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Strain-Specific and Sex-Specific Variation of Serum Biochemical Componensts of *Oreochromis niloticus*

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Abstract

The biochemical components in serum of four strains of *Oreochromis niloticus* were determined to study the strain-specific and sex-specific variations, including serum potassium, sodium, chlorine, calcium, cholesterol, urea nitrogen, total protein, albumin, globulin, lactate dehydrogenase, glutamic-oxaloacetic transaminase, alkaline phosphatase and α–amylase. Significant differences were observed among strains and between sexes in most of the biochemical components. These variations may be useful for the genetic characterisation of fish strains. The relationship between the biochemical components in serum and growth as well as feeding habits were discussed.

Introduction

A lot of studies have been done on fish hematology, but mostly dealt with interspecific differences between species and between hybrids and their parents. There are no particular reports on the strain-specific variation of serum biochemical components in fish. It was observed that the male tilapia grows faster than the female 16-47% (Li et al. 1997). It was also proven that

the GIFT strain of Nile tilapia has a better performance in growth, seinability and other aspects (Eknath et al. 1993, Li et al. 1997, 1999). The aim of this study was to reveal that there are inherent factors causing the above variation, and the possibility of utilizing these variations in genetic improvement. This paper reports the strain-specific and sex-specific variations in biochemical components in serum of four strains of Nile tilapia (*Oreochromis niloticus).*

Materials and Methods

Sources of fish

Four currently or potentially important strains of Nile tilapia were used in this study:

1. "GIFT" strain - genetically improved strain by ICLARM etc. Institutes (Eknath et al. 1993), introduced into China in 1994 from the Philippines.
2. "Egypt" strain - introduced into the Philippines from Egypt in 1992, introduced into China in 1994 from the Philippines.
3. "88" strain - introduced into China from Egypt in 1988, cultured in some locations in China.
4. "78" strain - introduced into China from Sudan in 1978, the most popular strain of tilapia in China.

Fish were sampled from the Huzhou Aquatic Seed Farm in the Zhejiang Province. Fifteen females and 15 males were taken from each strain respectively. The weights of "GIFT", "Egypt" , "88" and "78" strains were 195.6±12.4g, 140.2±8.6g, 176.8±11.3g and 181.5±13.1g respectively. The experimental fishes were healthy. Before draining off blood, all the fishes were cultured by strains in cages (2 x 2 m^2), set in a 2000 m^2 pond for 24 hours, and fed with compound food a ratio of 4% /day of body weight, to avoid possible changes of biochemical indexes in serum brought about by fishing and transportation.

Serum preparation

Blood was drawn from the caudal artery of live fish using a high-pressure sterilized syringe, then put into a scale centrifuge tube, laid obliquely and stored in a refrigerator at 4 ^{0}C. Four hours later, the blood samples were centrifuged for about 8 minutes at 4000rpm. Supernatants were put into 1.5ml plastic centrifuge tubes, and stored in liquid nitrogen.

Serum analysis

The contents of potassium, sodium, chlorine, calcium, cholesterol and urea nitrogen in serum were determined using Beckmen ASTRA–8 Automatic Stat/Routine Analyzer following the biochemical analytical instrument (Beckman Corp. produced). The activities of glutamic-oxaloacetic transami-

nase (GOT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), α–amylase and the content of total protein and albumin in serum were determined using Monarch-2000 Automatic Biochemical Analyzer following the analytical instrument (Monarch Corp. produced). Globulin content was obtained from the total protein content minus the albumin content.

Statistical analysis

Statistical significance of differences in biochemical components were carried out using t-test(Zar, 1974) and residual analysis(Neter & Wasserman, 1974) in terms of strain and sex.

Results

Differences between females and males

ELECTROLYTES

The contents of serum potassium and calcium among females were significantly higher than those in males in the four strains (P<0.05-0.01). There was a significant difference in chlorine between the females and the males in the “GIFT” and “78”strains but not in the “Egypt” and “88” strains. As for the serum sodium, there was no significant difference between the females and the males in the “GIFT”, “Egypt”, and “88” strains, but there was a slight diference in the “78” strain (Table (2).

CHOLESTEROL AND α–AMYLASE

There was no significant difference in the activity of serum α–amylase between females and males in the four strains (Table 3). The contents of serum cholesterol among females were higher (p<0.05-0.01) than those in males in all the strains.

PROTEINS

The contents of serum total protein, albumin, globulin and the ratio of albumin/globulin among females were significantly higher than those in males (P<0.05-0.01).

GOT AND UREA NITROGEN

The activity of serum GOT among females was lower than those in males in the four strains (Table 3). There was a significant difference in serum GOT activity between the females and the males of the “GIFT” and “Egypt” strains (P<0.01). The content of serum urea nitrogen among females was slightly higher than those in males in the four strains (Table 1). The difference

between females and males in all the strains except the "88" strain ($P<0.05$) was not significant.

LDH AND ALP

There was a significant difference ($P<0.05$) in the serum LDH activity between females and males in all the strains except in the "78" strain.

Table 1. Contents (mmol/l, mean±SD) of electrolytes, cholesterol and urea nitrogen in serum of four strains of Nile tilapia (the numbers in parentheses represent the number of fish analyzed).

Strain	Sex	Potassium	Sodium	Chlorine
"GIFT"	F	5.96±0.17(12)	155.6±6.1(12)	127.6±4.7(12)
"Egypt"	F	6.91±0.49(12)	156.3±6.8(12)	127.7±7.2(12)
"88"	F	5.26±1.11(12)	158.4±6.8(12)	132.5±6.8(12)
"78"	F	5.65±0.83(11)	150.8±1.9(11)	123.1±2.6(11)
Average	F	5.95	155.3	127.7
"GIFT"	M	3.19±0.61(12)	158.7±2.8(12)	134.9±3.9(12)
"Egypt"	M	4.47±0.46(12)	154.2±6.0(12)	131.9±6.2(12)
"88"	M	4.21±0.97(12)	156.1±3.3(12)	134.3±4.1(12)
"78"	M	1.09±0.75(11)	154.2±3.9(11)	133.1±4.4(11)
Average	M	3.24	155.8	133.6

Strain	Sex	Calcium	Cholesterol	Urea nitrogen
"GIFT"	F	4.15±0.17(12)	6.29±1.28(12)	52.1±3.2(12)
"Egypt"	F	4.45±1.01(12)	6.72±1.42(12)	46.8±3.5(12)
"88"	F	3.60±0.31(12)	7.56±1.44(12)	47.5±6.2(12)
"78"	F	4.92±0.44(11)	5.59±0.77(11)	40.8±1.7(11)
Average	F	4.28	6.54	46.8
"GIFT"	M	3.45±0.08(12)	5.36±0.67(12)	51.7±6.5(12)
"Egypt"	M	3.40±0.22(12)	4.87±0.60(12)	44.9±2.1(12)
"88"	M	3.32±0.16(12)	5.71±0.47(12)	43.6±1.6(12)
"78"	M	3.09±0.23(11)	5.01±0.92(11)	39.5±2.1(11)
Average	M	3.32	5.24	44.9

Table 2. Contents (g/l, mean±SD) of total proteins, albumin globulin and ratio of albumin/globulin in serum of four strains of Nile tilapia (the numbers in parentheses represent the number of fish analyzed).

Strain	Sex	Total proteins	Albumin	Globulin	Albumin/Globulin
"GIFT"	F	44.7±3.69(12)	12.4±0.56(12)	32.3±3.32(12)	0.38
"Egypt"	F	45.7±5.71(12)	14.8±2.18(12)	30.9±3.01(12)7	0.44
"88"	F	41.2±3.52(12)	11,8±1.46(12)	29.3±2.57(12)	0.49
"78"	F	37.4±2.24(11)	13.3±0.86(11)	24.8±1.35(11)4	0.51
Average	F	42.3	13.1	29.3	0.46
"GIFT"	M	33.3±2.73(12)	9.73±0.91(12)	23.9±1.82(12)	0.41
"Egypt"	M	33.8±3.14(12)	9.90±1.11(12)	23.8±2.06(12)	0.41
"88"	M	33.4±3.78(12)	9.73±0.69(12)	23.7±3.19(12)	0.41
"78"	M	28.1±2.12(11)	8.32±0.87(11)	19.8±3.27(11)	0.42
Average	M	32.2	9.42	22.8	0.41

Table 3. Activities (IU/l.mean±SD) of serum enzymes in four strains of Nile tilapia (the numbers in parentheses represent the number of fish analyzed).

Strain	Sex	GOT	LDH	ALP	α-amylase
"GIFT"	F	334±33(12)	2111±67(12)	43.3±3.79(12)	467±61(12)
"Egypt"	F	241±53(12)	1264±125(12)	52±5.66(12)	342±28(11)
"88"	F	270±10(12)	2357±161(12)	76.3±2.52(12)	361±46(12)
"78"	F	186±24(11)	995±103(11)	37±6.52(11)	272±38(12)
Average	F	258	1682	52.2	361
"GIFT"	M	378±17(12)	1465±153(12)	23±4.36(12)	489±55(12)
"Egypt"	M	357±90(12)	1453±59(12)	44±2.0(12)	363±21(12)
"88"	M	299±87(12)	1520±169(12)	29.5±4.95(12)	382±43(12)
"78"	M	192±36(11)	1109±183(11)	30.7±4.33(11)	284±35(11)
Average	M	307	1387	31.8	380

The serum ALP activity in females was much higher than in males of the four strains (Table 3).

Differences among strains, females only

ELECTROLYTES

The ranking of the serum potassium content among females in the four strains was "Egypt" > "GIFT" > "78" > "88" (Table 1; Fig.1). There was a significant difference ($P<0.05$-0.01) in the "GIFT" vs "Egypt", "Egypt" vs "88", "Egypt" vs "78", and "GIFT" vs "88" strains. But there was no significant difference in the "GIFT" vs "78", and "78" vs "88" strains.

The ranking of serum calcium content among females in the four strains was "78" > "Egypt" > "GIFT" > "88" (Table 1; Fig. 1). There was no significant difference in the "GIFT" vs "Egypt", "GIFT" vs "88", and "Egypt" vs "88" strains unlike in the "GIFT" vs "78", "Egypt" vs "78" ($P<0.05$), and "88" vs "78" strains ($P<0.05$-0.01).

CHOLESTEROL AND α–AMYLASE

The ranking of the activity of serum α–amylase among females in the four strains was "GIFT" > "88" > "Egypt" > "78" (Table 3; Fig. 3). There was a highly significant difference ($P<0.01$) among all the strains except in the "Egypt" vs "88" strain.

The ranking of serum cholesterol content among females in the four strains was "88" > "Egypt" > "GIFT" > "78" (Table 1; Fig. 1). There was a significant difference in the "GIFT" vs "88", "Egypt" vs "78", and "78" vs "88" strains ($P<0.05$-0.01), but there was no significant difference in the "GIFT" vs "Egypt", "GIFT" vs "78", and "Egypt" vs "88" strains.

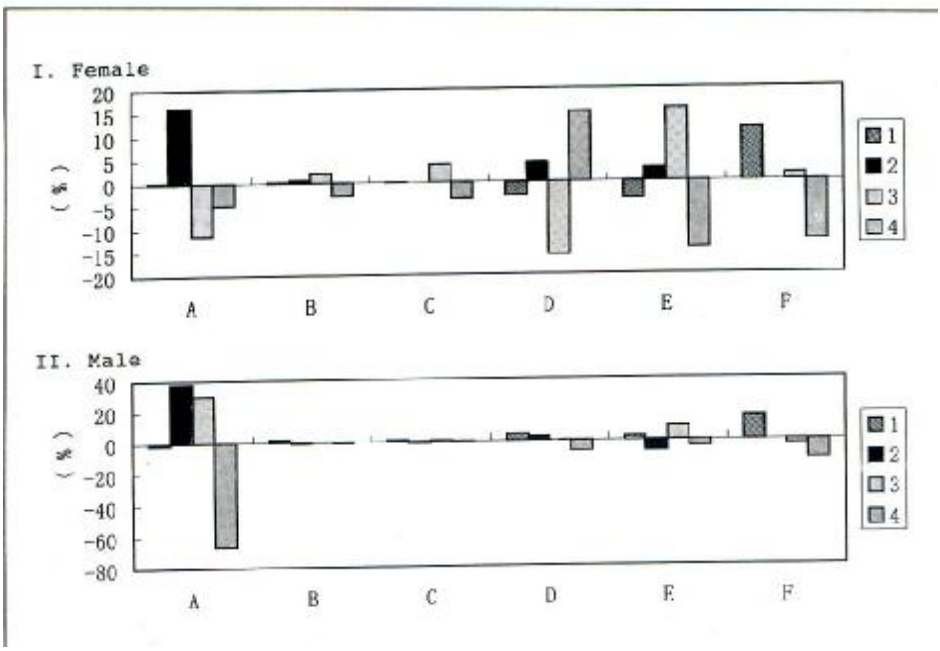

Fig.1. Comparison of contents of potassium, sodium, chlorine, calcium, cholesterol and urea nitrogen in serum among four strains of Nile tilapia (*Oreochromis niloticus*).
I. Female; II. Male
1. "GIFT" strain; 2. "Egypt" strain; 3. "88" strain; 4: "78" strain
A. Potassium; B. Sodium; C. Chlorine; D. Calcium; E. Cholesterol; F. Urea nitrogen

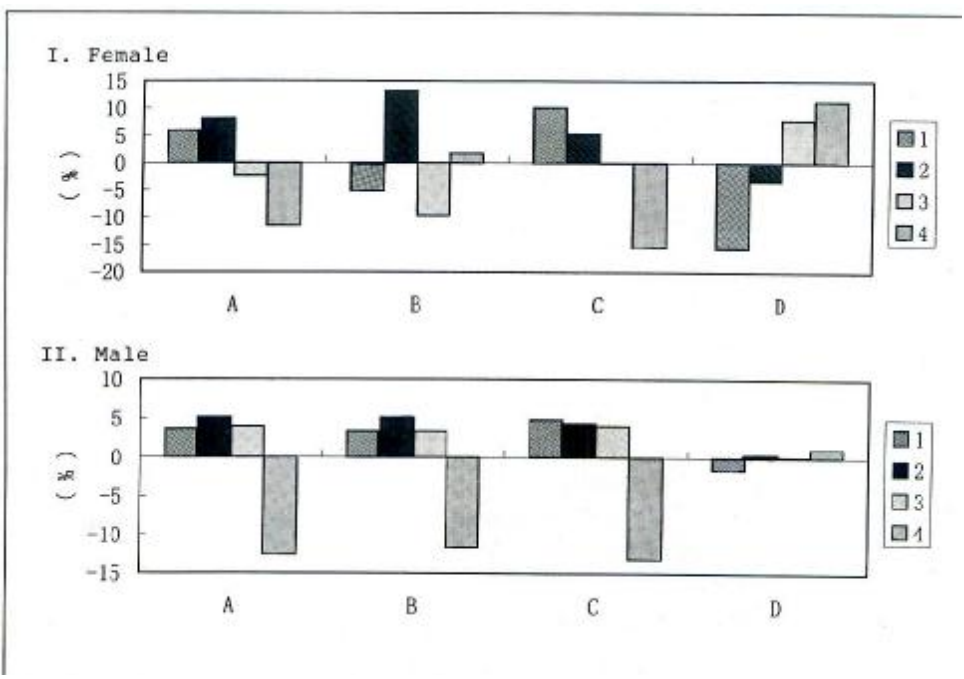

Fig. 2. Comparison of contents of total proteins, albumin, globulin and the ratio of albumin to globulin in serum among the four strains of Nile tilapia (*Oreochromis niloticus*).
I. Female; II. Male
1. "GIFT" strain; 2. "Egypt" strain; 3. "88" strain; 4. "78" strain
A. Total proteins; B. Albumin; C. Globulin; D. Ratio of albumin/globulin

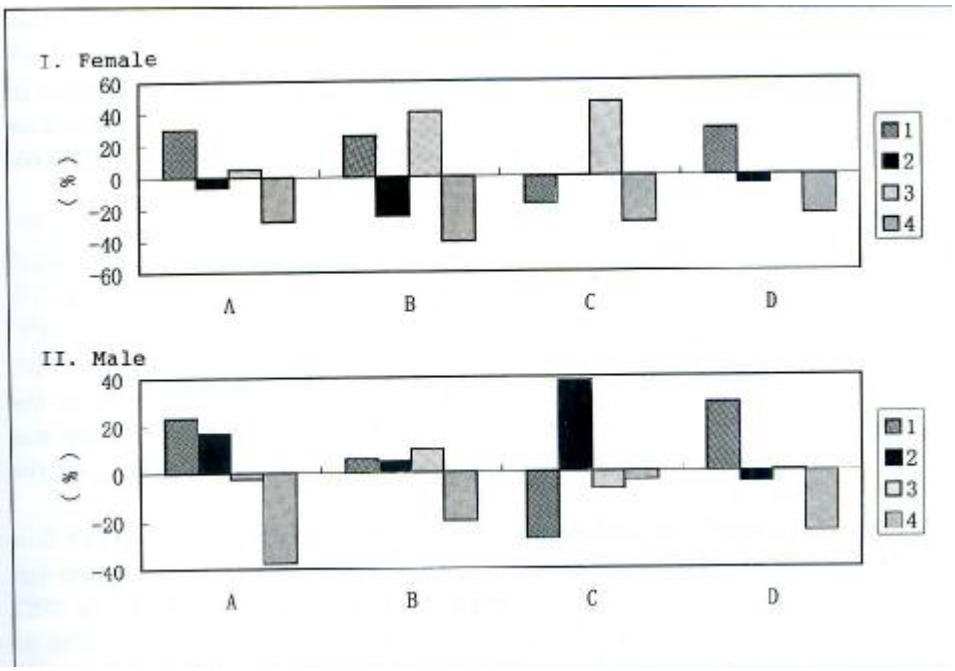

Fig. 3. Comparison of activities of serum enzymes among four strains of Nile tilapia (*Oreochromis niloticus*).
I. Female; II. Male
1. "GIFT" strain; 2. "Egypt" strain; 3. "88" strain; 4. "78" strain
A. Glutamic-oxaloacetic transaminase(GOT); B. Lactate dehydrogenase(LDH); C. Alkaline phosphatase(ALP); D. α-amylase

PROTEINS

The ranking of the contents of total protein, albumin and globulin among females in the four strains was "Egypt" > "GIFT" > "88" > "78"; "Egypt" > "78" > "GIFT" > "88", and "GIFT" > "Egypt" > "88" > "78" strains respectively (Table 2; Fig. 2).

There was a significant difference ($P<0.05$-0.01) in serum total protein contents in all strains except in the "GIFT" vs "Egypt" strain, while there was a significant difference ($P<0.05$-0.01) in the contents of albumin and the ratio of albumin/globulin in all the strains except in the "GIFT" vs "88" strain. There was a significant difference in the serum globulin contents in all the strains except in the "GIFT" vs "Egypt", and "Egypt" vs "88" strains.

GOT AND UREA NITROGEN

The ranking of the serum GOT activity and the content of serum urea nitrogen among females in the four strains was "GIFT" > "88" > "Egypt" > "78" (Table 3, 1; Fig.3, 1). There was a significant difference ($P<0.05$-0.01) in the four strains, except in the "Egypt" vs "88" strain, in the activity of serum GOT and the content of serum urea nitrogen.

LDH AND ALP

The ranking of the activities of serum LDH and ALP among females in the four strains was “88” > “GIFT” > “Egypt” > “78”, and “88” > “Egypt” > “GIFT” > “78” respectively (Table 3; Fig.3) with a significant difference (P<0.05-0.01).

Differences among strains, males only

ELECTROLYTES

The ranking of the content of serum potassium among males in the four strains was “Egypt” > “88” > “GIFT” > “78”(Table 1; Fig.1). There was a significant difference (P<0.05-0.01) in the four strains, except in the “Egypt” vs “88” strain.

The ranking of the content of serum calcium among males in the four strains was “78”> “GIFT” > “Egypt” > “88” (Table 1;Fig.1). There was a significant difference (P<0.05-0.01) in the “GIFT” vs “88”, “GIFT” vs “78”, “GIFT” vs “Egypt”, “Egypt” vs “78”, and “88” vs “78” strains. There was no significant difference between each two strains in males of Nile tilapia in the content of serum sodium and serum chlorine content (Table 1; Fig. 1).

CHOLESTEROL AND α–AMYLASE

The ranking of the activity of serum α–amylase and the content of cholesterol among males in the four strains of Nile tilapia was “GIFT” > “88” > “Egypt” > “78” (Table 3; Fig. 1), and “88” > “GIFT” > “78” > “Egypt” strain (Table 1; Fig. 3). The differences in the activity of serum α–amylase among the four strains was significant except in the “Egypt” vs “88” strain. There was no significant difference in the content of serum cholesterol among the four strains, except in the “Egypt” vs “88” (P<0.01), and “88” vs “78”(P<0.05) strains.

PROTEINS

There were no significant differences among the “Egypt”, “GIFT” and “88” strains in total protein, albumin and globulin contents. But, they were all significantly higher than that of the “78” strain (Table 2; Fig.2). There was no significant difference among the four strains in the ratio of albumin/globulin.

GOT AND UREA NITROGEN

The ranking of the activity of serum GOT and the content of urea nitrogen among males in the four strains was “GIFT” > “Egypt” > “88” > “78” (Table 3,1; Fig.3, 1). There were significant differences among the four strains, except in the “Egypt” vs “GIFT”, and “Egypt” vs “88” strains in GOT

activity. There were significant differences among the four strains except in the “Egypt” vs “88” strain in content of urea nitrogen.

LDH AND ALP

There was no significant difference among the “GIFT”, “Egypt” and “88” strains in males in serum LDH activity. But, they were all significantly higher than those in the “78” strain (Table 3; Fig.3). The ranking of serum ALP activity among males in the four strains was “Egypt”>”78" > “88”>”GIFT”. There were significant differences among the four strains, except in the “78” vs “88” strain.

Discussion

It is well known that the contents of potassium, sodium, chlorine and calcium play a significant role in the balance of electrolytes and osmotic pressure of the internal milieu. The appropriate ratio of sodium/electrolytes (potassium, calcium) maintains the normal neuron-muscular response (Shanghai First Medical College, 1979). This research showed that there were strain-specific and sex-specific variations among the four strains of Nile tilapia in the contents of serum electrolyte. This variation is one of the factors of sex and strain differentiations in the metabolism of tilapia.

It is also known that α–amylase acts at the internal glucosidic bonds of the starch chains. The significant difference between sexes in serum components except in the activity of α–amylase indicates a similarity in feeding habits between the females and males of Nile tilapia. On the other hand, regardless of sex, the activity of serum α–amylase in the “GIFT” strain is the highest among the four strains. This may explain the fast growth of the “GIFT strain (Li et al. 1996).

The cholesterol is an essential material in the synthesis of some enzymes and hormones. The content of serum cholesterol is related to liver metabolism. Among human beings, not only does the content of serum cholesterol differ between sexes, but also in the feeding habits (Shanghai Medical Analyzing Institute, 1984). This study revealed that the differences between sexes and among strains exist in the content of serum cholesterol, which was higher among females than in males. The above considerations coupled with the significant differences between females and males in the contents of serum of total protein, albumin and globulin, represent a variation in metabolism, e.g., synthesis of serum proteins of liver between females and males.

GOT is one of the two important transaminases. It can catalyze the amino-transportation between glutamic acid and oxaloacetic acid. The activity of serum GOT is probably relative to growth of fish. The higher the activity of serum GOT, the faster the fish grows (Lou et al. and 1995). The activity of serum GOT of the male tilapia is higher than that of the female, which is one of the physiological factors causing males to grow faster than

females. The study showed that the activity of serum GOT is highest in the "GIFT" strain and lowest in the "78" strain. This probably is one of the indicators of fast growth in the "GIFT" strain.

The contents of serum urea nitrogen in the four strains of Nile tilapia are all low. It may be related to the discharge mechanism of fish. Among crucian carp, the discharging ammonia is 73% of the total contents of nitrogen waste, in common carp it is 60%(Li et al. 1984). However, there are differences in sex and strain in the content of urea nitrogen, which is highest in the "GIFT" strain and lowest in the "78" strain. This may indicate an efficient metabolism of amino acid in the "GIFT" strain.

LDH is a glycolytic enzyme, which with the aid of coenzyme I, can turn lactic acid into pyruvic acid by dehydrogenation. ALP is a non-specific enzyme which catalyzes the hydrolysis of organic monophosphate ester (Qi, 1988). Serum LDH and ALP are related to metabolism. This study showed that there are differences in the enzyme activities between sexes and among strains.

It is known that the normal values of blood components have genetic and physiological variations. The genetic variation may due to interspecific factors between species and intraspecific within species. The physiological variations may be caused by age, sex and nutritional aspects. In this study, four strains of tilapia were of the same age and were sampled from the same culture environment. Therefore, the observed variations of serum biochemical components may reflect the genetic variations in nature.

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