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Role of Anise Seeds Powder (*Pimpinella anisum*) on Some Blood Aspects and Growth Parameters of Common Carp Fingerlings (*Cyprinus carpio* L.)

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Abstract: The study was conducted to investigate the effect of various addition levels of dietary Anise seeds powder on some immunological aspects and growth parameters of common carp fingerlings (*Cyprinus carpio* L.). Thirty two at mean Wt. 52.50 gm ranged 30-75 gm were randomly distributed into four duplicate treatments. No Anise powder adding to treatment 1 (control), while T2, T3, T4 had treated with 0.3%, 0.6% and 1.0% of anise seeds powder respectively. Fish were weighed biweekly for ten weeks experiment period. Diets and Flesh of experimental fish was analyzed chemically before and after the experiment. Growth parameters was calculated and blood samples had taken and tested for whole blood picture (Hb, PCV%, R.B.Cs. and differential W.B.Cs. Counts). All data were analyzed statistically by Complete Randomized Design (CRD) and tested with Least Significant Differences (LSD) at $p \geq 0.05$ Probability. The results showed the best response were in T3 and T4 with significant differences between all treatments, these seeds powder play a positive promoting agent by altering the levels of growth parameters, immunological performances by improving blood components and properties. Because Anise has anethole, Tyrosinase inhibitor activity and shikimic acid, which they slow the spread of pathogenic agents into fish bodies and keep fishes in a constant healthy state by stimulates their immunity.

Key words: Nutrition, common carp, Anise

INTRODUCTION

Anise is a dainty, sweet, spicy and white flowered umbrella-shape and annual plant (Wissam *et al*., 2009). The used part of anise is the thresh out fruit which called "seeds" which they are grayish brown, ovate, hairy about one-fifth of an inch long with ten crenate ribs. It's a Mediterranean plant and Asia Minor and cultivating in many European countries (Ozean and Jean, 2006). Many researchers mentioned that Anise has 90% anethole and aldehyde which play a role as antifungal (Tripathi and Shuklas, 1987; Bown, 2001 and Cifitic *et al*., 2005). Anise anethole has many activities as antifungal, antibacterial, antiviral, antioxidant, expectorant, antispasmodic, carminative, diaphoretic properties beside digestive system stimulation and diuretic stimulate (Al-Neamy, 2008), Anise also has Tyrosinase inhibitory activity which play as antifungal and antibacterial agent (Kubo and Himejima, 1991). In canned food Anise used as a preservative material to increase their half life and in human medicine anise used for kidney failure treatment (Al-Dajwy, 2006). Anise stimulates digestive system, appetizer by increasing digestibility of nutrients by increase digestible enzymes secretion and develop hepatic activity (Al-Neamy, 2008; Langhout, 2000; Muzahim, 2009 and Hamilton, 2003). Pods of anise contain chemical compounds called shikimic acid (Ozean and Jean, 2006). Which is used to manufacture Tamiflue that treat Bird and Swine influenza cited in (Hamilton, 2003) Shikimic acid is a neurominidase inhibitor which slows the interval spread of all viruses. Besides using it to treat many human disorders and stimulates immunity. Anise structure has vit. B and minerals (Ca, Mg, K and Fe) (Jasim, 2005). Antioxidant activity of anise took place by removal of Fatty Acids (FAs) Saturation which stimulates WBCs phagocytosis and antibodies formation (Good and Pop, 1964). Blood picture can show abnormalities of infected fishes, because there are many factors influence blood components like temperature, dissolved oxygen, age, sex, weight and health state of fishes (Al-Neamy, 2008). Pathologically lymphocytes decreased macrophages increased during infection (Ozean and Jean, 2006). Researchers agreed that lymphocytes responsible of humeral, cellular and quantitative immunity (Dalphy, 2004). Blood volume in fishes ranged 2-4 ml/100 gm of body weight (Good and Pop, 1964) and RBCs Count ranges 1-3 x 10^6 cell/ml while WBCs ranged between 5-7 x 10^3 cells/ml. The studies which explain the anise activity in fish nutrition were seldom, therefore the present work studying anise as promoting agent of growth parameters, blood and immunological performances by improving blood components properties.

MATERIALS AND METHODS

Diets: An experimental diet was manufactured in fish laboratory by grinding the ingredients (Animal concentrated protein, soya bean meal and rice barn,

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Table 1: Percentages of experimental diet ingredients

Items	%
Animal concentrated protein (40%)	10
Soya bean meal	25
Rice barn	20
Yellow corn	12
Barely	10
Wheat barn	20
Vit. and minerals premix	1
Soya bean oil	2

yellow corn, barely, wheat barn, soya bean oil, vitamins and minerals mixture) (Table 1) where added and mixed by electrical blender.

Experimental diets divided into four parts represent four treatments where anise seeds powder was added at 0.0% (control), 0.3% (T2), 0.6% (T3) and 1.0% (T4). Approximate chemical analysis of experimental diets was determined according to Peters and Hoss (1974) as in Table 4. Chromic oxide (Cr_2O_3) was added to each of four treatments diets at 2% for digestibility determination as worded by NRC (1994).

Thirty two common carp *(Cyprinus carpio L.)* fingerlings at mean weight 52.50 gm (weight ranged between 30-75 gm) were distributed randomly into eight glasses aquarium sized 30 x 40 x 60 cm (four fishes in each aquarium) in Fisheries Laboratory at Animal Resources Department, Agricultural College. Experiment expands 94 days. Fish were accumulated for two weeks before the experiment, aquarium supplied with air pumps for aeration. Water temperature was nearly constant on 20°C±2 by air conditioning the laboratory. Fish feaces and uneaten food were siphoned and 80% of aquarium water was replaced daily with dechlorinated tap water. Fish were fed 3% of their body weight which determined by weighing fish bi weekly in order to adequate their weight increase.

Chemical analysis for experimental diets and Fish flesh analysis chemically before and after the experiment. Also chemical analysis of fish feaces was done following AOAC, 2001 procedures. Blood samples were taken from caudal vein of tested fish by plastic heparinized syringe (1.0 ml). Blood samples were tested according to Blaxhall and Daislly, 1973.

Hb-Estimation (Hb): According to Blaxhall and Daislly, 1973 Hb percentage has tested according to cyanomethaemoglobin method. Take 5 ml of Drabkins reagent mixed with 0.02 ml of blood for ten minutes and centrifuged to get rid of superannuates. Then read its absorbance at wave length 540 nanometer in spectrophotometer and calculate Hb percentage by the formula:

$$Hb\% = \frac{\text{Standard Hb conc.}}{1000} \times \text{Diluting factor} \times \frac{\text{Sample reader}}{\text{Standard Hb reader}}$$

(Brown, 1957).

Red Blood Cells Count (RBCs count): According to Blaxhall and Daislly, 1973 method by mixing 0.98 ml of modified dacies solution with 0.02 ml of blood. Then put a drop on haemocytometer chamber and covered with a cover slide. R.B.Cs counted microscopically in 5 small squares (from 25 small squares) then calculates R.B.Cs. number by formula:

Calculator No. of R.B.Cs. (N) x 2500 =
No. of RBCs cell / 100 ml of blood
(Varley *et al.*, 1980).

Packed Cell Volume (PCV): According to Blaxhall and Daislly, 1973 by filling a microhaematocrit a tube (at 7.5 m in length and 1.1-1.2 ml in diameter) with blood then closed with artificial clay and centrifuge for 5 min bispeed 1500 cycle/minute. Then read PCV% for each 100 ml of blood by haematocrit reader (Brown, 1957).

Differential count of WBCs: According to Blaxhall and Daislly, 1973, Differential count of WBCs has done by take a blood smear on slides then dry by air and fixed by methanol and stained it with Gimza stain to examine it microscopically by oil immersion lense (x100) and count 100 cells to calculate the percent age of each kind of WBCs. Growth parameters also studied as following:

Growth rate:

GR = W2 - W1 (G.R.) (gm / fish) ..... (Uten, 1978)

W2 = Final weight of fish
W1 = Initial weight of fish

Daily Growth Rate (DGR):

$$DGR = \frac{W2 - W1}{T} (\text{gm/fish/day}) \text{ ..... (Uten, 1978)}$$

T = Period of experiment (70 days)
W2 = Final weight of fish
W1 = Initial weight of fish

Relative Growth Rate (RGR) %:

$$RGR\% = \frac{W2 - W1}{W1} \times 100 \text{ ..... (Gerking, 1971)}$$

W2 = Final weight of fish
W1 = Initial weight of fish

Food Conversion Ratio (FCR):

$$FCR = \frac{R}{G} \text{ ..... (Gerking, 1971)}$$

R = Amount of food consumed by fish (gm)
G = Weight gain (gm).

Specific Growth Rate (SGR %):

$$SGR = \frac{LnW2 - LnW1}{T} \times 100 \ldots\ldots (\%gm/day) \ldots\ldots \text{(Brown, 1957)}$$

Ln W1 = Ln of Initial weight of fishes.
Ln W2 = Ln of final weight of fishes.
T = Days of experiment (70 days).

Food Conversion Efficiency (FCE) %:

$$\%FCE = \frac{G}{R} \times 100 \ldots\ldots\ldots\ldots \text{(Gerking, 1971)}$$

G = Weight gain (gm).
R = Amount of food consumed by fish (gm)

Protein Efficiency Rate (PER):

$$PER = \frac{G}{F} (gm/day) \ldots\ldots\ldots\ldots\ldots \text{(Donald } et\ al.\text{, 1976)}$$

F = Amount of consumed protein by fish (gm).
G = Weight gain (gm).

Productive Protein Value (PPV) %:

$$PPV = \frac{B_n - B_o}{I} \times 100 \ldots\ldots\ldots\ldots \text{(Farukawa and Tsukahara, 1966)}$$

B_n = % body protein of fish at end of experiment.
B_o = % body protein of fish at start of experiment.
I = Protein intakes through experiment (gm).

Apparent Protein Digestible Rate (APDR):

$$APDR = 100 - \left(\frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in feaces}} \times \frac{\%\ \text{protein in feaces}}{\%\ \text{protein in diet}} \times 100 \right) \ldots\ldots$$

(Maynard *et al.*, 1979).

Apparent Digestible Rate (ADR):

$$ADR = 100 - \left(\frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in feaces}} \times 100 \right) \ldots\ldots \text{(Maynard } et\ al.\text{, 1979)}$$

Apparent Fat Digestible Rate (AFDR):

$$AFDR = 100 - \left(\frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in feaces}} \times \frac{\%\ \text{fat in feaces}}{\%\ \text{fat in diet}} \times 100 \right) \ldots\ldots$$

(Maynard *et al.*, 1979).

Statistical analysis was carried out by using Complete Randomized Design (CRD) and SAS (2001) programme to analyzed data. Duncans Multiple Range test for significant differences of all fourth treatments at ($p \geq 0.05$) probability level was followed (Duncan, 1955).

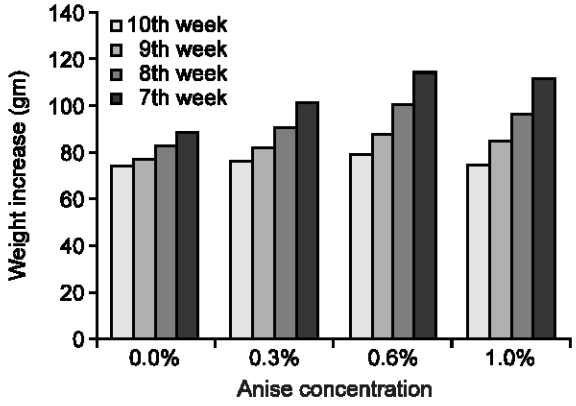

Fig. 1: Means weight increase at 7th-10th weeks of experiment for common carp fed various percentages of Anise

RESULTS AND DISCUSSION

Results showed that the addition of Anise to the common carp fingerlings feed promote the growth of the fish at various levels, where growth parameters was increased internally with increasing percentages of Anise. The most obvious increases of growth parameters cleared between 7th - 10th weeks of experiment as showed in Fig. 1.

Table 2 showed that there was no significant differences between T3 and T4 in GR (58.04 and 56.79 gm/fish) respectively, RGR % (102.91% and 103.047 respectively) FCR (2.35 and 2.28) respectively and FCE (42.8% and 45.59% respectively), SGR were (2.74%, 2.73%) respectively, whereas T3 and T4 differed significantly in DGR (0.83 and 0.81 gm/fish/day) respectively and PPV % (12.91% and 17.05) respectively, as showed in Fig. 2 and 3.

T1 significantly differs with all other treatments of studied growth parameters. ADR % results showed that T4 (74.52%) was the best and its percentage was far away from T2 (62.69) and T1 (54.36). Table 2 showed that APDR and AFDR were very close mathematically. Consequently, results showed that Anise addition can be a good growth promoter agent for common carp fingerlings, by stimulating stomach secretions and regulation of digestion (Al-Neamy, 2008). Many researchers mention that adding Anise to broilers diet gave the same results by increase body weight and FCE%, because Anise has antioxidant activity which preventing fatty acids oxidation and increase the benefits of fishes body from nutrient material as Al-Neamy (2008) mentioned and that fits our results in Table 3. In general weight increases referred to the ability of Anise to regulate absorption of metabolized amino acids across the intestinal wall to body tissues and cells and that agreed with the results of Isag and Ikuyo (1998).

Results of approximate analysis of fish flesh before and after period of experiment showed that the moisture

Table 2: Some physiological parameters of common carp fed various percentages of Anises

	Anise Conc.			
Phys. Pro.	T1 0.0%	T2 0.3%	T3 0.6%	T4 1.0%
Initial weight (gm/fish)	55.9	54.66	56.40	54.92
Final weight (gm/fish)	88.62	101.33	114.44	111.71
GR (gm/fish)	32.72±03.62c	46.67±05.01b	58.04±05.79a	56.79±03.07a
DGR (gm/fish/day)	0.46±00.04d	0.67±00.05c	0.83±00.07a	0.81±00.02b
SGR %	2.38±00.04b	2.60±00.05c	2.74±00.07a	2.73±00.02a
RGR %	58.53±19.37c	85.38±25.86b	102.91±25.79a	103.40±26.53a
FCR	3.94±01.20a	2.78±00.72b	2.35±00.32c	2.28±00.37c
FCE %	27.50±08.66c	38.07±10.41b	42.80±04.26a	45.59±07.54a
PER	1.26±00.40c	1.65±00.43b	1.84±00.15b	2.07±00.39a
PPV %	11.85±02.91b	10.45±01.98b	12.91±02.21b	17.05±03.58a
ADR %	57.36	62.69	74.19	74.52
APDR	89.31	92.14	96.09	96.98
AFDR	94.59	96.04	97.03	99.67

Different letters in table above means that there was a significant difference between treatments

Table 3: Approximate chemical analysis of common carp flesh fed various percentages of Anises

		Anise conc.			
Items	Before exp.	T1	T2	T3	T4
Moisture	77.92a	73.38b	73.33b	73.10b	72.34b
Protein	12.25b	15.36a	15.24a	16.28a	16.92a
Fat	5.05b	6.42a	6.42a	6.14a	6.23a
Ash	3.85c	3.67a	3.70a	3.26b	3.39b
Carbohydrates	0.93c	1.17b	1.31a	1.22a	1.12b

Different letters in table above means that there was a significant difference between treatments

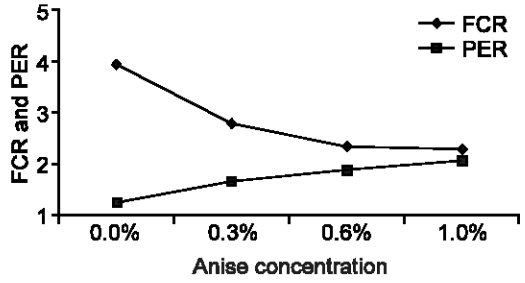

Fig. 2: FCR and PER values for common carp fed various percentages of Anise

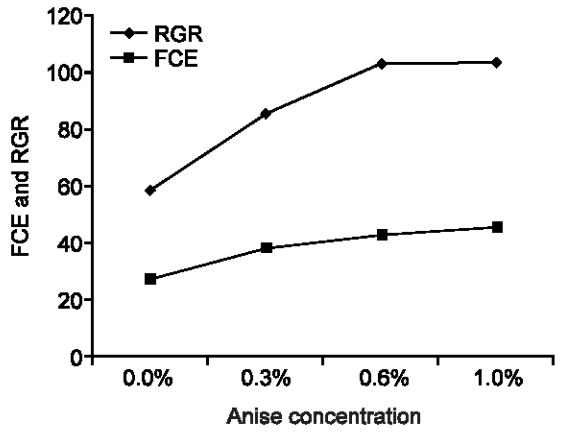

Fig. 3: FCE and RGR values for common carp fed various percentages of Anise

Table 4: Approximate chemical analysis of experimental diets

	Anise conc.			
Items	T1	T2	T3	T4
Moisture	4.08	3.78	3.71	3.79
Protein	22.78	22.03	22.72	22.04
Fat	7.49	8.59	8.20	7.97
Ash	7.58	7.19	7.08	7.40
Fibers	5.48	5.49	5.47	5.76
Carbohydrates	52.59	52.92	52.82	53.04
Energy (kcal)	409.07	416.31	416.14	411.21

Gross energy (kcal/100 gm) = (% pr. x 5.5) + (% fat x 9.1) + (% CHO x 4.1) .... (Tripathi and Shuklas, 1987)

decreased 77.92% to 72.34%, while fat levels and protein increased in T4 (6.23%, 16.92% respectively). Table 3 showed increases of protein and fat deposition and fat in fish flesh, as a result for increasing metabolized protein and fat, for Anise's role in fish diet. Anise added to broiler diet showed significantly increases in body weight, growth rate, food conversion ratio, food conversion efficiency (Al-Neamy, 2008).

Table 4 showed that experimental diets were isonitrogenous (protein percentages ranged 22.03-22.78%) and esocaloric energy (ranged 409.07-416.31 kcal/100 gm).

Blood parameters property increased within increasing anise additions to fish diet as showing in (Table 5). T3 and T4 showed significant differences in Hb (8.27, 8.9 gm/dL respectively). (RBCs) (2.75 x 10^6, 3.29 x 10^6 cell/ml respectively), Basophiles (0.15 (10^3, 0.14 x 10^3 cell/ml respectively), Eosinophiles (0.14 x 10^3, 0.13 x 10^3 cell/

Table 5: Some blood parameters of common carp food various percentages of Anise

	Anise Conc.			
Blood prop.	T1	T2	T3	T4
Hb (gm/dL)	6.51±0.39D	7.16±0.40c	8.27±0.54b	8.90±0.13a
PCV %	24.47±1.04B	25.01±0.67b	26.99±0.26a	27.71±0.30a
RBCs x 10^6 (cell/ml)	1.86±0.11C	2.55±0.23b	2.75±0.32b	3.29±0.30a
Lymphocyte x 10^3 (cell/ml)	47.20±0.27A	46.32±0.32b	45.91±0.90b	45.39±0.82c
Monocytes x 10^3 (cell/ml)	0.19±0.01A	0.18±0.01a	0.13±0.03b	0.15±0.01b
Neutrophiles x 10^3 (cell/ml)	5.77±0.36B	5.86±0.44b	5.80±0.21a	5.85±0.16a
Basophiles x 10^3 (cell/ml)	0.18±0.01A	0.16±0.02b	0.15±0.009b	0.14±0.004C
Eosinophiles x 10^3 (cell/ml)	0.18±0.03A	0.17±0.03b	0.14±0.02b	0.13±0.009C

Different small letters in table above means (presence of significant differences between results)

ml respectively) and lymphocytes (45.91 x 10^3, 45.39 x 10^3 cell/ml respectively), while PCV % (26.99%, 27.71% respectively) and Neutrophiles (5.80 x 10^3, 5.85 x 10^3 cell/ml respectively), which are showed no significant differences.

Observed differences between T3 and T4 related to anise contained Limonene and Fe in its structure which are affecting and activate blood circulation (Al-Neamy, 2008). Increases RBCs of fish fed anise, will increase Hb and PCV already because Anise activate spleen and liver (which are responsible for blood production in fish) to produce more blood proteins (Johnsson and Larrson, 1979). Karolazos *et al.* (2007) were proved that there is a connection between diet ingredients and blood picture of fish specially Hb and PCV. The differential count of (WBCs) was definitely best in T4 comparing to that of T1, T2 and T3 values in (Table 5) were higher. Granulomatous WBCs determine the availability of immunological system. Typically WBCs were lower in T4 which are stimulate the phagocytosis of any foreign bodies, bacteria viruses, parasites and even poisons by stimulation of cytokines secretion (Naji *et al.*, 2009). Counted lymphocytes revealed differences in their count among four treatments were T4 has the best level. On the other hand Monocytes showed lower count at higher percent of anise. Substantially T4 demonstrated lowest values of lymphocytes and Monocytes, Lymphocytes divided into B and T lymphocyte. They are granulomatous WBCs and taken as an indicator for immunological state of fishes (Bown, 2001). B-lymphocyte formed Antibodies (Ab) for all invasive pathogenic agents, which called immunoglobulin and symbolled Igs to form humeral immunity. T-lymphocyte maturated in thymus gland and formed cellular immunity by cytokines secretion that will stimulates macrophages, Eosinophiles, basophiles and Neutrophiles. Al-Neamy (2008) believed that anise stimulates immunological system which is noticed in results of this study. All results referred to anise role increasing immunological prognosis which developed all growth parameters. In AFDR results showed increase of digestible fat in tissues, that can be explained when there is high concentration of Fatty Acids (FAs) in blood and increasing lipoprotein and lipase to supply more fat deposition in body tissues (Naji *et al.*, 2009). Also An

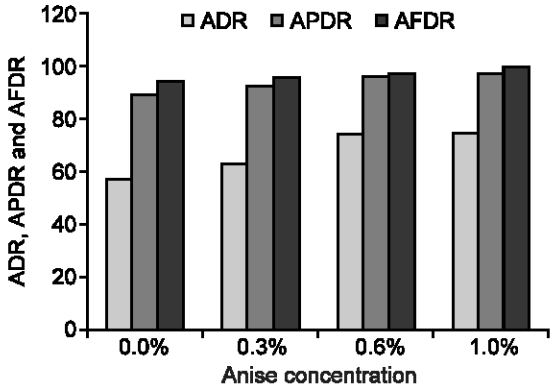

Fig. 4: ADR, APDR and AFDR values for common carp fed various percentages of Anise

interesting fact had detected, that (AAs) when increased in blood means increasing of PPV and APDR values which noticed in Fig. 4.

In pathological state the superiority of nutrient requirements of FAs and AAs will supply more to liver for producing more Igs and acute phase protein which are inhibit the invasive microbial growth (Ozean and Jean, 2006). At same time stimulating cytokines secretion from WBCs to regulate the function of immunological system with endocrine nodes and nervous system. The absence of infection in experimental fish related to continuous adding of anise into fish diet, which developed the immunity against pathogens and increase digestible fats and proteins which is clear in this study results. Naji *et al.* (2009) mentioned that there is antagonistic relationship between immunity and growth parameters, because when cytokines secreted from WBCs during infection will inhibit growth rates, which related to incomplete metabolism and physiological disorders during infection which are major reasons for at least 30% of growth rates decreases. A survey of the fourth individual treatments of fish fed various levels of anise showed developing in blood components and increases of growth rates within increase anise percent.

Abbreviations: AAs: Amino acids, FAs: Fatty acids, Ab: Antibodies.

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