www.als-journal.com/ ISSN 2310-5380/ September 2023

Full Length Research Article

Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 24/07/2023;
Date Revised: 21/09/2023;
Date Published Online: 30/09/2023;

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How to Cite:
Talabani SK, Shadia AA, Abdulrahman NM, Halshoy HSH, Karim SH (2023). Inoculation of wheat with *Azospirillum* sp. bacteria and study of their germinated portions effects on common carp performance and health aspects. Adv. Life Sci. 10(3): 464-471.

Keywords:
Wheat; *Azospirillum* sp.; Germinated portions; Common carp; Performance; Blood; Health

Note:
This version is changed from the originally published copy with minor typographical corrections.

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Inoculation of wheat with *Azospirillum* sp. bacteria and study of their germinated portions effects on common carp performance and health aspects

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Abstract

Background: As the germinated wheat regarded as a natural source of prebiotic, and the importance of prebiotic on performance and health of fish, so the effect of feed containing various amount of germinated wheat as a natural source of prebiotic, on growth performance, blood chemical, immunity indices, health and biological parameters in common carp (*Cyprinus carpio* L.).

Methods: The first experiment started by planting pots in the plastic house, the pots arranged according to a Randomized Complete Design (RCD) layout with three replications. In each replication, ten seeds planted in the pots. The replications divided and arranged on 2 treatments, a control treatment taken in which seeds will be not inoculated with *Azospirillum* bacteria, the second treatment inoculated seeds with *Azospirillum* species which isolated from soil and culturing on its selective media then incubated at 28 ^{0}C for 7 days, after that the colonies of bacteria activated in its broth culture media at 28 ^{0}C for 3 days and it will be used as a liquid inoculum for the seeds. The second experiment the rearing of fish conducted at the Fish diseases Laboratory, College of Veterinary Medicine/ University of Sulaimani. 100 common carp (Cyprinus. carpio L.) reared for70 days.

Result: Negative control T1 was higher significantly in Hepatic somatic index, T1 and T3 in Gills somatic index. T4 increased significantly in FCR, and Intestine Length index. T3 and T5 increased growth performance parameters and Feed and Protein efficiency ratio. T1, T3, and T4 increased the Intestine weight index. T4 and T5 increased the RBC. T3, T4, and T5 increased the ALT, and Globulin. MCH, MCHC, Lymphocytes, and CKI increased significantly in T5. All treatment groups increased each of Condition factor, and Granulocytes as compared to negative control. No significant differences seen in both Kidney, Spleen somatic index, Hb.

Conclusion: According to the results obtained the inoculation of wheat with *Azospirillum* sp. bacteria enhance the common carp performance and health aspects by means of some biological parameters.

Introduction

To meet the demand for fish with white meat intended for human consumption, commercially significant fish are raised in captivity under controlled conditions. In industrial fish farming, the weight of each fish was increased to maximize productivity. When artificial feed is used in aquaculture, fish grow faster because they can attain their maximum weight within a set time frame. New items are introduced to fish feed to increase feed conversion efficiency and, consequently, fish development. Numerous studies have demonstrated that putting herbs in a fish's diet can help it develop and keep it healthy [1].

Germinated barely and Earth Apple powder used as a natural source of prebiotic enhances the health aspects as biological parameters and meat indices ($P \leqslant 0.05$) of common carp [2]. The investigation of [3] was carried out to use *Vici sativa* (common vetch) to make diets for common carp *Cyprinus carpio* L. and concluded that in common carp diets, germinated common vetch seed can be successfully employed at a level of 45% (germinated seed for 5 days) as an affordable plant protein source without having a negative impact on fish performance.

It is thought that additional immunostimulants in feed will improve fish health and survival. It is well known that major changes in biochemical, dietary, and sensory properties occur during germination as a result of the respiration and synthesis of new cells in the growing embryos of legume and oil seeds. Similar to this, germinating lupins can also have a rise in phenol concentration. Tannins and alkaloids in peanuts were significantly reduced by fermentation, falling by 60% and 40% respectively, compared to germination, which had declines of 86% and 45%. Without impairing growth performance, the percentage of fermented peanut meal in the diet may be increased to 60%. However, fish fed 60% germinated peanut meal or untreated peanut reported slower development and increased cortisol levels [4].

Wheat is considered the key of cereal crops [5]. It is therefore dependent on the fertility of the soil and, consequently, the availability of nutrients to plants through fertilizers. It may be possible to reserve optimal agricultural yields by maintaining suitable and appropriate soil conditions. It is well known that the nitrogen nutrient is crucial for wheat production, particularly in soils with low-to-low fertility [6]. Chemical, physical, and biological soil qualities are altered by the use of chemical fertilizers. The biological excretion of enzymes responsible for nutrient transformation in soils is stimulated by biofertilization [7]. One of the most popular genera of plant growth-promoting rhizobacteria (PGPR) is the *Azospirillum* genus. This bacterium-plant association's ability to boost crop productivity has previously been documented. Root development is improved, growth regulators are produced, and nitrogen is fixed, among other positive effects on plant growth [8].

The active chemicals in seed treatments give the crop a benefit during germination and seedling growth without causing any internal alterations in the seed. Since consistent germination and high seedling vigor are key components of good establishment and crop performance, seed quality is a crucial component of contemporary cultivation tactics. Beneficial microbes from rhizobia, Trichoderma spp., arbuscular mycorrhizal fungus, and other bacteria can be added to seeds prior to planting to improve germination. Early interactions between them and plants have a bio stimulant impact, which includes higher plant growth, increased nutrient uptake, and increased plant resilience to abiotic stress. The most important findings for horticulture species include tomato, soybean, canola, sunflower, and wheat, maize, rice. Beneficial microbe treatments increased plant germination, seedling vigour, and biomass while alleviating restrictions associated with seeds (including abiotic stress), both during and after emergence. Although the results are generally favorable, further research is necessary to fully understand the effects of seed treatments across a range of crops and cultivation methods, as well as under a variety of climatic conditions and beneficial microbe species and strains [9].

Results from [10] show that the use of germinated wheat had a substantial impact on the majority of the attributes that were examined. The addition of germinated wheat increased some common carp growth performance and blood parameters in a substantial way.

The goal of this research was to evaluate the effect of feed containing various amount of germinated wheat as a natural source of prebiotic, on growth performance, blood chemical, immunity indices, health and biological parameters in common carp (*Cyprinus carpio* L.). The research problem is to investigate the uses of germinated wheat as a natural source of prebiotic and consequences of health, physiological and performance of common carp. The purpose of the study is to using a nature source product to enhance fish immunity and performance with the aim to increase fish performance and production in fish farms. We want to understand the subjective experience of using germination of wheat in order to: Fish growth performance, feed utilization, some blood and physiological parameters, and health indices.

Methods

Germination part:

The experiment conducted during the 2022-2023 growing season at College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Iraq.

The experiment started by planting pots in the plastic house, the pots arranged according to a Randomized Complete Design (RCD) layout with three replications. In each replication, ten seeds planted in the pots. The replications divided and arranged on 2 treatments, a control treatment taken in which seeds will be not inoculated with *Azospirillum* bacteria, the second treatment inoculated seeds with *Azospirillum* species which isolated from soil and culturing on its selective media then incubated at 28 0C for 7 days, after that the colonies of bacteria activated in its broth culture media at 28 0C for 3 days and it will be used as a liquid inoculum for the seeds.

Fish rearing part

This experiment was conducted at the Fish diseases Laboratory, College of Veterinary Medicine/ University of Sulaimani. 100 common carp individuals used with average body weight between 50-55 g, they fed with commercial pellets and acclimatized for about 10 days before the implementation of the experiment. The duration of the study was 70 days.

Research design and Data collection techniques

A totally randomized design organized in five groups and three replicates organized. Each group, containing eight individuals, receives different treatments, as follows:

T1 No supplements as control (Negative control),

T2 with 5 g/kg germinated wheat (inoculated with *Azospirillum*),

T3 with 10 g/kg germinated wheat (inoculated with *Azospirillum*),

T4 with 5 g/kg germinated wheat (without inoculated with *Azospirillum*; as positive control),

T5 with 10 g/kg germinated wheat (without inoculated with *Azospirillum*; as positive control).

Fifteen plastic tanks (70 L) used for the development of the trial, which involves testing of five groups, performed in three replicates. Each tank received sufficient continuous aeration from a Hailea ACO-318 air compressor (air flow: 75L/min and power: 45-watt). Eight people were randomly assigned to each tank in order to lessen treatment inequities. Daily cleaning by using the pumping method performed for removing the waste from the system.

The experimental ratio included common foods available in Sulaymaniyah's municipal markets, as well as germinated seeds. In order to obtain tiny pieces, the pellets were crushed using Kenwood Multi-processors after being treated and dried at room temperature for four days. Fish from each tank received food equivalent to 3% of their body weight twice daily at 9:00 a.m. and 2:00 p.m. during the first week, and they were weighed every two weeks. The feeding levels then recalculated using the new weights. The feeding experiment lasted 70 days.

Performance parameters (Growth and feed utilization)

Weight gain and daily weight gain calculated using the following equations:

Weight gain = W2−W1 (g /fish)

Where W1: Fish weight (g) at the beginning of the experimental period and W2: Fish weight (g) at the end of the experimental period.

Daily weight gain = Weight gain/ Experimental period, = W2−W1/ T (g/day)

Where T: time between W2 and W1 (70 days).

Relative growth rate calculated as follows:

Relative growth rate = Weight gain/Initial weight x 100 = W2−W1/ W1 x 100 (RGR %)

Specific growth rate calculated as follows:

Specific growth rate = (Ln W2−Ln W1)/ T x 100 (SGR) %

Where: Ln W2 - final body weight; Ln W1initial body weight); T - experimental period

Feed conversion ratio calculated as follows:

Feed conversion ratio (FCR) = Total feed fed (gm.)/ Total wet weight gain (g).

Feed efficiency ratio calculated as previously described by (11) as follows:

Feed efficiency ratio (FER) = Total weight gain (g)/Total feed administered (g)

Protein efficiency ratio calculated as follows:

Protein efficiency ratio (PER) = Total wet weight gain (g/fish)/amount of protein administered (g/fish).

Biological indices

At the end of the experiment, four fishes, by each tank, randomly chosen and anaesthetized with clove powder [12]. After determining the weight and length of each fish, the fish dissected and the liver, spleen, gills, viscera, kidney, and intestine weighed by dividing the organ weight by fish weight and multiply by 100. The following organ-somatic indices were calculated.

Fulton condition factor = 100 (fish weight, g)/ (fish length, cm)^3 [13]

Blood examination

Each experimental group withdrew five fish at the end of the trial. To collect blood samples, caudal peduncle suction was employed. CBC tests are done on the BC-2800 hematology analyzer. It is a small, fully automatic, American-made hematology analyzer.

Whole blood samples were obtained in heparinized vials for the examination of several blood markers.

Complete Blood Count

Erythrocyte (red blood cells recommended) count (RBC x 10^{12} cells/L), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), mean corpuscular volume (MCV, fL), hemoglobin (Hb, g/L) and platelets (PLT x 10^9 cells/ L. Leukocyte White Blood Cells (WBC x 10^9 cells/L), granulocytes (%), lymphocytes (%) and monocytes (%).
Biochemical Parameters: Alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST, units/L), Cytokines (CKI, units/mg), total proteins (g/dL), globulin (g/dL), and albumin (g/dL).

Data Analysis and Interpretation

Using the General Linear Model function of XLSTAT 2016 Version 02.28451, data were subjected to one-way analysis of variance (ANOVA). Duncan's multiple range test and at P 0.05 were used to compare mean differences.

Results

The growth performance in each of the two treatments—T3 with 10g/kg germinated wheat (with *Azospirillum*) and T5 with 10g/kg germinated wheat (without *Azospirillum*)—was significantly improved by inoculating the wheat with the bacterium *Azospirillum* sp. and using the germinated portion. As seen in table (1), all treatments greatly raised the specific growth rate in comparison to the control group.

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.

The T4 with 5g/kg germinated wheat (without *Azospirillum*) was higher significantly in feed conversion ratio. The T3 with 10g/kg germinated wheat (with *Azospirillum*), and T5 with 10g/kg germinated wheat (without *Azospirillum*) increased significantly in each of Feed and protein efficiency ratio as shown in table (2).

Table (3) show that the Negative control T1 was higher significantly in Hepatic somatic index, the Gills somatic index was higher in both Negative control T1 and T3 with 10g/kg germinated wheat (with *Azospirillum*). No significant differences seen in both Kidney and Spleen somatic index.

All the treatments were higher significantly in Condition factor as compared to the control group, T4 with 5g/kg germinated wheat (without *Azospirillum*) increased significantly the Intestine Length index according to fish weight, the Intestine weight index was higher significantly in each of Negative control T1, T3 with 10g/kg germinated wheat (with *Azospirillum*) and T4 with 5g/kg germinated wheat (without *Azospirillum*). The Intestine Length index according to fish length was increased significantly in T3 with 10g/kg germinated wheat (with *Azospirillum*), T4 with 5g/kg germinated wheat (without *Azospirillum*) andT5 with 10g/kg germinated wheat (without *Azospirillum*) as shown in table (4).

RBC x 10^{12} cells/L count was higher significantly in each of T4 with 5g/kg germinated wheat (without *Azospirillum*) andT5 with 10g/kg germinated wheat (without *Azospirillum*). MCH was higher significantly in T5 with 10g/kg germinated wheat (without *Azospirillum*). No significant differences seen in Hb, MCHC, MCV and PLT x 10^9 cells/ L count as shown in table (5).

The WBC x 10^9 cells/L count was higher significantly in T3 with 10g/kg germinated wheat (with *Azospirillum*) and T5 with 10g/kg germinated wheat (without *Azospirillum*). All the treatments groups increased significantly the Granulocytes count. T5 with 10g/kg germinated wheat (without *Azospirillum*) increased the Lymphocytes count. No significant differences observed in Monocytes counts as shown in table (6).

T3, T4 and T5 increased the ALT and Globulins. T3 increased significantly the AST level. T5 increased significantly each of CKI and Total proteins. No significant differences seen in Albumin as shown in table (7).

Discussion

The following effects can result from adding chitinolytic Azospirilla to soil: It can help control fungi and insects, fix nitrogen in the soil, and boost plant growth. When cereals and non-cereal species were inoculated with Azospirillum, aboveground plant responses frequently showed increases in germination rates [14] and this found in the results of the recent study in which increased the germinated wheat therefore enhancing its component availability leading to better efficient of the fish.

Azospirillum strains could be successfully administered to plants that had never before had Azospirillum in their roots. They exhibited no preference for crop plants or weeds, or for annual or perennial plants. This shows that Azospirillum is not a bacterium particular to plants, but rather a generic root colonizer. Azospirillum is a common bacterium that is almost always present. As a plant growth promoter and/or insect and fungus pest controller, it has a wide range of potential applications, including wheat as used in a recent study.

According to [15], Azospirillum isolates are more important as soil bacteria and as endophytes in the biological control of insects and pathogenic fungi since they can hydrolyze fungal chitin in just 28 hours.

Treatments	Initial Weight	Final Weight	Weight gain	Daily weight gain	Relative growth rate	Specific growth rate
T1 - Negative control	50.67 ±0.01a	77.76 ±0.02c	27.09 ±0.04c	2.26 ±0.03b	53.47 ±0.04c	187.80 ±0.02b
T2 5g/kg germinated wheat (with *Azospirillum*	54.45 ±0.03a	89.74 ±0.04b	35.29 ±0.03b	2.95 ±0.06b	64.82 ±0.02b	195.12 ±0.05a
T3 10g/kg germinated wheat (with *Azospirillum*)	57.84 ±0.03a	98.74 ±0.01a	40.9 ±0.01a	3.41 ±0.02a	70.72 ±0.01ab	195.54 ±0.05a
T4 5g/kg germinated wheat (without *Azospirillum*)	59.04 ±0.02a	82.38 ±0.04bc	23.34 ±0.06cd	1.95 ±0.04c	39.54 ±0.02d	187.65 ±0.03b
T5 10g/kg germinated wheat (without *Azospirillum*)	55.73 ±0.01a	97.67 ±0.02a	41.94 ±0.05a	3.50 ±0.02a	75.26 ±0.04a	195.09 ±0.01a

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.
Table 1: Effect of wheat germinated inoculation of with *Azospirillum* sp. bacteria on common carp performance

Treatments	Feed conversion ratio	Feed efficiency ratio	Protein efficiency ratio
T1- Negative control	1.66 ±0.01b	60.41 ±0.07b	96.75±0.03c
T2 5g/kg germinated wheat (with *Azospirillum*	1.48 ±0.04b	67.69 ±0.03b	126.04 ±0.03b
T3 10g/kg germinated wheat (with *Azospirillum*)	1.33 ±0.02b	75.71 ±0.03a	146.08 ±0.02a
T4 5g/kg germinated wheat (without *Azospirillum*)	2.07 ±0.02a	48.46 ±0.01c	83.36 ±0.04cd
T5 10g/kg germinated wheat (without *Azospirillum*)	1.31 ±0.04b	76.84 ±0.04a	149.79 ±0.07a

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.
Table 2: Effect of wheat germinated inoculation of with *Azospirillum* sp. bacteria on common carp feed utilization

Treatments	Hepatic somatic index	Gills somatic index	Kidney somatic index	Spleen somatic index
T1 - Negative control	3.18 ±0.04a	4.62 ±0.01a	0.83 ±0.02a	0.19 ±0.05a
T2 5g/kg germinated wheat (with *Azospirillum*	1.95 ±0.04c	2.65 ±0.01c	0.51 ±0.05a	0.19 ±0.01a
T3 10g/kg germinated wheat (with *Azospirillum*)	2.36 ±0.03ab	4.40 ±0.05a	0.70 ±0.07a	0.22 ±0.06a
T4 5g/kg germinated wheat (without *Azospirillum*)	2.53 ±0.02ab	3.25 ±0.03b	0.81 ±0.01a	0.14 ±0.02a
T5 10g/kg germinated wheat (without *Azospirillum*)	2.01 ±0.01b	3.18 ±0.04b	0.54 ±0.07a	0.16 ±0.07a

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.
Table 3: Effect of wheat germinated inoculation of with *Azospirillum* sp. bacteria on common carp physio-biological indices

Treatments	Condition factor	Intestine Length index	Intestine weight index	Intestine Length index (Fish Length)
T1 - Negative control	1.34 ±0.03b	34.73 ±0.03b	3.22 ±0.08a	1.5±0.01b
T2 5g/kg germinated wheat (with *Azospirillum*	2.18 ±0.02a	25.60 ±0.03c	1.88 ±0.01c	1.52±0.01b
T3 10g/kg germinated wheat (with *Azospirillum*)	2.01 ±0.03a	34.44 ±0.06b	3.91 ±0.03a	2±0.03a
T4 5g/kg germinated wheat (without *Azospirillum*)	2.02 ±0.01a	40.06 ±0.06a	3.46 ±0.03a	2.07±0.02a
T5 10g/kg germinated wheat (without *Azospirillum*)	2.66 ±0.02a	35.12 ±0.07b	2.10 ±0.04b	2.1±0.05a

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.
Table 4: Effect of wheat germinated inoculation of with *Azospirillum* sp. bacteria on common carp biological indices

Treatments	RBC x 10^{12} cells/L	Hb	MCH	MCHC	MCV	PLT x 10^9 cells/ L
T1 - Negative control	0.94 ±0.01b	10.98 ±0.03a	45.85 ±0.07d	32.54 ±0.08c	111.74 ±0.03a	2.8 ±0.02a
T2 5g/kg germinated wheat (with *Azospirillum*	0.99 ±0.03b	11.09 ±0.01a	52.76 ±0.02c	35.51 ±0.01c	109.38 ±0.02a	3.1 ±0.03ab
T3 10g/kg germinated wheat (with *Azospirillum*)	0.93 ±0.01b	10.92 ±0.04a	64.72 ±0.02b	41.73 ±0.07b	105.71 ±0.02a	3.9 ±0.02a
T4 5g/kg germinated wheat (without *Azospirillum*)	1.09 ±0.03a	12.27 ±0.02a	64.84 ±0.02b	42.98 ±0.02b	110.83 ±0.07a	3.7 ±0.01a
T5 10g/kg germinated wheat (without *Azospirillum*)	1.58 ±0.03a	11.94 ±0.03a	72.98 ±0.01a	48.02 ±0.02a	112.84 ±0.03a	3.1 ±0.02ab

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.
Table 5: Effect of wheat germinated inoculation of with *Azospirillum* sp. bacteria on common carp blood picture

Treatments	WBC x 10⁹ cells/L	Granulocytesx 10⁹ cells/L	Lymphocytesx 10⁹ cells/L	Monocytesx 10⁹ cells/L
T1 - Negative control	56.43 ±0.04c	15.43 ±0.01b	34.82 ±0.05d	7.34 ±0.01a
T2 5g/kg germinated wheat (with *Azospirillum*	63.98 ±0.06b	19.02 ±0.04a	49.25 ±0.01c	7.38 ±0.02a
T3 10g/kg germinated wheat (with *Azospirillum*)	69.01 ±0.01a	18.72 ±0.02a	58.99 ±0.02b	7.98 ±0.06a
T4 5g/kg germinated wheat (without *Azospirillum*)	63.78 ±0.01b	18.99 ±0.03a	62.87 ±0.01b	7.88 ±0.02a
T5 10g/kg germinated wheat (without *Azospirillum*)	70.83 ±0.03a	19.11 ±0.03a	69.12 ±0.03a	7.99 ±0.03a

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.

Table 6: Effect of wheat germinated inoculation of with *Azospirillum* sp. bacteria on common carp differential WBC

Treatments	ALTIU/L	AST IU/L	CKI	Totalproteins	Globulin	Albumin
T1 - Negative control	155.83 ±0.07b	211.56 ±0.08e	3234.64 ±0.01d	31.43 ±0.01d	2.98 ±0.01b	0.73 ±0.01a
T2 5g/kg germinated wheat (with *Azospirillum*	162.12 ±0.01b	232.56 ±0.07d	3584.9 ±0.04b	33.89 ±0.01c	2.87 ±0.03b	0.82 ±0.01a
T3 10g/kg germinated wheat (with *Azospirillum*)	169.92 ±0.02a	287.81 ±0.06a	3389.8 ±0.01c	38.92 ±0.03b	3.01 ±0.03a	0.89 ±0.04a
T4 5g/kg germinated wheat (without*Azospirillum*)	173.82 ±0.01a	265.67 ± 0.05c	3592.9 ±0.04b	41.89 ±0.02b	3.92 ±0.04a	0.85 ±0.03a
T5 10g/kg germinated wheat (without *Azospirillum*)	169.01 ±0.01a	275.65 ±0.05b	3482.87 ±0.01a	45.94 ±0.09a	4.22 ±0.05a	0.92 ±0.04a

Means with the same superscript (s) in each column do not differ significantly from each other, whereas those with different superscripts do.

Table 7: Effect of wheat germinated inoculation of with *Azospirillum* sp. bacteria on common carp serum biochemical parameters

Additionally, several volatile and non-volatile antifungal substances are present in Azospirillum isolates.This discovery might be especially important for organic farms, where soils are altered by organic plant and animal wastes, increasing the soil's cellulose content and enhancing the activity of microbial plant diseases. As a result, *Azospirillum* isolates have demonstrated their efficacy in controlling Fusarium infection in soil with the highest concentration of cellulose. As a result, they are advocated as an effective biological fungicide, particularly in organic farms where there is a higher danger of developing fungal infection.

Injecting bacteria that promote plant development is one of the most useful, economical, and long-lasting ways to boost agricultural productivity and lessen reliance on nitrogen fertilizers. In tropical savannah, seed inoculation with *Azospirillum brasiliense* and *Bacillus subtilis* increased grain yield and N accumulation in grains of irrigated wheat regardless of the levels of N fertilizer used [16].

Azospirillum and Bacillus are the most frequently mentioned helpful bacteria in microbial consortia for enhancing the sustainability, plant growth, and productivity of various cereal crops.A potential inoculant for encouraging plant development and elevating wheat yield, N absorption, and N use efficiency has been reported as *Azospirillum*. This inoculant can colonize the rhizosphere of the plant and alter the design of the roots, perhaps enhancing nutrition, water uptake, and N use efficiency [16].

Previously it was showed that employing germinated barley significantly improved growth performance, which agrees with the findings of a recent study. Growth performance and several blood parameters significantly improved (P 0.05) when 5.0 g/kg of germinated barley was added to aquafeed [18]. The protein efficiency ratio and intestine length index of hydroponic germination (5.0 g) and germinated barley (2.5 g), on the other hand, significantly improved as a result of the current findings.

According to this study, wheat can be added to meat pate in the right amounts to increase its organoleptic quality. It also demonstrated that meat pate containing 10% germinated wheat had a high protein content of 14.36%. Comparing pates made with additions of 15% and 20% of germinated wheat to those made with 10%, researchers found that the 10% pate had a higher amount of both essential and non-essential amino acids [19]. Boleta et al., [20] found that According to the findings of a recent study, the inoculation with *A. brasilense* improves the accumulation of various minerals like B, Cu, Fe, and Mn and has increased effectiveness of N shoot. This may be the explanation for the advantages of germinated wheat.

Sprout Germinated Barley (SGB) containing β-clucan and hemicellulose rich in dietary fiber [21], Prebiotics are nonviable natural food components that are resistant to digestion, absorption, and colonic fermentation.By changing the intestinal flora's constituents in the gut, it benefits the host's health. It alters the social formation of intestinal microflora in favour of beneficial bacteria (Probiotics) by providing an environment that is conducive to their development and reproduction as well as enhancing the immune response [22]. In the test group of [23] had significantly better growth performance, which was due to increases in nutrient digestibility and absorption,and this agree with the results of the recent study when using the germinated wheat. Prebiotics may have caused a change that has increased the dominance of helpful microbes and forced bad germs to compete for food and space.Or decreased their metabolism by triggering the non-specific immunological response of the host, when it comes to β-clucan, it helps to maintain the digestive system, avoid colitis, increase immunity, and it can change the intestinal flora to the host's advantage [24], However, prebiotics must either increase the host's health or have a positive physiological effect on it, or both, and these may also be the reasons for the findings of the current study. Hemicellulose fibres are one of the functional nutrients that can alter the development of the gut flora in a way that is advantageous to the host.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Shahen Kamil Talabani and Hawar Sleman Hama Halshoy doing the planting of the wheat and inoculation with *Azospirillum* sp. Bacteria; Shadia Ali Abid and Shram Hoshyar Karim writing and data analyses; Nasreen MohiAlddin Abdulrahman doing the fish rearing experiment.

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