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Comparison for Using Two Types of Digestive Enzymes with Fish Powder in the Diets of Common CarpL. *Cyprinus Carpio*and its Effect on Growth and Blood Traits

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Abstract

The study was conducted in the Fish Laboratory / College of Agriculture / Tikrit University to investigate the effect of using prepared fish waste powder supplemented with phytase and pepsin at rates of 1% and 2% on the growth rates and the biochemical characteristics of the blood. 80 cuffs were distributed with a starting weight of 250 ± 1.70 g / fish randomly. Repeat for each treatment at a rate of 5 fish per replicator on 16 glass tanks. The feed containing fishmeal was prepared, chemical analyzes were carried out, and it was used to feed common carp. Treatment T3 recorded the highest rates of weight gain of 96.77 g, daily growth of 1.88 g / day, relative growth of 51.59%, specific growth of 0.29 g / day, and efficiency of food conversion of 55.39% compared to the rest of the treatments, and significant differences were recorded at the level of ($P\leq0.05$) between the different experimental treatments. A positive improvement was observed in the blood parameters, as it included (total protein, albumin, globulin) in the experimental treatments that were supported by enzymes. On the other hand, the values of serum enzymes (ALT, AST and ALP) decreased varying between the treatments with significant differences between all treatments at the level of ($P\leq0.05$) in addition to the lower levels of cholesterol and triglycerides in the experimental treatments compared to the control treatment, and this is a good evidence of the health of the experimental fish and that they are not exposed to stress or pathological injuries.From this research, we conclude from this research the possibility of using prepared fish waste powder with phytase and pepsin at rates of 1% and 2% as a source of animal protein in the diets of common carp without negative effects on nutrition efficiency, growth rates, and biochemical blood characteristics.

Key words: common carp, fish residue powder, phytase and pepsin, growth rates, biochemical characteristics of the blood.

Introduction

The common carp *Cyprinus carpio*L. is one of the most important fish found in several regions of the world. The Iraqi consumer also has the distinction of being an omnivorous fish for food (Marković *et al*., 2016). Enzymes play an important role in the breakdown of carbohydrates, proteins and fats to make them easily digestible and absorbable materials in the intestine, then transported by the circulatory system and transformed into energy in the bodies of living organisms. Nutritional additives, the most important of which are external enzymes, were used as sources to improve fish

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diets (Kumar *et al*., 2012). Fish powder is one of the main sources of protein high in nutritional value, as it is easily digestible and rich in essential amino acids. It is produced from unused fish or from animal waste, including fish and poultry (Khan *et al*., 2013).

The present study aims to compare the addition of the enzyme phytase and pepsin at rates of 1% and 2% in fish powder prepared from different types of fish and added in the diets of common carp and study its effect on growth rates and the biochemical characteristics of blood.

Materials and Methods of Work

Preparation of powders and suspensions:

The powders were prepared by grating the remnants of the mixed fish species and adding to them the proportions (1 and 2)% of their weight enzymes (phytase, pepsin) for 24 hours to ensure the appropriate processes for decomposition, and then dried in an electric oven at a temperature of 60 ° C until the powder was obtained. The feeds were prepared after mixing the raw materials well (wheat 30% - yellow corn 30% - barley 20% - bran 9% - fish powder 10% - vitamins and minerals 1%), and boiling water was added to them at a rate that ranged between (35-40)% of the components of the diet. Five different relationships were used:

1- A control diet containing prepared fish powder devoid of enzymes.

2- An experimental diet containing fish powder prepared with the enzyme phytase 1%.

3- An experimental diet containing fish powder prepared with the enzyme phytase 2%.

4- An experimental diet containing fish powder prepared with the enzyme pepsin 1%.

5- An experimental diet containing fish powder prepared with 2% pepsin enzyme.

Chemical Analyzes:

The chemical analyzes were carried out by the methods mentioned by AOAC (2000) as follows:

Humidity Estimate:

The percentage of moisture was measured and determined in the samples of the powders, prepared diets and fish of the experiment, using 5 grams of each type of powder, which was dried in an electric oven at a temperature of (105) ° C until the weight stabilized.

Protein Estimation:

The nitrogen was estimated according to the Micro Kjeldahl method using concentrated sulfuric acid for the purpose of digesting the experimental samples. Then, boric acid was used with the Bromocresol green for distillation, then crushed with hydrochloric acid and the conversion factor (6.25) was used to extract the percentage of protein in the samples.

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Lipid Estimate:

The percentage of fat was estimated using a soxhlet device and a solvent of diethyl ether absolute. The samples were placed in the device for a period of 8 hours and the fat percentage was calculated**.**

Fiber Estimate:

The fibers were determined by adding 1.25 N standard sulfuric acid for the purpose of digesting the sample for half an hour after it was boiled, then the sample was washed from the acid with hot distilled water and a 1.25 N standard NaOH base was added for half an hour after boiling the sample and washed from the acid with hot distilled water and then with acetone. Then, the dry and empty vessel was weighed, the sample was placed in it, and the oven was entered at a temperature of 60 ° C. The above method was performed based on the method mentioned by AOAC (2000) in the Animal Production Laboratory of the College of Agriculture - University of Baghdad.

Percentage of crude fiber% = weight of fiber (g) / sample weight (g) x 100

Ash Estimate:

The percentage of ash was calculated by burning 1 g per sample at a temperature of 550 ° C in the Muffle Furnace (550 ° C / 4 hr) MLW electro-type LM 212-11 of German origin for three hours and obtaining a white or gray powder and the percentage of ash was estimated. After the weights are fixed at a temperature of 60 ° C.

Extract Nitrogen -Free:

The dissolved carbohydrates were calculated by difference by subtracting the percentage of nutrient components (moisture, protein, fat, ash, and fiber) from 100.

Studied Traits

Growth Measurements

Total Weight Increase:

Weight gain rates were calculated according to the following law:

Weight gain (g) = Final weight (g) - Starting weight (g).

Daily growth Rate:

According to the daily growth rate according to the following equation:

Daily growth rate in g / day = weight gain (g) / time increase (day)

(Schmalhausen, 1926)

Qualitative Growth Rate:

The specific growth rate was estimated according to the following equation:

Specific growth rate in g / day = log of final weight - logarithm of initial weight / duration of experiment x 100

(Brown, 1957)

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Relative Growth Rate:

The rate of food conversion is calculated according to the following equation:

Relative growth rate% = final weight (g) - initial weight (g) / initial weight (g) x 100

(Utne, 1978)

Food Conversion Efficiency:

The efficiency of the food conversion was estimated according to the following equation:

Feed conversion efficiency% = fish wet weight gain (g) / feed intake (g) x 100

(Utne, 1978).

Biochemical Blood Tests:

Blood Tests

Blood is drawn from fish (3 fish / treatment) from the tail vein with a 1 ml syringe and heparin, an anti-coagulant, was added to it (Matar, 2000). Tests to measure the percentage of compacted cells' size, the number of red and white blood cells, and the hemoglobin test of the blood of fish drawn were performed according to the methods mentioned by them (Blaxhall and Dalslly, 1973).

Measurement of the Percentage of Hematocrit:

It has been used capillary tubes, length of 75 mm and a diameter of 1.1 mm, which were filled with blood and one end was closed with artificial clay. The prepared tubes were placed in a micro-centrifuge for 5 minutes at a speed of 10,000 rpm. The reading was obtained by means of a ruler, which represents the volume of stacked cells / 100 milliliters of blood.

Calculating the number of red and white blood cells:

Use 0.98 milliliters of Dices pivot solution consisting of 10 mL (Formaldehyde 4%, 31.3 g of Trisodium citrate, 1 g of Brilliant cresyl blue, and 1 liter of distilled water) in a test tube and add 0.02 ml of blood drawn with a pipette. Sahli, then the contents of the tube were mixed well and I took a drop of the mixture and placed it on a special slide called Neubaur Improved Haemocytometer Slide (Chamber) covered with a special glass cover (Slide), then a red blood cell counting process was performed in 5 small squares out of a total of 25 small squares located on Slide.

The calculated number N × 2500 = the number of red blood cells (cells / mm3) of blood and as in the following equation:

Calculated erythrocyte count x dilution factor / volume

The calculation of the WBC is counted in the four large side squares, and the counted number is N × 125, and the result represents the number of white blood cells (cells / mm^3), as in the following equation:

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Calculated leukocyte count x dilution factor / volume

Hemoglobin Concentration Measurement:

The method of cyanometric hemoglobin and described by Coles (1986) was used to estimate the hemoglobin concentration using the Drabkin's reagent, as 20 microliters of blood were drawn using a capillary pipette special for this purpose and mixed with 5 ml of this reagent to make the dilution factor 251 and leave for a period of 5 minutes and then put in a centrifuge at a speed of 5000 revolutions / min for 15 minutes to get rid of all the nuclei and coatings of red blood cells, then read using a spectrophotometer after zeroing it with the same detector and read at a wavelength of 540 nanometers. After that, it has been recorded the standard hemoglobin reading and then read the original pattern expressed by (Grams / 100 milliliters of blood) and according to the formula:

Hb concentration in grams / 100 ml of blood = sample reading / standard Hb reading x dilution factor

Blood Serum Tests

Glucose Measurement:

The concentration of glucose was measured using a ready-made kit by LiNEAR, by taking 1 ml of the reagent, placed in a test tube, and adding 10 micro-liters of serum to it, where the tubes are mixed well and left for 10 minutes, then the absorption is read in a Spectro photometer at a temperature of 37 M˚ at a wavelength of 500 nm.

Serum glucose concentration (mg / 100 mL) = sample reading / standard cholesterol reading x 100

Measurement of Cholesterol Concentration

The leaflet followed has attached to the standard kit produced by LiNEAR, which uses the method of enzymatic analysis to determine the level of cholesterol, according to the method of (Allain *et al*., 1974). The absorption was read at a wavelength of 505 nm using a spectrophotometer.

Serum cholesterol concentration (mg / 100 mL) = sample reading / standard cholesterol reading x 200

Triglycerides:

The determination of triglyceride in serum depends on the method of enzymatic hydrolysis mentioned by Fossati and Prencipe (1982). After preparing the solutions in the manual attached to the standard kit, the tubes are mixed well and left for 15 minutes at room temperature or 5 minutes at temperature. The absorbance of the standard solution and the sample solution was measured at a wavelength of 500 nm by a spectrophotometer. The following equation was applied to estimate the concentration of triglyceride mg / 100 ml = dl / mg.

Triglyceride concentration mg / 100 mL = sample reading / standard solution

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reading x 200

Measurement of Total Protein Concentration:

The kit produced by the company BIOLABO was used, depending on the method referred to by Falkner and Meites (1982) as this method relies on the interaction of copper ions in the presence of the base medium with the peptide bonds to produce a complex compound with a violet color, after which the absorbance was read by the spectrophotometer along The wavelength is 550 nm and the unit and determination of blood plasma proteins are g / 100 ml according to the following equation:

Total protein concentration = sample absorbance / standard solution absorbance x standard solution concentration

Albumin Concentration Measurement:

The measurement of albumin in fish serum was carried out by a ready-made kit from the American Randox company using a spectrophotometer, with a wavelength of 630 nanometers, according to the following equation:

Albumin concentration (mg / 100 mL) = sample absorbance / absorbance of standard solution x 4.5

Measurement of Globulin Concentration:

The concentration of globulin in fish serum was measured by subtracting the albumin value from the total protein value in the samples (Wolf and Darlington, 1971).

Globulin concentration = total protein concentration - albumin concentration

Liver Enzyme Test:

Liver enzymes, Aspartate amino Transferase (AST), Alanine amino Transferase (ALT) and (ALP) Alkaline phosphatase were determined by the Mindray ofGermany.

Statistical Analysis

The Statistical Analysis System -SAS (2012) program was used in analyzing the data to study the effect of different parameters on the studied traits according to a complete random design (CRD). The significant differences between the averages were compared with the Duncan (1955) polynomial test (Duncan multiple range test on the probability level (P.>0.05).

Results and discussion

Table (1) shows that the percentage of moisture increased in the treatment T4, reaching 8.91%, and the treatment T5 differed significantly ((P≤0.05) compared to the

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rest of the treatments, and no significant differences were recorded between the treatments in the percentage of protein, fiber and carbohydrates. The percentage of fat decreased in the two treatments. T3 and T5 were (8.68 and 8.73)%, respectively.Al-Bassam (2009) reached a fat percentage that ranged between (7.11-9.24)% when using powder prepared from small and large mouthed binini fish and adding it to diets of common carp. The percentage of ash ranged between (5.62 and 5.95)% in the diets of the experiment, and these results are close to what Voorhees et al. (2018) found, where the percentage of ash reached 6.68% when using fish powder in diets of trout fish.

Table (1) Chemical analysis of mixed fish residues powder

Soluble carbohydrates %	Ash %	Fiber %	Fat %	Protein %	Moisture %	Transactions %
0.134±35.03 a	0.506±5.95 a	0.562±4.88 a	0.27±8.92 a	0.371±36.59 a	0.062±8.63 a	Raw powder T1
0.223±35.87 a	0.498±5.62 b	0.214±4.97 a	0.357±8.85 a	0.060±36.31 a	0.01±8.38 a	powder with phytase 1% T2
0.468±35.33 a	0.437±5.83 a	0.271±4.92 a	0.116±8.68 b	0.432±36.81 a	0.082±8.43 a	Powder with phytase 2% T3
0.323±34.99 a	0.121±5.67 b	0.172±4.71 a	0.220±8.90 a	0.213±36.82 a	0.202±8.91 a	powder with pepsin enzyme1% T4
0.127±35.72 a	0.160±5.79 a	0.124±4.82 a	0.021±8.73 b	0.313±36.69 a	0.215±8.25 b	Powder with pepsin enzyme 2% T5

Growth Criteria for Fish Fed on Mixed Fish Waste Powder Diets:

Table (2) shows the rates of weight gain of fish during the weeks of the experiment, as we notice a fluctuation in the weight gain and significant differences between the treatments ($P \leq 0.05$), where the treatment T3 and T5 outperformed (96.77 and 94.19) g, respectively, and the enzymes improved the weight gain of the fish and the results were consistent with What was found by (Khan, 2016) when studying the growth requirements of food and energy in prepared feed for marine fish, and it was noticed that there were significant differences between the parameters in the daily, relative and qualitative growth rates and the efficiency of the food conversion with the superiority of the treatment T3 containing the phytase enzyme by 2%, where the rate of The daily growth is 1.88 g / day, the relative growth rate is 51.59%, the specific growth rate is 0.29 g / day, and the food conversion efficiency is 55.39%. The results of the study are similar to the findings of (Al-Bassam, 2009) in the growth rates when using diets of common carp fish containing powders prepared from small and large-mouthed *Cyprinion macrostomum*, and the results of Chowdhury (2015) when adding protease enzyme in powders of some aquatic organisms, where they increased Growth

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rates and feed conversion efficiency with this addition in the experimental fish. Dabrowski (2001) indicated that the continuous increase in growth rates is mainly related to the continuous increase in fish weight for all treatments, especially its increase with the increase in the level of additives in the diet for its positive effects in increasing the daily growth rates, qualitative growth and relative growth as a result of its association with all vital activities in the body, especially its important role in the operations of Metabolism and in improving the health and physiological condition of fish, and the improvement in growth rates may be due to the enzymes entering and activating the metabolism processes and achieving optimum utilization of the components of the diet, and that benefit is reflected in the weight increases (Ganji *et al*., 2003).

Table (2) Some growth characteristics of common carp fed on experimental diets

Conversion efficiency Nutritional%	growth rate Specificity in g / day	growth rate Relative%	Daily growth rate g / day	Weight gain g	Final weight g	Initial weight g	Transactions
40.533±0.447 c	**0.164±0.003 c**	**26. 36±0.319 c**	**1.34±0.04 c**	**75.221.135± c**	**1.622±321.45 b**	**255.45±0.735 a**	Rennet container for raw fish powder (control) T1
0.741±35.16 d	**0.002±0.13 d**	**0.461±20. 40 e**	**0.03±1.12 d**	**1.391±61.43 d**	**1.201±327.13 b**	**1.465±206.65 c**	A diet containing fish powder fortified with phytase 1% T2
0.591±55.39 a	**0.003±0.29 a**	**0.692±51.59 a**	**0.01±1.88 a**	**1.301±96.77 a**	**1.212±362.19 a**	**1.546±241.86 b**	A diet containing fish powder fortified with phytase 2% T3
0.213±29.213 e	**0.005±0.160 c**	**1.030±22.19 d**	**0.03±1. 13 d**	**1.260±89.32 b**	**1.213±317.32 c**	**1.345±235.42 b**	A diet containing fish powder fortified with pepsin enzyme 1% T4
0.126±50.11 b	**0.002±0.25 b**	**0.166±47.22 b**	**0.02±1.59 b**	**1.281±94.19 a**	**1.293±356.22 a**	**1.374±259.65 a**	A diet containing fish powder fortified with 2% pepsin enzyme T5

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*Different letters indicate the presence of significant differences within the same column below the level of significance (P≤0.05)

Table (3) indicates for blood serum analyzes (glucose, ALT, AST, ALP, albumin, globulin, cholesterol, and triglycerides) for fish fed on diets containing fish waste powder that treatment T1 showed a significant increase (P≤0.05) in the average blood glucose concentration, reaching 49,238 mg / DL compared to the rest of the treatments, with significant differences recorded between all treatments. Zhu *et al*. (2011) found that the glucose pathogen was not affected when fish meal was used with other animal proteins in Siberian sturgeon diets. Güllü et al. (2014) found no change in the presence of blood glucose at Their use of fish silage in the diets of iridescent trout. The results of the current study came close to the glucose values in the study (Al-Jubouri, 2018), as they ranged between (67.21-87.87) mg / 100 ml in common carp fish, and the glucose ranged between (51.74-55.87) mg / 100 ml when using fish powder with poultry powder. In the diets of dark sea denis fish (Karapanagiotidis *et al*., 2019). An increase in ALT was observed in treatment T3, reaching 19.113 IU / liter, while the two treatments T4 and T5 containing pepsin enzyme at concentrations of 1% and 2% recorded a significant decrease in ALT (15.423 and 15.438) IU / liter, respectively, and the AST value increased in treatment T4. 70,215 IU / L compared to the rest of the treatments, and T1 54,253 IU / liter recorded a significant decrease in the AST rate. T2 and T3 (59.738 and 57.642) IU / L gave the highest value of ALP.

An increase in liver enzyme levels is an important indicator of the occurrence of stress, and significant changes in the activities of these enzymes indicate tissue weakness (Svoboda, 2001). The increase in liver enzymes may be due to the high fat content of these feeds and cultured fish foods, which leads to Damage to liver tissue. It led to the release of the enzyme from the infected cells and raised its level in the blood, and any increase in the serum enzyme concentrations resulted from physiological, pathological, or nutritional states (Ebeid, 2005). Shivaknmar (2005) indicated that the activity of the liver enzymes AST and ALT acted as linkages between protein and carbohydrates. These enzymes are known to change under physiological and pathological conditions. Salaei (2006) found that the activity values of AST and ALT in the blood plasma of common carp raised in earthen ponds were significantly higher than the values recorded for fish reared in glass ponds. The fish are stressed too much, which leads to high values. Ghodratizadeh *et al*. (2011) observed an increase in ALT and AST values in the blood of common carp when fed diets containing Saccharomyces cerevisiae and Bacillus substilis. Al-Ash'ab et al. (2017) found that common carp were not pathologically affected when probiotics were added to feeds with higher efficacy of imported bioups compared to local bio promoter.

The two transactions T2 and T4 (0.911 and 0.801) mg / dL gave the highest albumin concentration, and the two treatmentsT1 and T2 recorded the highest concentration of globulin (4.812, 4.712) mg / dL, and it was noticed that there were no significant

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differences between the treatments containing fish waste powder and supported by enzymes for total protein. Zhou *et al*. (2010) obtained significantly higher concentrations of total protein, albumin, and globulin when using probiotics in the diets of Nile tilapia. Zhu *et al*. (2011) found that the use of fishmeal with animal protein concentrates improved the total protein concentration in Siberian sturgeon. Güllü et al. (2014) confirmed an improvement in overall protein intake of iris trout when using experimental diets containing fish silage compared to control diets without it. The total protein level increased from 5.37 mg / dL of common carp fed on a control diet to 7.78 mg / dL of fish fed on diets fortified with probiotics in the study (Al-Ash'ab *et al*., 2017).

A decrease in cholesterol levels was observed in the trial parameters compared to control treatment T167.229 mg / dL, respectively, with significant differences at ($P \leq 0.05$). Karapanagiotidis *et al*. (2019) upon partial use of fish powder in seabream fish found a significant reduction in cholesterol from 111.31 mg / dL to 85.16 mg / dL at the end of the experiment. One of the reasons for the decrease in cholesterol synthesis rates within cells may be due to the lack of cholesterol absorption in the intestine as well as the lack of cholesterol in the food intake (Kennish *et al*. 1992), which coincides with the decrease in triglycerides, and the reason may be due to the high value of cholesterol in the control treatment. It may be due to the energy contained in heavy fats containing saturated fats (Williams, 2007). Also, among the reasons for the decrease in cholesterol in experimental treatmentcould be the increase in the secreted organic acids due to the use of enzymes as biomimers in diets, which in turn may inhibit the building of fatty acids (Chen). *et al*., 2014).

These results give an indication of the absence of pathological conditions, breakage and damage to the liver cells of the experimental fish, as the chances of exposure to the factors causing hepatitis are reduced, as well as an increase in secondary metabolic products such as the production of short-chain fatty acids, including butyric acid, which is a source of energy for intestinal cells, which in turn increases Their synthesis and constant renewal (Ringø et al., 2010).

Table (3) serum analyzes (glucose, ALT, AST, ALP, albumin, globulin, cholesterol, and triglycerides) for fish fed on diets containing fish waste powder

Triglycerides Mg / dL	**Cholesterol Mg / dL**	**Clobulin Mg / dL**	**Two albums Mg / dL**	**Total protein Mg / dL**	**ALP IU / liter**	**AST IU / liter**	**ALT IU / liter**	**Glucose Mg / dL**	Transactions
47.750	62.686	4.507	0.536	4.942	75.437	97.154	21.936	79.961	**Fish Before Experience**
±59.367 0.546 a	±67.229 0.598 a	±4.812 0.072 a	±0.715 0.039 b	0.022±5.221 a	±52.711 0.513 b	0.190±54.253 e	±17.357 0.214 c	±49.238 0.411 a	**Raw powder T1**

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±58.447 0.612 a	±51.416 0.522 b	±4.712 0.072 a	±0.911 0.029 a	0.037±5.213 a	±59.738 0.511 a	0.392±63.754 c	±18.726 0.352 b	±43.668 0.382 b	**Phytase 1% T2**
±59.621 0.574 a	±56.361 0.518 b	±4.536 0.059 b	±0.725 0.036 b	0.029±5.272 a	±57.642 0.612 a	0.392±61.642 d	±19.113 0.239 a	±37.972 0.321 d	**Phytase 2% T3**
±50.741 0.330 b	±52.721 0.424 b	±4.327 0.046 c	±0.801 0.049 a	0.041±5.601 a	±52.591 0.473 b	0.264±70.215 a	±15.423 0.443 d	±40.813 0.426 c	**Pepsin enzyme 1% T4**
±46.479 0.260 c	±52.628 0.411 b	±4.421 0.062 b	±0.742 0.062 b	0.048±5.735 a	±51.572 0.468 |b	0.351±69.284 b	±15.438 0.463 d	±42.941 0.391 b	**Pepsin enzyme %2 T5**

Conclusion

This research concludes that the possibility of using prepared fish waste powder with the enzyme Phytisse and Pepsin as a source of animal protein in the diets of common carp without negative effects on feeding efficiency, growth rates, vital blood characteristics, in addition to reducing the cost of production.

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