Original Article

Effect of Water Temperature on Hematology and Virulence of *Aeromonas hydrophila* in Hybrid Catfish (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther)

Weena Koeypudsa* Malinee Jongjareanjai

Abstract

Water temperature was set up at 2 levels; high (Ht, 29.5±0.5°C) and low temperature (Lt, 19.5±0.5°C). Experimental catfish body weight and total length were 12.07±1.41 g and 11.57±0.62 cm respectively. Catfish were divided into 4 groups: high temperature with 0.1% sodium chloride (NaCl) (HtWs), high temperature without 0.1% NaCl (HtW/s), low temperature with 0.1% NaCl (LtWs), and low temperature without 0.1% NaCl (LtW/s). Catfish hematocrit (Hct) and clinical chemistry including serum glutamic oxaloacetic transminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine (Cr) were measured. The results showed that water temperature, Ht (HtWs and HtW/s) and Lt (LtWs and LtW/s), disturbed homeostasis. The Hct values showed that fish from LtW/s and LtWs group potentially were anemic fish much more than fish from HtWs and HtW/s group. The BUN to Cr ratio reveals that all fish in HtWs and LtWs group were dehydrated when compared to fish in HtW/s and LtW/s group. Ten isolates *Aeromonas hydrophila* (*A. hydrophila*) were intra-peritoneal injection to catfish which exposed to HtW/s and LtW/s. All *A. hydrophila* were isolated from kidney and liver of sick fresh-water fish in Thailand from 2005 to 2008. The virulence of *A. hydrophila* to catfish exposed to LtW/s and HtW/s were 100% and 40% respectively. The results demonstrated water temperatures play a major role to catfish hematology and *A. hydrophila* virulent levels. The evaluation of blood chemistry parameters provide a baseline and as a tool in fish health management.

Keywords: *Aeromonas hydrophila,* fish blood, fish health, hybrid catfish, virulence, water temperature

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บทคัดย่อ

ผลของอุณหภูมิน้ำต่อค่าโลหิตวิทยาและความรุนแรงของ *Aeromonas hydrophila* ในปลาดุกบิ๊กอุย (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther)

วีณา เคยพุดซา* มาลินี จงเจริญใจ

งานวิจัยนี้แบ่งอุณหภูมิน้ำออกเป็น 2 ระดับคืออุณหภูมิสูง (Ht, 29.5±0.5 ^{O}ซ) และอุณหภูมิต่ำ (Lt, 19.5± 0.5 ^{O}ซ.) ปลาดุกน้ำหนัก 12.07±1.41 กรัม ความยาว 11.57±0.62 ซม. แบ่งออกเป็น 4 กลุ่มดังนี้ เลี้ยงในน้ำที่มีอุณหภูมิสูงและมี 0.1% NaCl (HtWs) น้ำที่มีอุณหภูมิสูงและไม่มี 0.1% NaCl (HtW/s) น้ำที่มีอุณหภูมิต่ำและมี 0.1% NaCl (LtWs) และน้ำที่มีอุณหภูมิต่ำและไม่มี 0.1% NaCl (LtW/s) ปลาจะถูกเจาะเลือดเพื่อศึกษาค่าทางโลหิตวิทยาดังนี้ ค่าเม็ดเลือดแดงอัดแน่น (Hct), serum glutamic oxaloacetic transminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) และ creatinine (Cr) ผลการทดลองพบว่าปลาที่เลี้ยงในน้ำ Ht และ Lt ทั้ง 4 กลุ่ม (HtWs, HtW/s, LtWs และ LtW/s) มีค่าทางโลหิตวิทยาที่เปลี่ยนแปลง อันเนื่องมาจากการปรับสมดุลของร่างกายสัตว์ แต่การเจริญเติบโตของร่างกายเป็นไปตามปกติ ค่า Hct ของปลาในกลุ่ม LtW/s และ LtWs มีแนวโน้มเป็นโรคเลือดจางมากกว่าปลาในกลุ่ม HtWs และ HtW/s อัตราส่วนของ BUN/Cr แสดงให้เห็นว่าปลาในกลุ่ม HtWs และ LtWs เกิดภาวะขาดน้ำเมื่อเทียบกับปลาในกลุ่ม HtW/s และ LtW/s สำหรับการศึกษาระดับความรุนแรงของ *Aeromonas hydrophila* (*A. hydrophila*) ในปลาดุกนั้น ใช้ *A. hydrophila* ที่แยกได้จากตับและไตของปลาน้ำจืดป่วยในประเทศไทยระหว่างปี 2005-2008 จำนวน 10 isolations ฉีดเข้าช่องท้องปลาที่เลี้ยงในน้ำ LtW/s และHtW/s ผลการทดลองพบว่าระดับความรุนแรงของ *A. hydrophila* ในปลาดุกที่เลี้ยงในน้ำ LtW/s และHtW/s คือ 100% และ 40% ตามลำดับ จากผลการทดลองทั้งหมดนี้จะพบว่าอุณหภูมิน้ำมีความสำคัญยิ่งต่อค่าโลหิตวิทยาและระดับความรุนแรงของ *A. hydrophila* ในปลาดุก การแปรผลค่าทางโลหิตวิทยาสามารถนำไปใช้ในการประเมินสุขภาพสัตว์น้ำได้

คำสำคัญ: *Aeromonas hydrophila* เลือดปลา สุขภาพปลา ปลาดุกบิ๊กอุย ความรุนแรง อุณหภูมิน้ำ

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Introduction

Catfish is poikilothermic animal, which is environmental temperature dependent. This results in temperature coefficient (*Q10*) values of 2-3 for the rate of metabolism (Carlson et al., 1995; Taylor et al., 1999). Temperature is also known to have strong influences on enzyme reaction, growth efficiency, reproduction and immune response in fish (Tanck et al., 2000). Fish immune functions have important on the progression of infectious diseases. *Aeromonas hydrophila* (*A. hydrophila*), plays opportunistic bacteria in catfish. The deterioration of self-defense mechanism leads to vulnerable fish.

Sodium chloride (NaCl) or salt is commonly used in aquaculture for microbiological control, i.e. bacteria, parasite and fungus. In addition, salt is applied to improve fish survival during transportation (Velasco-Santamaria and Cruz-Casallas, 2008). Harpaz et al. (2005) further stated that addition 4% salt to the fish diet lead to better feed utilization. Surplus 0.1% salt to water is recommended for fresh-water fish as stress reducing from low temperature (Koeypudsa and Kitkamthorn, 2009).

Wells et al. (1986) addressed that circulating blood is accounted for 3-6% fish body weight. Fish blood is usually taken via caudal vein. Blood chemistry evaluations have been used to assess the health status of most animal (Carvalho and Fernandes, 2006). For example, serum glutamic oxaloacetic transminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are associated with hepatic injury, acute injuries in trunk kidney, bacterial infection and myocardial infarction (Chen et al., 2004). Wells et al. (1986) reported that SGOT increments have a high activity in both fish gills and in cardiac tissue. Alkaline phosphatase (ALP) gives information about liver dysfunction (Wells et al., 1986). Blood urea nitrogen (BUN) and creatinine (Cr) are related to

kidney lesions (Chen et al., 2004; Trumble et al., 2006). Hematocrit (Hct) is often interpreted as general health (Trumble et al., 2006). Hence, hematological changes may reflect vital organs function, internal organ equilibrium and homeostasis.

The aims of this work focused on hematological changes and somatic growth when fish exposed high and low temperature, with and without sodium chloride. Furthermore, virulent levels of *A. hydrophila* on catfish were also investigated. The results of this study lead to aquatic animal health assessment.

Materials and Methods

Hybrid catfish (Clarias gariepinus Burchell x C. macrocephalus Gunther): Hybrid catfish (*C. gariepinus* Burchell x *C. macrocephalus* Gunther) were purchased from private farm at Supanburi Province and were acclimatized in laboratory for 15 days. Fish weight and total length were 12.07±1.41 g and 11.57±0.62 cm, respectively. Water change was done 10% every morning. Catfish were fed with commercial pellets (36% protein) at a rate of 1.0% body weight each afternoon. All experimental fish were fasted for 1 day before starting experiment and blood sampling. All fish were only one time practiced.

Experimental design

Water temperature in this experiment was set up at 2 levels; high (Ht, 29.5±0.5°C) and low temperature (Lt, 19.5±0.5°C). Each two hundred catfish was conducted into 2 parts, 1 day and 30 days experiment. Water characteristics analyzed from each experimental group as shown in Table 1.

The first part was 1 day experiment, 200 catfish. Four experimental groups were set up as following: high temperature with 0.1% NaCl (HtWs), high temperature without 0.1% NaCl (HtW/s), low temperature with 0.1% NaCl (LtWs), and low temperature without 0.1% NaCl (LtW/s). Each experiment was comprised of 5 replicates. One replicate was 10-fish per 60 l-glass aquarium. One catfish was randomly selected from each replicate at 0 min, 15 min, 45 min, 2 hrs, 4 hrs, 6 hrs and 24 hrs.

The second part was 30 days experiment, 200 catfish. Four experimental groups were conducted as same as the first part, HtWs, HtW/s, LtWs, and LtW/s. Each experiment had 5 replicates, 10-fish per 60 l-glass aquarium. One catfish was randomized from each replicate at 0, 1, 5, 10, 15, 20 and 30-day. Fish body weight and total length were also recorded at the same time of blood drawing.

Table 1 Water quality parameters in experimental groups.

Parameters	Unit	HtW/s	HtWs	LtW/s	LtWs
pH		8.00	8.10	7.80	7.90
Dissolved Oxygen	mg/l	7.00	7.00	7.30	7.50
Air Temperature	°C	31.00	31.00	21.00	21.00
Water Temperature	°C	29.00	30.00	20.00	19.00
Alkalinity	mg/l	78.00	92.00	144.00	150.00
Hardness	mg/l	119.00	595.00	144.00	680.00
Ammonia	mg/l	0.07	0.24	0.55	0.75
Nitrite	mg/l	1.60	1.50	1.80	1.10
Nitrate	mg/l	0.33	0.44	0.44	0.31
Salinity	g/kg	0.00	1.00	0.00	1.00
Osmolarity	osmol/l	0.00	0.14	0.00	0.14

HtWs: high temperature with 0.1% NaCl, HtW/s: high temperature without 0.1% NaCl, LtWs: low temperature with 0.1% NaCl, LtW/s: low temperature without 0.1% NaCl.

Hematological analysis: Blood was drawn from caudal vein of tranquilized fish, which is exposed to 5 mg/l clove oil. Hematocrit (Hct) values were obtained by centrifuging samples in 75 µl microhematology tubes (Hematology centrifuge, SR10000, Thailand). Plasma enzyme activities were measured by automatic analyzer (BT 1000/2000 Plus, Biotecnica instrument, Italy). These included serum glutamic oxaloacetic transminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine (Cr).

Virulence of Aeromonas hydrophila (A. hydrophila)

Catfish were divided into 4 groups as following: 2 control groups (NsLtW/s and NsHtW/s) and 2 treatment groups (AhLtW/s and AhHtW/s). Each group composed of 5 replicates. Each replicate had 10 fish stocked in 60-l glass aquarium.

Ten isolates *A. hydrophila* were selected (Table 2). All isolates, one by one, were intraperitoneal injection to catfish in treatment groups, (AhLtW/s and AhHtW/s). Control fish, (NsLtW/s and NsHtW/s), were injected with 0.1 ml sterile saline water. PROBIT analysis was applied to calculate number of *A. hydrophila* that can kill 50% of catfish (median lethal dose, LD_{50}) at 24 hrs (Perera et al., 1997).

The LD50 is used to indicate the degree of virulence (Angka et al., 1995). The virulent levels of *A. hydrophila* on catfish at 24 hrs were defined as follows. If LD_{50} is between $1.0x10^0$-$9.9x10^2$ colony forming unit/ml (cfu/ml) as severely virulent, values between $1.0x10^3$-$9.9x10^5$ cfu/ml as strongly virulent, and values $1.0x10^6$-$9.9x10^8$ cfu/ml as virulent, values between $1.0x10^9$-$9.9x10^{11}$ cfu/ml as weakly virulent, and values between $1.0x10^{12}$-$9.9x10^{14}$ cfu/ml as very weakly virulent.

Table 2 Ten isolates of experimental bacteria (*A. hydrophila*).

Isolates	Fish	Organ	Province	Year
AH-51	Black tilapia	Liver	Chachoengsao	2005
AH-62	Fancy carp	Kidney	Bangkok	2006
AH-63	Hybrid catfish	Liver	Roi-ed	2006
AH-64	Hybrid catfish	Kidney	Roi-ed	2006
AH-82	Red tilapia	Liver	Chachoengsao	2008
AH-83	Red tilapia	Kidney	Chainart	2008
AH-84	Black tilapia	Kidney	Angthong	2008
AH-85	Black tilapia	Kidney	Nongkhai	2008
AH-86	Black tilapia	Kidney	Pracheenburi	2008
AH-87	Black tilapia	Kidney	Ubonratchathani	2008

Results

Figure 1 showed the values of fish hematology for 1-day exposure among HtWs, HtW/s, LtWs and LtW/s groups. The means of SGOT, SGPT, BUN and ALP values were statistical significance ($p \leq 0.001$). No significant change in Hct and Cr were found.

Figure 2 showed the trend lines direction for 30-day exposure. All groups, trend lines of SGOT and SGPT were increased. The tentative of BUN trend lines were decline in HtWs, LtWs and LtW/s groups. For ALP, the direction of trend lines in HtWs and LtWs groups were dropped. The BUN to Cr ratio were no significant different for 30-day exposure in HtW/s, LtWs and LtW/s groups. The values in Ws groups were higher than values in W/s groups.

Growth rate was increasing all experiments, both body weight and total length (Figure 3). Degree of virulence between LtW/s and HtW/s groups were 100% and 40%, respectively (Table 4).

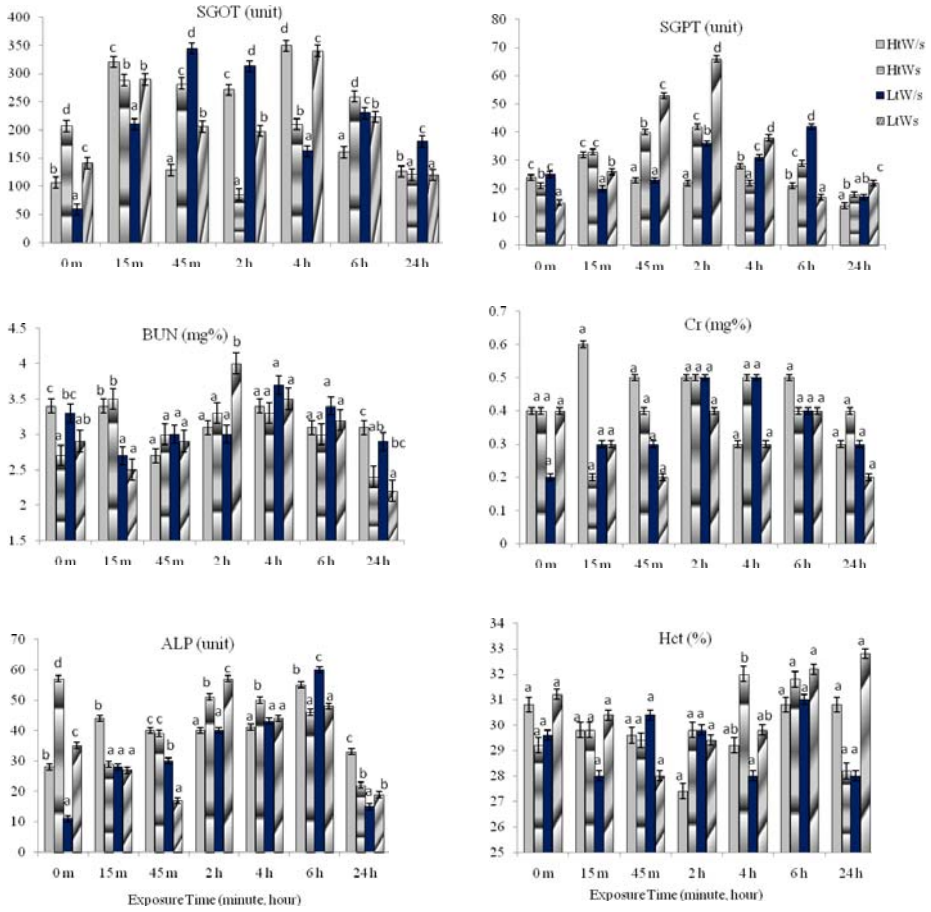

Figure 1 Fish hematology (mean of 5 replications) among 4 experimental groups for 1 day experiment. Different letters indicate statistical significance ($p \leq 0.001$)

SGOT: serum glutamic oxaloacetic transminase, SGPT: serum glutamic pyruvic transaminase, BUN: blood urea nitrogen, Cr: creatinine, ALP: alkaline phosphatase, Hct: hematocrit, HtWs: high temperature with 0.1% NaCl, HtW/s: high temperature without 0.1% NaCl, LtWs; low temperature with 0.1% NaCl, LtW/s: low temperature without 0.1% NaCl.

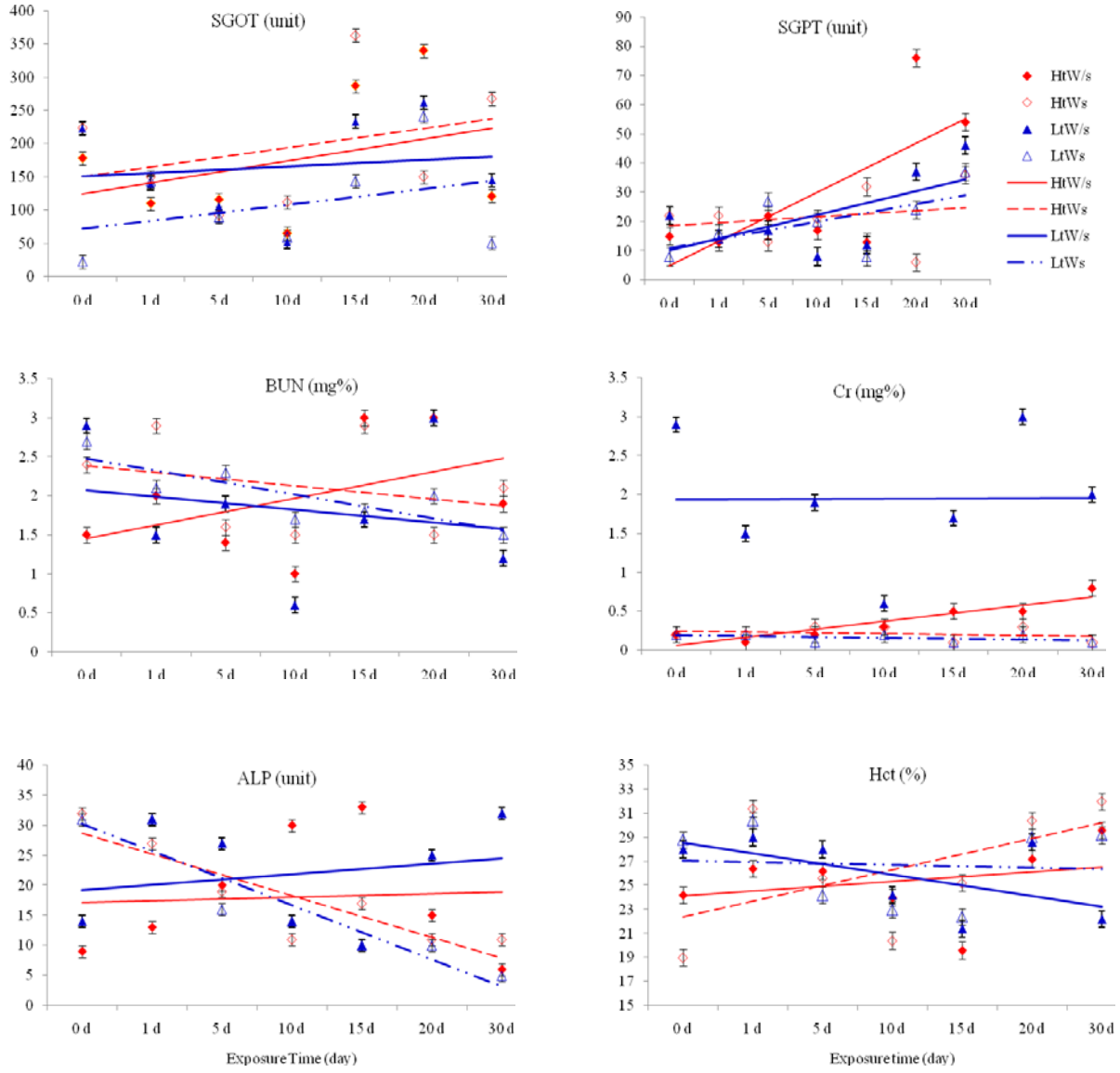

Figure 2 Fish hematology (means of 5 replications) among 4 experimental groups for 30 days experiment.

SGOT: serum glutamic oxaloacetic transminase, SGPT: serum glutamic pyruvic transaminase, BUN: blood urea nitrogen, Cr: creatinine, ALP: alkaline phosphatase, Hct: hematocrit, HtWs: high temperature with 0.1% NaCl, HtW/s: high temperature without 0.1% NaCl, LtWs; low temperature with 0.1% NaCl, LtW/s: low temperature without 0.1% NaCl.

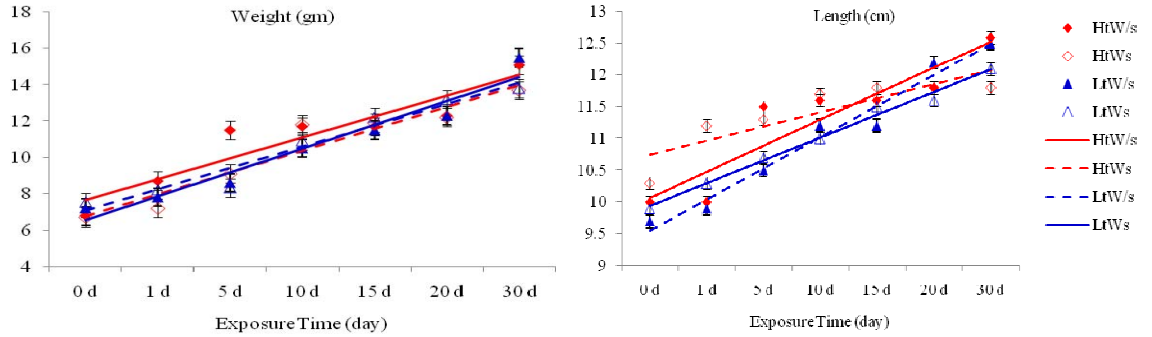

Figure 3 Somatic growths (means of 5 replications) among 4 experimental groups for 30 days

HtWs: high temperature with 0.1% NaCl, HtW/s: high temperature without 0.1% NaCl, LtWs: low temperature with 0.1% NaCl, LtW/s: low temperature without 0.1% NaCl.

Table 3 BUN/Cr ratio (mean of 5 replications ± SD) among 4 experimental groups for 30 days experiment.

Exposure Time (day)	Experimental groups			
	HtW/s	HtWs	LtW/s	LtWs
0	10.90±5.70[a]	15.09±9.84[a,b]	1.05±0.26[a]	19.06±10.99[a]
1	16.70±13.06[a]	19.26±11.56[a]	1.07±0.31[a]	10.75±5.51[a]
5	10.70±5.14[a]	6.13±2.18[a]	1.04±0.20[a]	21.00±5.83[a]
10	4.58±3.67[a]	6.15±2.72[a]	1.01±0.57[a]	10.53±6.86[a]
15	7.72±4.64[a]	23.5±8.66[b]	0.99±0.12[a]	15.90±4.12[a]
20	6.16±1.03[a]	8.7±6.32[a,b]	0.99±0.09[a]	14.90±7.89[a]
30	2.52±0.60[a]	17.1±6.24[a,b]	0.58±0.15[a]	13.90±4.85[a]

Different letters at the same column indicate statistical significance ($p \leq 0.001$).
HtWs: high temperature with 0.1% NaCl, HtW/s: high temperature without 0.1% NaCl, LtWs: low temperature with 0.1% NaCl, LtW/s: low temperature without 0.1% NaCl.

Table 4 Virulent levels of *A. hydrophila* on catfish when exposed to high temperature without 0.1% sodium chloride (HtW/s), and low temperature without 0.1% sodium chloride (LtW/s).

Isolates	Bacterial Concentrations (cfu/ml)		Virulent levels*	
	HtW/s	LtW/s	HtW/s	LtW/s
AH-51	$3.83x10^9$	$2.06x10^8$	Weakly virulent	Virulent
AH-62	$5.93x10^9$	$4.56x10^8$	Weakly virulent	Virulent
AH-63	$2.02x10^9$	$1.39x10^8$	Weakly virulent	Virulent
AH-64	$5.29x10^9$	$7.39x10^8$	Weakly virulent	Virulent
AH-82	$8.16x10^8$	$4.07x10^7$	Virulent	Virulent
AH-83	$8.31x10^8$	$1.84x10^7$	Virulent	Virulent
AH-84	$2.65x10^9$	$1.21x10^8$	Weakly virulent	Virulent
AH-85	$4.52x10^8$	$2.63x10^7$	Virulent	Virulent
AH-86	$1.60x10^7$	$1.96x10^6$	Virulent	Virulent
AH-87	$3.30x10^9$	$1.90x10^8$	Weakly virulent	Virulent

* Levels	Bacterial concentrations (cfu/ml)	Interpretation
1	$1.0x10^0$-$9.9x10^2$	Severely virulent
2	$1.0x10^3$-$9.9x10^5$	Strongly virulent
3	$1.0x10^6$-$9.9x10^8$	Virulent
4	$1.0x10^9$-$9.9x10^{11}$	Weakly virulent
5	$1.0x10^{12}$-$9.9x10^{14}$	Very weakly virulent

Discussion

Catfish is warm water fish (Carlson et al., 1995) and optimal temperature range for growth is 28-32°C (Buentello et al., 2000). Water quality in term of temperature (Table 1, 29.5±0.5°C) is acceptable range for this specie. At low temperature (19.5±0.5°C), growth rate also increased because fish was well-adapt to environmental changes. The growth increments, weight and length, were used as an indicator of whole organism level performance (Taylor and Miller, 2001).

Water temperature is known to be an important regulator of fish immune response (Langston et al., 2002). Teleosts body temperature typically displays ±1°C of the ambient water temperature (Morvan-Rocher et al., 1995). Low environmental temperature (Table 1, 19.5±0.5°C) enhances immune response suppression both specific and nonspecific defenses in catfish. Pathological situations in catfish depend on their own temperature-dependent immune system expression. Hence, the virulence degree of *A. hydrophila* to catfish at 24 hrs exposed to LtW/s was higher than HtW/s (Table 4).

The increment of SGOT and SGPT trend line directions (Figure 2) related to activity of liver. Fish liver is a major organ involve in metabolic process (Pacheco and Santos, 2001), e.g. glucose-utilizing, glucose-producing and glucose-storing (Lermen et al., 2004). Buentello et al. (2000) suggested that the more energy production, the more increase growth rate (Figures 2&3). The increased SGOT and SGPT values in fish reveal enzymes exporting from liver into bloodstream (Yang and Chen, 2003; Perez-Rostro et al., 2004). This implies increase energy consumption in catfish (Carvalho and Fernandes, 2006) and indicates hepatic metabolism hyperactivity (Barcellos et al., 2003).

Trend line direction of BUN in HtW/s group is increasing (Figure 2). This shown that fish utilizes protein as a hepatic gluconeogenesis (Barcellos et al., 2003) and associated with increased protein intake (Trumble et al., 2006). The consequence of protein intake is an elevated of ammonia excretion rate which is caused blood urea increment. Cr is the product of muscle creatine catabolism and is excreted by trunk kidney (Trumble et al., 2006). Cr values are fairly constant (Figure 1). Well et al. (1986) addressed that the raised serum creatinine (HtW/s, Figure 2) is indicative of decreased glomerular filtration rate of fish posterior kidney.

The values of BUN to Cr ratio (Table 3) in Ws groups (HtWs and LtWs) are higher than W/s groups (HtW/s and LtW/s). This indicates that fish in HtWs and LtWs groups were dehydrated. Normally, fish excreted ammonia through the gills. In condition of medium hyper-ammonia (Table 1), fish excreted urea by the urine (Barcellos et al., 2003). The high BUN/Cr ratio suggests less effective removal of urea than creatinine via trunk kidney. Catfish was ammonia accumulation and increasing of nitrogen excretion as urea form.

Two trend lines direction of ALP in W/s groups (Figure 2, HtW/s and LtW/s) were progressive. Wells et al., (1986) reported that a raised activity of ALP is generally associated with stress. Furthermore, they suggested applying ALP as an index of stress in fish. The reduction of 2 trend lines in Ws groups (Figure 2, HtWs and LtWs) was supported by Koeypudsa and Kitkamthorn (2009). They recommended adding 0.1% salt as anti-stress in fresh-water fish.

All groups, the variations in Hct show no statistical significance but at 4 hr (Figure 1). The directions of trend line in Lt (Figure 2, LtWs and LtW/s) were dropped. Langston et al. (2002) stated that low Hct influenced by low temperature. Reduced Hct levels and anemia have been noted in flatfish and winter flounder, *Pseudopleuronectes americanus* (Ziskowski et al., 2008). The reduction in Hct may be caused by blood loss, hemodilution and osmoregulatory dysfunction. Decelerated of Hct concentrations may be a disruption of anterior kidney function, since the head kidney is a major organ in hemopoiesis.

In conclusion, water temperature does play the major role to catfish health, both infectious and vital organ functions. Interpretation of hematological data is complicated. The possibility of evaluation considered as values of healthy fish under natural condition (HtW/s). The changes in hematological variables (Figure 1) in response to water temperature indicate fish attempt to restore homeostasis. This imply catfish has developed physiological and biochemical adaptations to survive in extreme environmental conditions (HtWs, LtWs and LtW/s). Surplus 0.1% NaCl to water is recommended for catfish general health.

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