Effect of partial replacement of Palm Kernel powder, Oyster Mushrooms and commercial enzyme instead of corn in the diets on the blood traits of common carp fingerling fish (Cyprinus carpio L.)

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Abstract. This study was conducted at Al-Anbar University/ College of Agriculture/ Department of Animal Production / Fish Laboratory for the period from 19/11/2018 to 27/1/2019 to know the effect of adding different levels of Crude palm kernel meal powder and which treated with oyster mushrooms and commercial enzyme mixed as a substitute for corn to the diets of common carp on the hematological parameters blood traits. In the experiment used 20 glass of aquariums basins with dimensions of 70 x 40 x 30 cm with a capacity of 84 liters and it filled with 70 liters were used, in which 140 fish were randomly distributed at a rate of individual weight 2 g ± 19.5 to 10 different treatments and by tow replicate per treatment (7 fish / repeated). The Fish were fed with 4% of their body weight on a pellets diameter of 4.5 mm, on the diets were made in laboratory with a protein content of 27.44% - 30.82%. T2, T3 and T4 coarse dates of raw palm kernel meal 33%, 66%, and 100% respectively were used instead of corn 25%. T5, T6 and T7 dates were treated with Oyster mushrooms with 33, 66, and 100% respectively instead of corn 25%. T8, T9, and T10 dates powder cores were treated with commercial enzymes mixture of 33, 66, and 100% respectively, instead of corn 25%. The results of the study showed, through statistical analysis of the percentage of Packed Cell Volume (PCV) and hemoglobin (Hb) significantly increased (P≤0.05) for T8 (45.50%) compared to T1, T2 and T5, which recorded 38.00, 35.00 and 35.00%, respectively. As for white blood cells, the highest value was in T9, which achieved a rate of 985 ×10³ cells/mm³. For red blood cells, treatment T8, which reached 3,830×10⁶ cells / mm³, recorded a significant increase (P≤0.05) compared to T9 (2,930×10⁶ cells/mm³), no significant differences were recorded with other treatments, and a significant increase (P≤0.01) was observed for T3, recorded at 81.50 ×10³ cells / mm³ In comparison with other treatments, there was also a significant increase (P≤0.01) for the T5 and T6, as it recorded 38.50 and 38.00 ×10³ cells / mm³, respectively, compared to the T2, T3 and T9, a significant superiority (P≤0.05) was achieved for the Monocyte for T4 and T8 coefficients that recorded 7.5 ×10³ cells/mm³ respectively, compared to T6, T7 and T9. The lowest value, which numbered 4.0 ×10³ cells / mm³, was recorded for each treatment and no significant differences (P≤0.05) were observed with T1, T2, T3, T5 and T10. As for the level of ALT enzyme in the blood plasma, significant elevation (P≤0.01) for the T5 and T7 treatments, as it recorded 32.00 IU / L, 33.0 IU / L, respectively, compared to the T1, T2, T3, T9, and T10 coefficients. As for the efficacy of the AST enzyme in the blood plasma, it was found that the T7 It was significantly superior (P≤0.01), as it reached 35.00 IU / liter, compared to the rest of the other treatments. We conclude from the study that there is a positive role for adding Crude palm kernel meal powder treated with the commercial enzyme mixed to the diet to improve blood traits and health status of common carp fish.

1. Introduction
Fish occupies an essential and effective center in filling part of the nutritional needs of humans in Iraq, it is considered economically an important source of animal protein, and the importance of fish in some regions as it constitutes a high percentage of daily food for humans as well as its exploitation in many industries such as feed, fertilizers and some medical preparations [9] [12]. Feed production is the main problem in fish farming projects at this time, as feed costs about 60-85% of the cost of operating on fish farms, and therefore
it is preferable to use inexpensive, unconventional feed [24]. The use of substitutes for high-priced materials such as corn will reduce the costs of diets as well as date palm powder (palm kernel meal), which is one of the main wastes from different industrial factories of dates, including alcohol and molasses [19]. It was noted that the nuclei seeds of dates have a high energy content [1]. 74% carbohydrates, 7.5% crude protein, 56.03% fiber, 7.3% moisture, 9.21% ether extract and 0.84% ash of [22]. From this we note that the palm kernel of dates are a material rich in carbohydrates, fats and protein, as well as the good ratio of calcium to phosphorus compared to the residues of vegetable products, and this is why nutritional scientists have been interested in it as one of the alternatives in animal feed, and but the limitation of it uses first determinant in its use was the hardness of the nuclei and how it is prepared for the animal. The reason for the stiffness is that it contains compounds of lignins, a strong substance found in the cell wall as well as cellulose and hemicellulose in the cell walls, which It contributes to reducing fish's digestibility, so there are many treatments available to analyze the cellulose chain in the dates nucleus powder making it more digestible. The chemical and biological treatments of date nucleus powder improve the nutritional value [17]. Cellulose is the main component of the cell wall of date nuclei, as it consists of a series of monosaccharides (glucose), linked together by a beta-cyclodextrin bond and when analyzing cellulose produces monosaccharides [3]. Which is a good source of energy from the microorganisms that analyze cellulose, especially bacteria and fungi [10]. The oyster mushroom Pleurotus ostreatus is the largest commercially produced mushroom in the world. Oyster mushrooms possess a complex enzymatic system, which made them characterized by a high ability to grow on many types of plant residues rich in cellulose [15], such as wheat straw, rice straw, yellow corn kernels, and agricultural residues of date palm [4],[11],[18]. Enzymes play an important role in digesting animal feed by adding them to the diet that raise the value of food -glucanase. As for (protease), it is very important for it to combine proteins and convert them into small peptides and free amino acids that absorb easily and there are other enzymes such as lipase Which works to digest fatty substances in intestine [19], it found few studies on the evaluation of date nuclei as a substitute for yellow corn in the diet of common carp and it not carried out in Iraq. For these reasons, this study is to evaluate the growth and physiological performance of common carp of feeding on diets at different levels of date nucleus powder and in three forms of date nucleus powder crushed as raw, processed with oyster mushrooms and treated with the commercial enzyme mixture as a partial and total substitute for yellow corn.

2. Materials and methods:

The fingerlings of common carp were brought from the fish farms in Essaouira on 15 October 2017, with different weights ranging from 13 to 34 g. The experimental fish were collected after being immersed in a 0.5% salt bath until signs of stress on the fish for sterilization. Then were transferred to ponds three cement ponds with a capacity of 1200 liters of water. The water temperature was 23 ± 1.5 °C, a water pump (Chinese origin) used for aeration and this continued for 30 days to adapt to environmental conditions. The fish were fed in this period on the commercial diet until they were distributed to the glass aquariums of the experiment. The weights started with the experiment ranged from 17 g to 22 g in each repeat at an average weight of 19 g.

The experiment was conducted in the fish laboratory - College of Agriculture - Anbar University for the period from 19 December 2018 to 27 January 2019. (70) days. Designed and equipped with 20 glass aquariums with dimensions of 70 cm x 40 cm x 30 cm, the basins were washed with water and sterilized with coarse salt of sodium chloride at 5 g / 1 and left for a week after it was cleaned and filled with 70 liters of water until the fish were placed in there. (W) for heating in the case of low temperature, and is equipped with air by two pumps, each of which is half a horse power operating alternately using a timer hour for each pump and the air flow is distributed evenly to all as well as temperature gauges, filling and discharging With a super pump of Chinese origin, the laboratory is equipped with a water tank 1000 Liters with 3 heaters for heating water for filling ponds and storing liquefied water with 4000 L tanks for a period of not less than 24 hours to get rid of chlorine and maintain an appropriate water temperature before using it for experiment basins.

One hundred and forty common carps were used at a rate of 19.28 g/ fish and a live mass of 134.96 g/ basin aquariums. They were distributed over 20, filled 70 liters of water and placed in each 7 fish with 10 treatments per treatment. Each treatment was represented by a diet where ten diets were used, the first diet without additives was promised a comparative treatment, and the second, third and fourth nuclei were added Palm Kernel Meal, and the fifth, sixth and seventh dates were added. Dates are treated with commercial enzyme mixture of 33, 66 and 100%, respectively, replacing yellow corn 25%.

2
Fish were fed on these diets for the duration of the 70-day trial. The localization period was 15 days, feeding from 1% to 4% gradually at three meals per day. The fish were weighed every 15 days and the feed weight was adjusted according to the new weight of the fish. The water temperature was measured daily with a Chinese mercury thermometer and the pH value of the water was measured. Aquariums are cleaned and the water is changed by 50-60% daily. Feeding the first three meals starting at eight in the morning and the second meal starts after 5 hours after the first meal and the third meal at six in the evening.

Statistical Analysis System was used to analyze the effect of different coefficients in the studied characteristics on a complete Randomize Design (CRD), and the significant differences between the averages were compared with [8],[21]. Polynomial at the level of significance (P≤0.05), depending on the mathematical model.

\[ Y_{ij} = M + T_i + e_{ij} \]

Table 1. Chemical Analysis of experimental Treatment

| Treatment | Moisture% | Protein% | Fat% | Ash% | Fiber% | Nitrogen free extract % |
|-----------|-----------|----------|------|------|--------|------------------------|
| T1        | 9.74      | 30.57    | 7.05 | 15.1 | 4.27   | 33.28                  |
| T2        | 10.8      | 30.63    | 7.17 | 16.44| 2.57   | 32.44                  |
| T3        | 7.22      | 30.82    | 7.24 | 17.11| 4.64   | 32.97                  |
| T4        | 6.73      | 29.65    | 6.83 | 16.96| 5.99   | 33.89                  |
| T5        | 7.53      | 28.14    | 10.74| 12.99| 9.72   | 30.88                  |
| T6        | 7.56      | 27.44    | 7.65 | 13.11| 10.98  | 33.26                  |
| T7        | 5.34      | 27.98    | 7.53 | 14.39| 12.97  | 31.79                  |
| T8        | 7.78      | 28.79    | 8.32 | 16.59| 4.00   | 34.6                   |
| T9        | 8.69      | 29.3     | 12.17| 17.14| 4.98   | 27.72                  |
| T10       | 8.54      | 29.93    | 9.08 | 17.00| 6.00   | 29.44                  |

3. Results and Discussion

3.1 Packed Cell Volume (PCV) and Hemoglobin (Hb)

Table 2 shows the effect of the experimental treatments on the hematological parameters of the fish. From the results of the statistical analysis of the percentage of packed cell volume and hemoglobin showed a significant increase (P≤0.05) for the T8 that recorded a rate of 45.50% compared to the T1, T2 and T5, which recorded 38.00, 35.00 and 35.00%, respectively, while no significant differences (P≤0.05) were observed between treatment T8 compared to other treatments, as well as for hemoglobin, the normal PCV ratio for fish is from 20 to 40% and the numbers of red blood cells, packed cell volume and hemoglobin can change with season, nutritional status and health status of fish [13].

3.2 White blood cells (WBC)

The results of the statistical analysis of white blood cells in Table 2 show that the highest level was in T9, which recorded a rate of 985 ×10³ cells / mm³, and through the results it was found that the treatments of the experiment gave different values for the numbers of white blood cells, which can be arranged ascending as follows: T4, T8, T6, T10, T3, T7, T1, T5, T2 and T9, respectively, as rates were recorded 101, 101, 108, 111, 113, 670, 735, 885, 900 and 985 ×10³ cells/ mm³. Table 2 shows the effect of the experimental treatments on the blood traits of the fish. From the results of the statistical analysis of the percentage of packed cell volume and Hemoglobin showed a significant increase (P≤0.05) for the T8 that recorded a rate of 45.50% compared to the T1, T2 and T5, which recorded 38.00, 35.00 and 35.00%, respectively, while no significant differences (P≤0.05) were observed between treatment T8 compared to other treatments, as well as for hemoglobin, the normal PCV ratio for fish is from 20 to 40% and the numbers of red blood cells, packed cell volume and hemoglobin can change with season, nutritional status and health status of fish [13].
Treatments of the experiment, as the number in the bony fish is less than 150,000 cells/mm³, which is one of the cells of the immune system that is important to protect the body from diseases [13].

3.3 Red Blood Cell (RBC)

Through the results of the statistical analysis in Table 3, T8 increased significantly (P≤0.05) recorded $3.830 \times 10^6$ cells/mm³, compared to the T9 treatment that recorded $2.930 \times 10^6$ cells/mm³, and did not differ significantly with other treatments, as the number in most bony fish Less than $2 \times 10^6$-$3 \times 10^6$ cells/mm³.

Table 2. Effect of different experiment parameters on blood traits of common carp fingerlings C. carpio L. ± standard error during the trial period

| treatments | PCV $\times 10^6$ cells/mm³ | HB G/100ml | WBC $\times 10^3$ cells/mm³ |
|------------|-----------------------------|------------|-----------------------------|
| T1         | 38.00±2.0                   | 11.95±0.65 | 735±1750.0                  |
| T2         | 35.00±2.0                   | 10.95±0.65 | 900±1100.0                  |
| T3         | 40.50±1.5                   | 12.80±0.5  | 113±2200.0                  |
| T4         | 40.00±1.0                   | 12.65±0.35 | 101±700.0                   |
| T5         | 35.00±2.0                   | 10.95±0.65 | 885±3150.0                  |
| T6         | 39.00±2.0                   | 12.30±0.7  | 108±1900.0                  |
| T7         | 39.00±4.0                   | 12.30±1.3  | 670±1600.0                  |
| T8         | 45.50±2.5                   | 14.45±0.85 | 101±500.0                   |
| T9         | 39.50±0.5                   | 12.45±0.15 | 985±1550.0                  |
| T10        | 38.50±0.5                   | 12.15±0.15 | 111±600.0                   |

Level of significance (P≤0.05)

a, b, c, d, e: Different letters within one column indicate significant differences between the parameters at the level of significance (P≤0.01) and (P≤0.05)

T1: Control, T2: A diet containing 33% Palm Kernel Meal powder instead of yellow corn. T3: A diet containing 66% Palm Kernel Meal powder, a substitute for sorghum. T4: A diet containing 100% Palm Kernel Meal, a yellow corn substitute. T5: Adiet containing Palm Kernel Meal, treated with P. Ostreatus, with a 33% substitute for yellow corn. T6: A diet containing Palm Kernel Meal, treated with P. Ostreatus, with a 66% alternative to yellow corn. T7: Adiet containing Palm Kernel Meal powder treated with P. Ostreatus at 100% yellow corn substitute. T8: A diet containing Palm Kernel Meal powder treated with a commercial enzyme mixture of 33% as a substitute for yellow corn. T9: Adiet containing Palm Kernel Meal powder treated with a commercial enzyme mixture of 66% as a substitute for yellow corn. T10: A diet containing Palm Kernel Meal powder treated with a commercial enzyme mixture of 100% as a substitute for yellow corn.
3.4 Heterozygous blood cells
The results of the statistical analysis in Table 3 indicate the effect of experimental treatments on the percentage of heterozygous blood cells, as there was a significant increase (P≤0.01) for T3, as it recorded $81.50 \times 10^3$ cells / mm$^3$, compared to other treatments.

3.5 Lymphocyte Cell
The results of the statistical analysis in Table 3 showed a significant increase (P≤0.01) in T5 and T6, as recorded 38.50 and 38.00 $\times 10^3$ cells / mm$^3$, respectively, compared to T2, T3 and T9, and no significant differences (P≤0.05) were found. With T1, T4, T7, T8 and T10 compared to other treatments.

Table 3. Effect of different experiment parameters on blood traits of common carp fingerlings C. carpio L. (± SD)

| treatments | RBC $\times 10^6$ cells /mm$^3$ | HETRO | LYMPH $\times 10^3$ cells /mm$^3$ |
|------------|--------------------------------|--------|---------------------------------|
| T1         | 3.430±0.240 ab                 | 55.00±1.0 cd | 36.00±1.0 ab |
| T2         | 3.230±0.140 ab                 | 66.00±3.0 bc | 26.50±1.5 b  |
| T3         | 3.580±0.230 ab                 | 81.50±1.5 a  | 13.00±1.0 c  |
| T4         | 3.400±0.320 ab                 | 58.50±6.5 bcd| 32.00±5.0 ab |
| T5         | 3.125±0.055 ab                 | 53.00±2.0 cd | 38.50±2.5 ab |
| T6         | 3.165±0.225 ab                 | 54.50±5.5 d  | 38.00±7.0 a  |
| T7         | 3.320±0.210 ab                 | 57.00±1.0 cd | 36.50±1.5 ab |
| T8         | 3.830±0.360 ab                 | 62.00±1.0 bc | 27.50±0.5 ab |
| T9         | 2.930±0.010 ab                 | 68.50±1.5 bcd| 25.50±0.5 ab |
| T10        | 3.260±0.330 ab                 | 63.00±3.0 bcd| 29.50±4.5 ab |

Level of (P<0.05) (P<0.01) (P<0.01)

a, b, c, d, e: Different letters within one column indicate significant differences between the parameters at the level of significance (P<0.01) and (P<0.05)

T1: Control. T2: A diet containing 33% Palm Kernel Meal powder instead of yellow corn. T3: A diet containing 66% Palm Kernel Meal powder, a substitute for sorghum. T4: A diet containing 100% Palm Kernel Meal, a yellow corn substitute. T5: Adiet containing Palm Kernel Meal, treated with P. Ostreatus, with a 33% substitute for yellow corn. T6: A diet containing Palm Kernel Meal, treated with P. Ostreatus, with a 66% alternative to yellow corn. T7: Adiet containing Palm Kernel Meal powder treated with P. Ostreatus at 100% yellow corn substitute. T8: A diet containing Palm Kernel Meal powder treated with a commercial enzyme mixture of 33% as a substitute for yellow corn. T9: Adiet containing Palm Kernel Meal powder treated with a commercial enzyme mixture of 66% as a substitute for yellow corn. T10: A diet containing Palm Kernel Meal powder treated with a commercial enzyme mixture of 100% as a substitute for yellow corn.
3.6 Monocyte Cells.
The results of the statistical analysis in Table 4 showed a significant increase (P≤0.05) for T4 and T8 which recorded the highest value (7.5 ×10³ cells/mm³) compared to T6, T7 and T9 that recorded the lowest value (4.0 ×10³ cells/mm³) No significant differences were observed compared with treatment T1, T2, T3, T5 and T10.

3.7. Liver enzymes (ALT, AST)
The results of the statistical analysis in Table 4 indicate the effect of treatments on the level of ALT enzyme in the blood plasma of common carp fish 70 days after the start of the experiment, a significant difference (P≤0.01) was observed for T5 and T7 as they recorded 32.00 IU/L and 33.0 IU/L, respectively, compared to T1, T2, T3, T4, T9, T10, and T5 and T7 showed no significant differences (P≤0.05) in ALT value in blood plasma with T6 and T8.

As for the effectiveness of the AST enzyme in blood plasma, it was found that T7 achieved significant superiority (P≤0.01) compared to other treatments, as it recorded 35.00 units/liter and no significant differences (P≤0.05) were recorded compared with T4 which recorded 16.50.

The values for these enzymes are within the normal range and are indicative of the normal state of fish. They are from 17 - 136 IU/L for ALT, and from 51 - 443 IU/L for AST, and an indication of absence of disease and stress [7].

Table 4. Effect of different experiment diets on blood traits of common carp fingerlings C. carpio L. (± SD)

| treatments  | Monocyte ×10³ cells/mm³ | ALT UR/L | AST UR/L |
|-------------|-------------------------|----------|----------|
| T1          | 7.0±0.00                | 22.5±1.5 | 24.5±1.5 |
|             | ab                      | cde      | bc       |
| T2          | 6.0±1.0                 | 18.5±0.5 | 16.0±2.0 |
|             | ab                      | de       | d        |
| T3          | 4.5±0.5                 | 24.0±3.0 | 24.0±2.0 |
|             | ab                      | bcde     | bc       |
| T4          | 7.5±0.5                 | 17.5±1.5 | 16.5±1.5 |
|             | a                       | e        | d        |
| T5          | 5.5±0.5                 | 32.0±1.0 | 28.5±1.5 |
|             | ab                      | a        | ab       |
| T6          | 4.0±2.0                 | 30.0±2.0 | 27.5±3.5 |
|             | b                       | ab       | b        |
| T7          | 4.0±0.00                | 33.0±2.0 | 35.0±2.0 |
|             | b                       | a        | a        |
| T8          | 7.5±0.5                 | 27.0±2.0 | 22.5±2.5 |
|             | a                       | abc      | bcd      |
| T9          | 4.0±1.0                 | 25.0±3.0 | 25.0±1.0 |
|             | b                       | bcd      | bc       |
| T10         | 5.0±1.0                 | 20.5±1.5 | 18.0±3.0 |
|             | ab                      | cde      | cd       |

Level of (P≤0.05) (P≤0.01) (P≤0.01)

See Table 2 for details

Oyster mushrooms are distinguished for containing most essential and non-essential amino acids, especially lysine and tryptophan, as well as for their good vitamins B1, B2, Niacin and C and mineral salts [6],[20], and this may be the reason behind the improvement of these characteristics. And that the
distinctive content of the oyster mushroom from folic acid [5]. [20]. may be a catalyst for increasing the number of red blood cells (RBC) for the treatment of adding oyster mushrooms to the diet.

The rise in the numbers of red blood cells leads to an increase in the packed cell volume (PCV), because there is a positive correlation between them [23]. The results of this study were in agreement with the researcher’s study [16]. which included the effect of adding different levels of Palm Kernel (0, 0.5, 1, 2.4%) to the diet in the physiological characteristics of the common carp fish. T2 increased significantly (P≤0.05) in both hemoglobin value and Packed Cell Volume compared with other treatments. The results also did not agree with the researcher himself, it showed a significant decrease (P≤0.05) in the value of ALT and AST in the blood plasma.

Oyster mushrooms contain an antiprotease that some bacteria use to invade the host [2]. Antiprotease also helps increase the immunity of animals due to its antibacterial and anti-inflammatory role [14]. Studies have shown that high antiprotease activity protects fish from infection and disease, and improves their health status. And increase its survival.

It is also noted from Table (4) that both the liver enzymes ALT and AST were decreased in all treatments, and even control, and this indicates that the cause is not the treatments but rather it is related to the fish themselves, and there is no indication of the presence of fish stress or the effect of the treatment on the liver.

4. Conclusions

Crude palm kernel meal can be added with 100% substitute for yellow corn 25% in the diets of Caprinus carpio fish. palm kernel meal treated with oyster mushrooms can be used in 33% substitute for yellow corn 25% in the diets of common carp fish. Palm kernel meal powder treated with commercial enzymes mixture can be used at 66% substitute for yellow corn 25% in diets of common carp as it is considered a good source of protein as well as contribute in improving of blood traits and thus increasing health of fish. palm kernel meal can be used as a culture medium for oyster mushrooms suitable for human consumption for more than three weeks.

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