Studying the effect of oral administration for Agaricus bisporus fungus on immunological parameters for anemia-induced rats

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ABSTRACT

The study was conducted in the laboratories of the Department of Food Sciences, College of Agriculture, Animal House, College of Veterinary Medicine, University of Tikrit for the period from 20 February to 15 April 2018, in order to determine the effect of oral administration of Agaricus bisporus, with a percentage of 10 and 20% alone or with a concentration of (1, 2 and 3 $mg.kg^{-1}$ iron) from the weight of laboratory animals bred for 42 days to observe their effects on the immunological parameters of laboratory rats. The results showed that the oral administration of A. bisporus alone or with iron at concentrations of (1, 2, and 3 $mg.kg^{-1}$) from the weight of laboratory animal caused a significant increase ($P < 0.05$) in the total white blood cell count and the percentage of white cells, the type of neutrophils and the percentage of lymphocytes. It was also found that the oral administration of A. bisporus alone or with iron concentrations caused a significant increase in the levels of immunoglobulins (IgM and IgA) and the concentration of interleukin-6 (IL-6). it concluded from the data that using fungus in oral administration improved the immunological parameters for rates in which the anemia was induced.

Keywords: Agaricus bisporus, White blood cell, IL-6, IgM, IgA.
Research paper from the Ph.D. thesis for the first Author.

دراسة تأثير الإعطاء الفموي للفطر *Agaricus bisporus* في المعايير المناعية للجرذان المستحث فيها الأنيميا

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الخلاصة

أجريت الدراسة في مختبرات قسم علوم الأغذية / كلية الزراعة، والبيت الحيواني / كلية الطب البيطري - جامعة تكريت للمدة 20/ شباط لغاية 15/ نيسان/ 2018، بهدف تحديد تأثير الإعطاء الفموي من فطر العرهون *Agaricus bisporus* بنسبة 10 و 20 % لوحده أو مع تركيز 1 ، 2 و 3 ملغم من الحديد/كغم من وزن الحيوانات المختبرية المرباة لمدة 42 يوماً لملاحظة تأثيراتها في المعايير المناعية للجرذان المختبرية. بينت النتائج أن الإعطاء الفموي من الفطر *A. bisporus* لوحده أو مع الحديد بالتراكيز 1 ، 2 و 3 ملغم/كغم من وزن الحيوانات المختبرية قد سبب في الزيادة المعنوية ($P<0.05$) في العدد الكلي لخلايا الدم البيض ونسبة الأعداد للخلايا البيض نوع العدلات Neutrophils ونسب الخلايا اللمفاوية Lymphocytes، كما تبين أن الإعطاء الفموي من الفطر *A. bisporus* لوحده أو مع تراكيز الحديد سبب ارتفاع معنوي في مستويات الكلوبيولينات المناعية IgM وIgA وتركيز الانترلوكين-6 (IL-6). ونستنتج من المعطيات ان استخدام الفطر في الاعطاء الفموي قد ادى الى تحسين المعايير المناعية للجرذان المستحث فيها الانيميا.

الكلمات المفتاحية: *Agaricus bisporus*، خلايا الدم البيض، IL-6 ، IgM، IgA
البحث مستل من أطروحة دكتوراه للباحث الأول.

1. INTRODUCTION

bisporus is one of the most suitable fungi for human consumption, with high nutritional value and biological activity. Numerous studies have mentioned its primary and sub-metabolic traits responsible for the therapeutic activity for the prevention of diseases such as cancer, hyperlipidemia, microbial diseases, cardiovascular problems, liver disease, and immune-related diseases [1, 2]. This type of fungus has been classified by [3] with the following classification:

Kingdom - Fungi

Divison - Basidomycota

Class - Agaricomycetes

Order - Agaricales

Family - Agaricaceae

Genus - Agaricus

Species - Agaricus bisporus

Agaricus bisporus has a long history in many traditional therapeutic uses, where the use of A. bisporus extracts for their bioactivities such as antioxidant [4], anti-bacterial activity [5], anti-cancer, and anti-inflammatory increases with recent advantages in controlling Coronary artery disease, diabetes, bacterial and fungal inflammatories, immune system disorders and cancers [6]. Dhamodharan and Mirunalini, [7] indicated to the therapeutic properties for this fungus and its biomedical applications in caring for human health and treating inflammatory and chronic cancers. There is a growing interest in extracting bioactive ingredients from fungus to develop functional foods. Studies have shown that A. bisporus has a good history of being used in many traditional treatments. The use of its extracts and bioactive compounds have also increased as an antioxidant and anti-inflammatory in the world against many human diseases such as coronary heart disease, diabetes, bacterial and fungal inflammatories, and disorders of the human immune system [8].

2. MATERIALS AND METHODS

Preparation of laboratory animals

Healthy and disease-free laboratory animals were obtained from the College of Veterinary Medicine, University of Tikrit, and they are 65 adult rats (Albino type), with an age of (8 - 9 weeks), and their weights ranged from (160 - 163 g). They were distributed randomly into thirteen groups with similar weights. Each group included five animals. Iron was given orally in three concentrations (1, 2, and 3 $mg.kg^{-1}day^{-1}$) and the Agaricus bisporus fungus was also given orally at two concentrations of (10 and 20%) for a period of 42 days. The animals were placed in cages made of plastic, after covering their floors with sawdust, which was replaced four times a week. The animals were fed regularly using ready-made feed according to (9) and the basic diet contained casein 84.95 g, pure protein 158.5 g, 100 g of sterols, 5 g of vitamin mixture, 50 g of the mixture of mineral salts, 50 g of cellulose, 100 g of glucose, 536 g of starch. The animals were raised under the supervision of a specialized veterinary staff taking into account the aspect of hygiene, and the groups of animals included the following:

1- The control group.
2- A group of animals affected by anemia.
3- The group of animals given iron only at a concentration of (1 $mg.kg^{-1}$).
4- The group of animals given iron only at a concentration of (2 $mg.kg^{-1}$).
5- The group of animals given iron only at a concentration of (3 $mg.kg^{-1}$).
6- The group of animals given the Agaricus bisporus fungus at a concentration of 10%.
7- The group of animals given the Agaricus bisporus fungus at a concentration of 10% + iron at a concentration of (1 $mg.kg^{-1}$).
8- The group of animals given the Agaricus bisporus fungus at a concentration of 10% + iron at a concentration of (2 $mg.kg^{-1}$).
9- The group of animals given the Agaricus bisporus fungus at a concentration of 10% + iron at a concentration of (3 $mg.kg^{-1}$).

10- The group of animals given the Agaricus bisporus fungus at a concentration of 20%.
11- The group of animals given the Agaricus bisporus fungus at a concentration of 20% + iron at a concentration of (1 mg.kg^{-1}).
12- The group of animals given the Agaricus bisporus fungus at a concentration of 20% + iron at a concentration of (2 mg.kg^{-1}).
13- The group of animals given the Agaricus bisporus fungus at a concentration of 20% + iron at a concentration of (3 mg.kg^{-1}).

Inducing anemia

Anemia was induced for all groups except for the positive control group through bleeding from the retro-ocular vein, using capillary tubes containing heparin according to (10), which led to bleeding in the range of 15-20 drops. This process was repeated on the third and fifth days before the start of the experiment. The process is induced anemia by iron deficiency and it was inferred by measuring the concentration of Hb, s-iron, ferritin and P.C.V.

Preparation of the Agaricus bisporus fungus:

The A. bisporus fungus, which was provided from the local market, was dried by using a hot air dryer and then grinding into a powder and it was prepared in two concentrations:

1- 10% concentration, where 1 g of fungus powder was weighed and dissolved in 10 ml sterile water.
2- 20% concentration. where 2 g of fungus powder was weighed and dissolved in 10 ml sterile water.

White blood cell count:

The white blood cell count was calculated by diluting the blood sample with a ratio of 1: 2 using Turkey's solution, after mixing the blood well with a solution, it left for 15 min, after which drops were placed on the slide and the total White blood cell count was calculated in four squares according to the following equation (11):

Total white blood cell count (cells.mm^{-3}) = calculated cell count / 10x20x4

White blood cell differential

The method described by (12) was followed by making a smear for the blood sample, staining it with Leishman stain, then examining it under a microscope and the types of white cells were calculated separately.

Estimating Immunoglobulins.

Immunoglobulins were estimated using an ELISA (Enzyme Linkage Immune System Assay) device as mentioned in (13). The kits and solutions obtained from the French company Bio labo were left at laboratory temperature for several minutes to ensure the evaporation of the water on its surface. The estimation was made by filling the pit in the instrument plate with 5 mL of each of the samples as well as the control sample and left to complete the adsorption process, where it was left for 15 minutes before being closed. it was then transferred to the incubator of the device at 35 C for 72 hours in the case of estimating both IgA globulins, and for 96 hours in the case of the IgM type.

Estimating interleukin-6 (IL-6) concentration.

The concentration of Interleukin-6 (IL-6) was estimated using an ELISA (Enzyme Linkage Immune System Assay) device as mentioned in (14), where the kits and solutions obtained from the French company (Bio labo) were left at laboratory temperature for several minutes to ensure the evaporation of the water on its surface. The estimation was made by filling the pit in the instrument plate with 5 mL of each of the samples as well as the control sample and left to complete the adsorption process, where it was left for 15 min before being closed.

Statistical Analysis

The results of the experiments were analyzed using the General Linear Model within the ready-made statistical program (15) to study the effect of the factors according to the completely randomized design (CRD). The Duncan test (16) was also conducted to

determine the significant differences between the averages of the affected factors on the studied traits at the level of ($p < 0.05$).

3. RESULTS AND DISCUSSION

The effect on the total and differential of white blood cells counts in the infected group of animals

Table (1) shows the efficacy of oral administration of A. bisporus in the parameters of total and differential for white blood cells counts in anemia-induced rats. The results revealed that the oral administration of iron at concentrations of (1, 2, and 3 $mg.k^{-1}$) caused a significant increase ($p < 0.05$) in the total of white blood cells counts, which amounted to (5.03, 5.81, and 6.35 ($\times 10^6$). / mm^3)), respectively, compared to its counts in the blood of the animals of the positive control group in which anemia induced, which was (4.27 ($\times 10^6$ / mm^3)). while it decreased significantly in the case of control with the total of white blood cells counts in the negative control group, which was (7.15 × 106 / mm^3). As for the effect of oral administration for A. bisporus at concentrations of 10 and 20% with iron concentrations of 1, 2, and 3 $mg.kg^{-1}$, it was found that there was a significant increase in the total of white blood cells counts (WBC). The use of a concentration of 10% of A. bisporus led to an increase in the total of white blood cells counts (WBC) amounted to (5.3, 9.9, and 11.4 ($\times 10^6$ / mm^3)), respectively, compared to their counts in the group of animals that were given fungus without iron, which amounted to (4.6 ($\times 10^6$ / mm^3)). In the case of oral administration of A. bisporus at a concentration of 20%, it led to an increase the total of white blood cells counts (WBC) which amounted to (7.46, 11, and 12.7 ($\times 10^6$ / mm^3), respectively, compared to their counts in the group of animals given from the fungus without iron, which their counts amounted to (6.0 ($\times 10^6$ / mm^3)). The white blood cells count in the case of administration for rats at concentrations of 10 and 20% were significantly higher compared to their numbers in anemia-induced rats. As for the percentage of lymphocytes, the oral administration of iron at concentrations of (1, 2, and 3 $mg.kg^{-1}$) led to a significant increase in its percentage, which amounted to (55.3, 55.8, and 57.5%), respectively, compared to the percentage in the animals of the group in which anemia was induced amounted to (48.5%). Oral administration of A. bisporus at concentrations of (10 and 20%) with iron at concentrations of (1, 2, and 3 $mg.kg^{-1}$) had a significant effect on the percentage of lymphocyte for rats where 10% of A. bisporus caused the significant increase in the percentage of lymphocytes amounted to (53.6, 55, and 55.7%), respectively, compared to the group of animals given fungus without iron which amounted to 51.5%. In the case of oral administration of A. bisporus at a concentration of 20%, it led to a significant increase in the percentage of lymphocytes, which amounted to (55.9, 56.4, and 57.1%), respectively, compared to the percentage in the group of animals given fungus without iron, which amounted to 53.5%. The status of administration for rats at concentrations of 10 and 20% was significantly higher compared to the percentage of lymphocytes for rats in which anemia was induced. The results showed that the oral administration of iron at concentrations of (1, 2, and 3 $mg.kg^{-1}$) had an effect on the percentage of neutrophils, where it led to a significant increase amounted to (37.9, 38.6, and 39.5%), respectively, compared to their percentage in animals group in which the anemia was induced which amounted to 33.7%. Oral administration of A. bisporus at concentrations of (10 and 20%) with iron at concentrations of (1, 2, and 3 $mg.kg^{-1}$) had a significant effect on the percentage of neutrophil in rats, where 10% of A. bisporus led to a significant increase in the percentage of neutrophils which amounted to (43.7, 43.9 and 44.5%), respectively, compared to their percentage in the group of animals given the fungus without iron, which amounted to 41%.In the case of oral administration of A. bisporus at a concentration of 20%, it led to increase in the percentage of neutrophil which amounted to (43, 44.6, and 45%), respectively, compared to their percentage in the group of animals given fungus without iron, which amounted to 41.8%. The effect of administration for rats at concentrations of (10 and 20%) led to a significant increase compared to rats in which

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anemia was induced. The increase in the total counts of white blood cells (WBCs) could be due to the fungus containment of many compounds that have a role in improving the immune system, such as antioxidants and free radical inhibiting factors such as (Superoxide dismutase, Peroxidase), which caused that when a source Oxidative stress in the body was removed, it has led to the return of the white blood cells counts, even if they are few, to the normal numbers, thus, this increase in the white blood cells counts after treating them with the fungus is due to the containment of the fungus on the multiple compounds that are considered medicinally and effective, such as polysaccharides, for example, β -glucans (17). The fungus also has the property of aggregation and suppression of leukocyte cell death (18).

Table 1: Effectiveness of oral administration of Agaricus bisporus on the white blood cells counts for rats in which anemia was induced.

Treatments	Iron concentration ($mg.kg^{-1}$)	WBC	Lym	Net
		$10^6/mm^3$	%	
Control	**0**	7.15 ^{cd} ±0.18	54.3 ^{bc} ±2.51	38.1 ^{de} ±2.26
Infected with Anemic	**0**	4.27 ^{e} ±0.75	48.5 ^{ef} ±1.14	33.7 ^{fg} ±0.73
Iron	**1**	5.03 ^{de} ±0.44	55.3 ^{b} ±1.14	37.9 ^{e} ±0.92
	2	5.81 ^{de} ±0.2	55.8 ^{b} ±1.12	38.6 ^{de} ±4.36
	3	6.35 ^{d} ±0.18	57.5 ^{a} ±1.87	39.0 ^{d} ±0.79
fungus 10%	**0**	4.6 ^{e} ±0.18	51.5 ^{d} ±1.9	41.0 ^{c} ±0.66
fungus 10%	**1**	5.3 ^{de} ±1.5	53.6 ^{c} ±0.98	43.7 ^{b}±0.86
	2	9.9 ^{bc} ±1.15	55.0 ^{b} ±1.38	43.9 ^{b} ±1.36
	3	11.4 ^{ab}±0.93	55.7 ^{b} ±1.21	44.5 ^{ab} ±2.17
fungus 20%	**0**	6.0 ^{d} ±1.15	53.5 ^{de} ±1.04	42.5 ^{bc}±1.04
fungus 20%	**1**	7.46 ^{cd} ±1	55.6 ^{b} ±2.3	43.0 ^{b} ±1.06
	2	10.9 ^{b} ±2.21	56.4 ^{ab} ±2.12	44.6 ^{ab}±2.35
	3	12.7 ^{a} ±0.81	57.1 ^{a} ±0.23	45.0 ^{a} ±1.05

* The similar characters in one column mean that there are no significant differences between them at the probability level of (0.05)

Effectiveness of oral administration of Agaricus bisporus on immunoglobulins (IgM and IgA) for rats in which anemia was induced.

Table (2) shows the effectiveness of oral administration of A. bisporus on some immunological parameters for rats in which anemia was induced. The results revealed that the oral administration of iron at concentrations of (1, 2, and 3 $mg.k^{-1}$) caused a significant increase ($p < 0.05$) in the concentration of immunoglobulin (IgM), where its concentration amounted to (79.7, 80.4, and 82.5 ng / L), respectively, compared to its concentration in the animals of the positive control group in which anemia induced, which was (73.5 ng / L). The oral administration of A. bisporus at concentrations of (10 and 20)% with iron at concentrations of (1, 2, and 3 $mg.kg^{-1}$) had a significant increase in the concentration of immunoglobulin (IgM), where the use of concentration 10% of A. bisporus led to an increase in the concentration of immunoglobulin (IgM) which amounted to (84.9, 85.2 and 87.1 ng / L), respectively, compared to its value in the group of animals given fungi without iron, which amounted to (81.2 ng / L). In the case of oral administration of A. bisporus at a concentration of 20%, it led to an increase the concentration of immunoglobulin (IgM) which amounted to (86.6, 87.4, and 89.3 ng / L), respectively, compared to its value in the group of animals given fungus without iron, which amounted to (83.5 ng / L). The oral administration of rats at concentrations of (10 and 20%) caused a significant increase in the concentration of immunoglobulin (IgM) compared to the rats in which anemia was induced. The results showed that the oral administration of iron at concentrations of (1, 2, and 3 $mg.kg^{-1}$) caused the significant increase ($p < 0.05$) in the concentration of immunoglobulin (IgA), where its

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concentration amounted to (92.3, 92.4, and 93 ng / L), respectively, compared to its concentration in the blood of animals group in which anemia was induced, which amounted to (85.2 ng / L). While the oral administration of A. bisporus at concentrations (10 and 20%) with iron at concentrations of (1, 2 and 3 $mg.kg^{-1}$) led to a significant increase in the concentration of immunoglobulin (IgA), where the concentration of 10% of A. bisporus led to an increase in the concentration of immunoglobulin (IgA) which amounted to (92.8, 93 and 94.2 ng / L), respectively, compared to the group of animals given fungus without iron, where the IgA concentration amounted to (90.4 ng / L). the oral administration of A. bisporus at a concentration of (20%) has also led to increase the concentration of immunoglobulin (IgA) which amounted to (93.6, 94.5, and 95.3 ng / L), respectively, compared to the group of animals given fungus without iron, where the IgA concentration amounted to (92 ng / L). The status of administration for rats with concentrations of (10 and 20%) was significantly higher compared to the concentration of immunoglobulin (IgA) for rats in which anemia was induced.

Table 2: Effectiveness of oral administration of Agaricus bisporus on the concentration of immunological parameters (IgM and IgA)for rats in which anemia was induced.

No.	Treatments	Iron concentration ($mg.kg^{-1}$)	**IgM**	**IgA**
			ng/L	
1	Control	**0**	80.8 ^{e} ±1.4	91.8 ^{c} ±1.88
2	Infected with Anemic	**0**	73.5 ^{h} ± 0.85	85.2 ^{f} ± 1.14
3	Iron	**1**	79.7 ^{ef} ±1.55	92.3 ^{bc} ±1.46
4		**2**	80.4 ^{e} ±0.71	92.4 ^{bc}±0.24
5		**3**	82.5 ^{d} ±0.28	93.0 ^{b} ±0.3
6	fungus 10%	**0**	81.2 ^{de} ±1.54	90.4 ^{cd} ±1.23
7	fungus 10%	**1**	84.9 ^{c} ±0.66	92.8 ^{bc} ±0.93
8		**2**	85.2 ^{bc} ±0.75	93.0 ^{b} ±1.47
9		**3**	87.1 ^{ab} ±1.33	94.2 ^{ab} ±0.45
10	fungus 20%	**0**	83.5 ^{cd} ±1.32	92.1 ^{bc} ±2abc
11	fungus 20%	**1**	86.6 ^{b} ±2.52	93.6 ^{b} ±3.32
12		**2**	87.4 ^{ab} ±0.74	94.6 ^{ab}±1.13
13		**3**	88.3 ^{a} ±2.82	95.3 ^{a} ±2.35

* The similar characters in one column mean that there are no significant differences between them at the probability level of (0.05)

and the effectiveness of multiple sugars in the fungus is controlled by their association With receptors of immune cells (21).

Effectiveness of oral administration of Agaricus bisporus at concentration of interleukin-6 (IL-6) for rats in which anemia was induced

Figure (1) show the effect of oral administration of A. bisporus on the concentration of interleukin-6 (IL-6) for rats in which anemia was induced. The results showed that the oral administration of iron at concentrations (2 and 3 $mg.kg^{-1}$) caused a significant increase ($p < 0.05$) amounted to (4.19 and 4.32 ng / L), respectively, compared to its concentration in animals group for rats in which the anemia was induced, which The results agree with (19, 20) who indicated that the significant increases in the values of IgM and IgA globulins. It could be due to the fungus containing polysaccharides that activated and stimulated the immune system, which led to the significant increase in immune globulins, and the use of fungus also activated other components of the immune system such as natural killer cells, neutrophils and macrophages, and activating the expression and secretion of cytokines in the immune system. the sugars and active substances in mushrooms are also unable to penetrate into the immune cells directly to activate them, so the mechanism for stimulating sugars includes various cell receptors such as $dectin^{-1}$, complementary receptors (CR3), Lactosylceramide and TLR,

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and 6.36 ng / L), respectively, compared to the group of animals given fungus without Iron which amounted to (4.35 ng / L). In the case of oral administration for rats at concentrations of (10 and 20%), the value of IL-6 was significantly higher compared to that of the anemia-induced rat. These results agree with (22, 23) who showed that supplementation with the use of fungus enhances the level of immunity because it contains many substances that stimulate the immune system. The reason for this immune-boosting can be attributed to the presence of many active substances, including polysaccharides derived from a fungus, which activate macrophages that in turn stimulate the secretion of cytokines such as IL-6 (24), where Macrophages play an important role in the host's immune defense response, and they eliminate pathogens and recruit other cells to sites of persistent inflammation through the secretion of regulatory cytokines (25).

amounted to (3.22 ng / L), while no significant differences were obtained at oral administration of A. bisporus with iron at a concentration of (1 $mg.kg^{-1}$), where the concentration amounted to (3.93 ng / L). It was also found that the effect of oral administration of A. bisporus at concentrations (10 and 20%) with iron at concentrations of (1,2 and 3 $mg.kg^{-1}$) in the concentration of interleukin-6 (IL-6) for rats led to a significant increase, where the concentration of 10% bisporus with iron at concentrations of (1, 2 and 3 $mg.kg^{-1}$) led to increasing the concentration of interleukin-6 (IL-6) amounted to (4.46, 5.74 and 6.02 ng / L), respectively, compared to its value in the group of animals given fungus without iron which Its concentration amounted to (3.91 ng / L). Likewise, in the case of oral administration of A. bisporus at a concentration of (20%) with iron led to a significant increase in the concentration of interleukin-6 (IL-6) amounted to (5.20, 5.82,

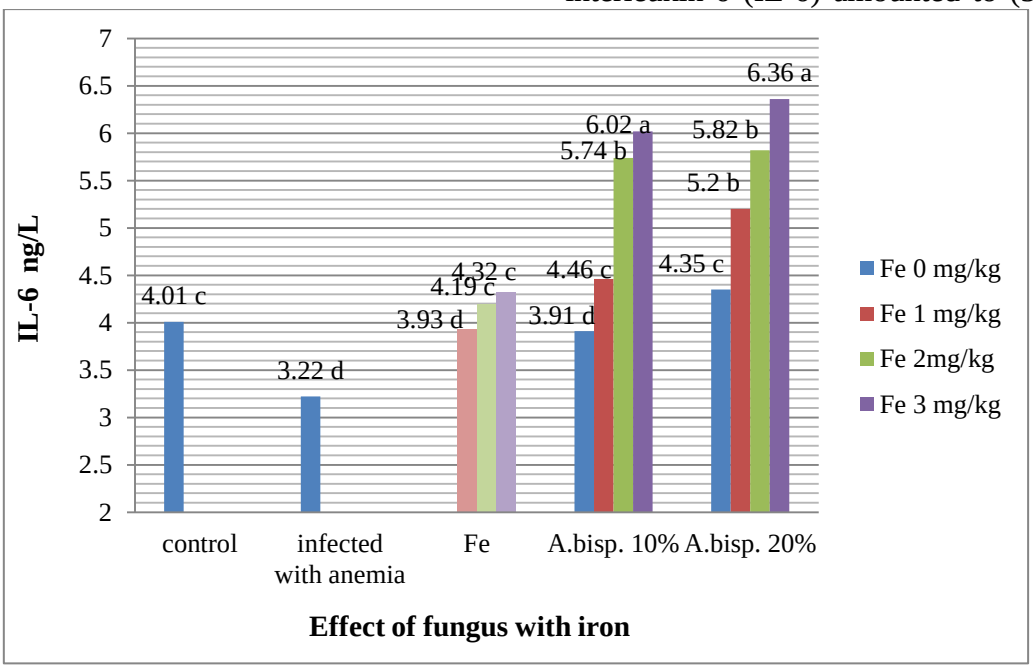

Figure 1: Effect of oral administration of A. bisporus and iron concentrations on interleukin-6 concentrations.

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