boosters have been increased in aquaculture [8]. There is urgent requirement to utilize natural products as an alternate to lessen the use of chemical drugs to improve the host immune response, prevent bacterial resistance, and reduce free radicals [9].

Herbal extracts can be used not only for diseases but also enhance growth, and favoring the reproduction, fish nutrition of aquaculture [7]. Turmeric (*Curcuma longa*), a medicinal aromatic plant belonging to the family Zingiberaceae, widely cultivated in tropical regions. Turmeric plant, along with its active compound, shows a wide spectrum of pharmacological effects in the field of aquaculture such as growth-enhancing, immune-modulating, anti-inflammatory, antioxidative, liver protecting, stress-alleviating and antimicrobial properties [10-12]. Its compounds inhibit oxidation as well as it is cost effective [13]. Present natural medicines insist that the powder of turmeric has been used for gastrointestinal diseases, especially for biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation sinusitis, and cough [14]. The findings reported by Tacon et al. [15] showed that addition of curcumin in rainbow trout's diet can effectively improve haematological parameters. The major biologically active component of turmeric is curcumin in addition to flavonoids, phenolic compounds, Vitamin B, C and β-carotene [16, 17]. The global production of fisheries and aquaculture in 2022 was expanded to 223.2 million tonnes according to "The State of World Fisheries and Aquaculture (SOFIA)" 2024 edition. Asia contribute 92 % in global aquaculture production [18]. In 2020, Asia produce 27.58 million tonnes of Carp with China being the higher producer [19].

Common carp (*Cyprinus carpio*) from cyprinidae family is also called European carp. It is a fresh water fish widespread globally. The production of common carp expanded gradually from 2.41 million tons in 2000 to 4.08 million ton till 2013 [7]. It is the major cultured fish, which make up the 72% of total fresh water fish production [20]. Due to rich proteinaceous source and increased demand of common carp, this study was examining the effects of turmeric powder supplementation on the growth performance, proximate composition and hematology profile of common carp.

2. Materials and methods

2.1. Experimental site

The study was carried out in the laboratory of Department of Zoology GC Women University Sialkot. Total 180 *Cyprinus carpio* fingerlings (mean weight= 30.23±0.04 g) brought from a commercial fish hatchery, Sialkot were transferred to the experimental site and acclimatized for one week.

2.2. Experimental setup

Fish were transferred to glass aquaria having capacity of 100 litre water. Experimental trial carried out for 12-week. at a rate of ten fish per aquarium. Each treatment having three replicates, fingerlings were distributed into six groups having ten fish each replicate fed with graded levels of turmeric powder diet (0, 1, 2, 3, 4, and 5%) and carried out in a completely randomized design (CRD). Fish were fed twice a day (08:00-16:00) to satiation at 3% body weight. Physico-chemical parameters were monitored regularly including the water temperature (30±0.21 °C), pH (6.07±0.14), dissolved oxygen (6.70±0.25 mg/L), and total dissolved solids (1230±0.10 mg/L) using the Hanna HI 9828/4-01

multiparameter (Chelmsford, UK).

2.3. Diet preparation

Commercial basal diet of 30% CP digestible protein per kcal is used and feed was enriched with different quantity of turmeric powder. Turmeric, obtained from the local market in its natural form, grated and soaked in water to be added in commercial feed. Then, the test feed was sun dry and stored at -20 C^0. The first group was designated as control (TE0), the remaining groups i.e. TE1, TE2, TE3, TE4 and TE5 received basal diet with 1%, 2%, 3%, 4% and 5% turmeric powder respectively.

2.4. Measurement of growth parameters and feed efficiency

The growth parameters were measured and recorded at stocking and after one-week interval. The weight gain of the fish was calculated as the difference between the final weight and the initial weight (Eq. 1). Similarly, total length gain was determined by subtracting the initial total length from the final total length (Eq. 2). Fork length gain was computed by subtracting the initial fork length from the final fork length (Eq. 3). Feed conversion ratio (FCR), an essential indicator of feed efficiency, was calculated as the ratio of feed intake (in grams) to weight gain (in grams) (Eq. 4). Specific growth rate (SGR) is another growth parameter that can be calculated by subtracting natural log of initial weight from natural log of final weight, dividing it with total number of experimental days and then multiplying it with 100 to get SGR in percentage (Eq. 5). Feed efficiency ratio was determined by taking ratio of weight gain (gram) to feed intake (gram) (Eq. 6). By dividing wet body weight with cube of total length of body and multiplying it with 100, condition factor (K) that is a feed utilization parameter can be computed (Eq. 7). Survival rate in percentage was determined according to Eq. 8. All growth parameters were measured by following equations (Eq. 1-8) of Tacon et al. [15] .

Weight gain (g) = Final weight - initial weight (Eq-1)

Total length gain (cm) = Final total length – Initial total length- (Eq-2)

Fork length gain (cm) = Final fork length – Initial fork length (Eq- 3)

Feed conversion ratio = Feed intake (g) / Weight gain (g) (Eq-4)

Specific growth rate SGR (%) = 100 x (Ln final weight– Ln initial weight) / Total number of days (Eq-5)

Feed efficiency rate = weight gain (g) / Feed intake (g) (Eq-6)

Condition factor (K g/cm^3) = Wet body weight / Total length of $body^3$ × 100 (Eq-7)

Survival rate % = Number of fish survived / Initial number of fish × 100 (Eq-8)

2.5. Measurement of hematological parameters

Following the feeding phase, hematological parameters were determined for this purpose the blood samples were obtained from the caudal vein of common carp with sterilized syringe and were preserved in K3EDTA anticoagulant coated vials to assess it for the determination of different hematological parameters viz. hemoglobin test (Hb), hematocrit (Hct), total erythrocyte count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Blood is diluted using commercially available dilution solutions (RBC, 1: 200, and WBC, for 1:20) to dilute blood cells. Blood cells were determined by using a neubauer hemocytometer under light microscope and examining the smallest sections of the large center square, where the red cells lie at 10X magnification for better blood cell distribution. Hemoglobin (Hb) counts were determined using the Hemoglobin Analyzer (URIT-12) [21, 22]. Different hematological parameters such as MCH (pg), MCHC (g/dL), MCV (fL) and PCV (%) were determined by following Eq no. 9, 10, 11, 12 respectively.

MCH (pg) = Hb / RBS ×10 (Eq-9)

MCHC (g/dL) = Hb / Hct × 100 (Eq-10)

MCV (fL) = Hct / RBC × 10 (Eq-11)

PCV (%) = MCV × RBC / 10 (Eq-12)

2.6. Measurement of Immunity parameters

Total and Differential leukocyte count (WBC) of blood samples were also determined. Blood cells were diluted by

dilution (WBC, 1:20). WBCs were counted, and the percentage of each WBC type was calculated by method of Ghanbary [23].

2.7. Statistical analysis

The data of the various variables obtained from this experiment were statistically analysed by the microcomputer using the method of Steel [24]. Results were measured using one-way ANOVA (Analysis of variances). The results were presented as mean ± standard deviation and considered significant at $p<0.05$.

3. Results and discussion

3.1. Growth performance and feed utilization

Effect of different concentrations of turmeric on growth performance of *C. carpio* were showed significant ($p<0.05$) effect in terms of weight gain, fork and total lengths, feed conversion ratio (FCR), specific growth rate (SGR), feed efficiency ratio (FER), condition factors (K) and survival rate as mentioned in table 1. At the end of experiment after supplementation with different concentrations of turmeric results in significant increase in weight gain ($p<0.05$). Feeding 4% Turmeric TE4 diet, showed significant growth in terms of weight gain (9.94±1.53), total length gain (1.79±0.27) and fork length gain (1.46±0.23) as compared to TE0 diet. Lowest FCR value was noticed in both TE3 and TE4 diet followed by TE diet. The highest SGR was observed maximum in TE3 (2.84±0.39). Feed efficiency ratio increased with graded levels of turmeric highest in both TE3 (1.03±0.15) and TE4 diet (1.04±0.15) respectively followed by TE5 diet (1.01±0.14). Condition factor increase with increasing in curcumin intake with highest recorded in TE4 (1.08±0.01) and minimum in control (0.89±0.01). Survival rate was observed 100% in all treatments.

3.2. Hematological parameters

Hematological indices of *C. carpio* fed with different concentrations of curcumin are given in table 2. The red blood cell count (RBC), hemoglobin concentration (Hb), haematocrit (Hct), mean corpuscular volume (MCV), Packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are hematological indices. RBCs count showed a significant improvement in the treatment groups (TE1, TE2, TE3, TE4 and TE5) as compared to TE0 at the end of experiment.

Table 1. Growth performance of *Cyprinus carpio* fed supplemented with graded levels of turmericfor 12-weeks.

	TE_0 (0%)	TE1 (1%)	TE2 (2%)	TE3 (3%)	TE4 (4%)	TE5 (5%)
Weight gain (g)	$6.37±1.00^{e}$	$7.68±1.26^{d}$	$8.19±1.33^{c}$	$9.93±1.53^{ab}$	$9.97±1.54^{a}$	$8.85±1.40^{b}$
Total length gain (cm)	$1.43±0.16^{d}$	$1.45±0.23^{d}$	$1.62±0.26^{c}$	$1.66±0.27^{b}$	$1.79±0.27^{a}$	$1.66±0.26^{b}$
Fork length gain (cm)	$1.20±0.14^{c}$	$1.23±0.16^{c}$	$1.30±0.23^{b}$	$1.36±0.24^{ab}$	$1.46±0.23^{a}$	$1.42±0.21^{a}$
FCR	$1.73±0.65^{a}$	$1.65±0.65^{b}$	$1.64±0.59^{b}$	$1.47±0.52^{d}$	$1.46±0.52^{d}$	$1.55±0.42^{c}$
SGR %	$1.88±0.29^{f}$	$2.23±0.36^{e}$	$2.35±0.37^{d}$	$2.84±0.39^{a}$	$2.74±0.42^{b}$	$2.64±0.42^{c}$
FER	$0.96±0.13^{d}$	$0.99±0.15^{d}$	$1.00±0.15^{c}$	$1.03±0.15^{a}$	$1.04±0.15^{a}$	1.01±0.14ab
K g/cm^3	$0.89±0.01^{d}$	$0.99±0.01^{c}$	$0.98±0.02^{c}$	$1.04±0.02^{b}$	$1.08±0.01^{a}$	$1.04±0.01^{b}$
Survival rate %	100±0.02	100±0.12	100±0.13	100±0.03	100±0.34	100±042

Means sharing similar letters are statistically non-significant ($P>0.05$).

Table 2. Hematology profile of *Cyprinus carpio* fed diets supplemented with graded levels of turmeric after 12-weeks.

	TE_0 (0%)	TE1 (1%)	TE2 (2%)	TE3 (3%)	TE4 (4%)	TE5 (5%)
RBCsC ($\times 10^6/\mu L$)	1.49 ± 0.01^{d}	1.64 ± 0.01^{c}	1.69 ± 0.01^{bc}	1.73 ± 0.01^{bc}	1.83 ± 0.01^{ab}	1.88 ± 0.05^{ab}
Hb (g/dL)	6.11 ± 0.01^{e}	6.78 ± 0.01^{d}	7.10 ± 0.05^{c}	7.29 ± 0.05^{c}	8.43 ± 0.05^{b}	8.86 ± 0.06^{b}
Hct (%)	17.0 ± 0.05^{e}	17.9 ± 0.09^{d}	18.1 ± 0.05^{d}	18.4 ± 0.09^{c}	19.3 ± 0.05^{b}	19.5 ± 0.05^{a}
MCV (fL)	104.2 ± 0.58^{c}	106.7 ± 1.40^{c}	107.1 ± 0.62^{bc}	114.6 ± 1.26^{a}	109.2 ± 1.20^{b}	106.2 ± 0.64^{bc}
PCV%	17.0 ± 0.04^{d}	17.9 ± 0.11^{c}	18.1 ± 0.06^{b}	19.4 ± 0.20^{a}	19.5 ± 0.23^{a}	19.1 ± 0.07^{a}
MCH (pg)	41.0 ± 0.28^{b}	41.3 ± 0.39^{b}	42.0 ± 0.57^{b}	42.1 ± 0.62^{b}	47.1 ± 0.53^{a}	46.8 ± 0.10^{a}
MCHC (g/dL)	35.7 ± 0.39^{c}	37.8 ± 0.48^{bc}	39.2 ± 0.58^{b}	39.4 ± 0.49^{b}	45.2 ± 0.45^{a}	44.0 ± 0.29^{a}

Means sharing similar letters are statistically non-significant (*P>0.05*).

Table 3. Immunity parameters of *Cyprinus carpio* fed diets supplemented with different concentrations of turmeric for 12-weeks.

Parameters	TE_0 (0%)	TE1 (1%)	TE2 (2%)	TE3 (3%)	TE4 (4%)	TE5 (5%)
WBCs ($\times 10^3/\mu L$)	9.85 ± 0.07^{f}	11.5 ± 0.12^{e}	12.4 ± 0.11^{d}	13.6 ± 0.14^{c}	15.8 ± 0.10^{a}	14.6 ± 0.12^{b}
Lymphocyte (%)	61.0 ± 0.71^{d}	620 ± 0.75^{d}	64.0 ± 0.51^{c}	66.0 ± 1.05^{ab}	67.0 ± 1.20^{ab}	69.0 ± 1.00^{a}
Neutrophils (%)	17.05 ± 0.42^{a}	16.86 ± 0.74^{a}	17.02 ± 0.65^{a}	16.90 ± 0.71^{a}	17.01 ± 0.47^{a}	17.3 ± 0.52^{a}
Monocytes (%)	6.04 ± 0.05^{e}	6.04 ± 0.05^{d}	6.12 ± 0.03^{c}	6.05 ± 0.08^{b}	6.08 ± 0.11^{a}	6.05 ± 0.06^{b}
Eosinophils (%)	0.90 ± 0.07^{d}	0.87 ± 0.01^{c}	0.87 ± 0.06^{c}	1.01 ± 0.02^{a}	0.98 ± 0.07^{b}	1.00 ± 0.03^{a}
Basophils (%)	0.97 ± 0.00^{a}	0.94 ± 0.01^{a}	0.95 ± 0.00^{a}	1.00 ± 0.01^{a}	1.00 ± 0.01^{a}	1.00 ± 0.05^{a}

Means sharing similar letters are statistically non-significant (*P>0.05*).

Highest mean value of RBCs count, was observed in TE4 (1.88±0.00) fed with diet supplemented with 4% turmeric powder when compared with control and other treatments. The hemoglobin content gradually increased with turmeric concentrations, and is maximum in the TE4 diet (8.86±0.06). It was least observed in the control group (6.11±0.01) with no turmeric supplementation in the diet. Hematocrit also increased with the increase of turmeric concentration in the diet. TE4 had highest (19.5±0.05) of hematocrit followed by TE5, TE3, TE2 and TE1. Mean corpuscular volume (MCV) was observed highest (114.6± 1.26) in the TE3 followed by TE4 (109.2±1.20), TE5 (107.1±0.62), TE2 (106.7 ± 1.40^{bc}), TE1 (106.2±0.64) and TE0 (104.2±0.58).

Packed cell volume (PCV) increased with increased level of turmeric concentrations in diet i.e. highest in TE3 and TE4. It was observed that Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) showed positive trend with the increase of curcumin level in basal diet. TE4 had the highest MCH (47.1±0.53) while TE0 had the minimum value (41.0±0.28). MCHC was recorded highest in TE4 (45.2±0.45) and lowest in TE0 (35.7±0.39).

3.3. Immunity parameters

White blood cells (WBCs) are the cells of immune system that protect the organism from infectious diseases and foreign invader. With the increase in turmeric concentration, a

remarkable increase in WBCs was observed, mentioned in table 3. That shows an increase in the immunity of the fish as white blood cells are directly related to the immunity.

White blood cells count was highest in TE4 (15.8±0.12) followed by TE5, TE3, TE2 and TE1 respectively, while TE0 had the minimum number of WBCs (9.85±0.07).

Lymphocytes are the type of white blood cells that contribute in the immunity of the body. Number of lymphocytes increased with an increase in turmeric concentration with highest recorded in TE5 (69.0±1.00) with 5% turmeric concentration and in least amount in TE0 (61.0±0.71) with no turmeric supplementation. While neutrophils were found non-significant ($p>0.05$). The percentage of monocytes noticed significantly greater in TE4 dietary group (6.08±0.11). The eosinophils value is highest in both TE3 (1.01±0.02) and TE5 (1.00±0.03). Basophils content remain non-significant ($p>0.05$) as given in Table 3.

4. Discussion

The most useful method to control disease in aquaculture is through the use of antibiotics but these are criticized widely for their side effects, as a result to minimize the risk of disease the level of immunity or resistance against infection in the organisms must be elevated through the use of herbal feeds, immunostimulatory and vaccines [25]. Immunomodulation is considered as a remarkable alternative strategy to antibiotics due to their high range of actions, low cost and environment friendly disease controlling factors. The immunostimulator can be constituent of biological agent or artificial drug. Herbal extracts work as immunostimulator, are the most useful agents for disease controlling because of its economic importance and variety in nature [6].

Result of this present study showed that 4% turmeric supplementation as herbal additive in the feed of common carp (*Cyprinus carpio*) had a positive effect on growth performance parameters and WBCs count [5]. Turmeric has an attractive flavour that may increase the feed intake and accelerate growth [26]. The effects of turmeric and different types of pepper separately on different types of fish were investigated in various studies. Mohmoud reported that the highest growth performance and the lowest feed conversion ratio (FCR) were obtained in common carp (*Cyprinus carpio*) fed with 1.5% of turmeric this range found lower than our results [12]. In addition, [27] study reported 5% turmeric content in diet could improve the growth of *C. carpio*. This range slightly higher than present work. This fact may be due to the nutrient utilization by gut microbiota affected by curcumin [12].

In rainbow trout (*Oncorhynchus mykiss*) providing diet with 2% curcumin showed higher weight gain, SGR and lower FCR [28]. The effect of turmeric supplementation on the growth performance, survival rate, nutrition profile and blood parameters in the ornamental fish *Andinocara rivulatus*. They observed improved growth performance, survival rates and blood profile in fish fed with 3% of turmeric diet [29]. This range is slightly lower than present results.

Turmeric supplementation (200 mg/kg) improved the growth rate in *Channa argus* fish [30]. Abdelrazek stated that turmeric supplemented diets had shown to be immune stimulatory that can enhance non-specific immune response and functioning of B cells and T cells. The 4% turmeric supplementary diet could be suggested as feed additive for increasing growth performance and for higher productive responses in intensive fish farming [31]. This range inclined with present work. In addition, high graded level may have side effects and further study is needed to evaluate the effect of low level of turmeric as dietary supplementation.

Dietary supplement can have a profound effect on hematological profile of fish and these parameters could be used to determine the health status of a fish [32]. The parameters help in assessing the anaemic condition in fish. The results of our study showed that supplementation of turmeric improve the hematology profile in fish. Increase in MCV, MCH and MCHC was observed showing that the potential of blood for carrying oxygen is good enough. The results were concurrent with other study, showed increase the Hb, Hct and

RBCs in Gilthead seabream when fed with turmeric supplements as compared to growth [6]. The results reported by [33] also observed an increase in haematological indices of *Clarias gariepinus* fed with onion peel powder and pawpaw seed supplemented diet.

Indeed, the determination of immunological indices are the best way to evaluate the impact of feed additive for the health of fish. In the following study an increase in WBCs number was observed. WBCs defend body against infections. The reason for this increase is curcumin which is the major bioactive compound of turmeric having antibacterial, antipathogenic properties.

The curcumin supplementation at the level of 3 % in the basal diet could increase white blood cells that improved fish resistance against diseases and increased the quantity and movement of red blood cells, which reduces red blood coagulation. These results also suggest that time period of feed consumption, its quantity and method of dosage for the use of herbal additive needs more researches [29].

In addition, turmeric supplementation in the commercial diet influenced growth of common carp and fish health by improving immunity. This quality of turmeric is noteworthy for use in fish farming. But as this research is performed under controlled laboratory conditions, for a short duration of time on a single fish species with limited number of doses. Further research can be done to determine the separate and synergistic effects of turmeric supplemented diet on fish under field conditions.

5. Conclusion

The study results showed that addition of turmeric supplementation in the diet of common carp showed marked improvement in fish growth performance, feed utilization parameter, haematological indices and immunity. Therefore, the use of turmeric as dietary supplement in common carp feed is recommended specially at 4% inclusion level as it is proven to have potential to be act as growth promoter and immune stimulator.

Data availability statement

The data supporting the results of this study can be obtained from the corresponding author upon request.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Authors contribution

Moazama Batool, Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Supervision, Writing-review & editing. Howaina Asghar, conducted the research, collected, and analyzed the data, and wrote the manuscript. Qurat-ul-ain, formal analysis, edited, and reviewed. Sheeza Bano, Sadia Maalik, formal analysis, edited and reviewed. Sajida Mushtaq, Mamoona Mahmood, writing, editing, and review.

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