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การเปรียบเทียบค่าทางเคมีของเลือดปลาดุกบิ๊กอุย *(Clarias gariepinus x C. macrocephalus)* ระหว่างปลาที่ติดเชื้อแบคทีเรีย *Aeromonas hydrophila* และปลาไม่ติดเชื้อแบคทีเรีย *A. hydrophila*

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บทคัดย่อ: ปลาดุกบิ๊กอุย *(Clarias gariepinus x C. macrocephalus)* ซื้อจากฟาร์มเอกชน มีความยาวจากปลายปากถึงปลายหาง 12.2 ± 1.1ซม. น้ำหนัก 11.2 ± 3.1 กรัม แบ่งการเลี้ยงปลาออกเป็น 4 กลุ่มคือ เลี้ยงปลาที่อุณหภูมิน้ำต่ำ (19 - 20 ^{O}ซ) เป็นเวลา 1 วัน (Lt1d) เลี้ยงที่อุณหภูมิน้ำต่ำเป็นเวลา 4 วัน (Lt4d) เลี้ยงที่อุณหภูมิน้ำสูง (29 - 30^{O}ซ) เป็นเวลา 1 วัน (Ht1d) และเลี้ยงที่อุณหภูมิน้ำสูงเป็นเวลา 4 วัน (Ht4d) ปลาในแต่ละกลุ่มจะมีทั้งปลาที่ติดเชื้อแบคทีเรีย *Aeromonas hydrophila* และไม่ติดเชื้อแบคทีเรีย ปลาที่ไม่ติดเชื้อจะฉีดน้ำเกลือเข้าช่องท้องของปลาตัวละ 0.1 มล. สำหรับปลาที่ติดเชื้อแบคทีเรีย *A. hydrophila* จะฉีดเชื้อแบคทีเรียที่ทำให้ปลาตายร้อยละ 30, 50 และ90 (LD30, LD50 และ LD90) เข้าช่องท้องของปลา จำนวน 2.5 x 10^{3} - 5.1 x 10^{6} โคโลนี/มล. ทั้ง 2 กลุ่มของปลาที่เลี้ยงในอุณหภูมิน้ำต่ำ (Lt1d และ Lt4d) สำหรับปลาที่เลี้ยงในอุณหภูมิน้ำสูงทั้ง 2 กลุ่ม (Ht1d และ Ht4d) จะฉีดเชื้อแบคทีเรียเข้าช่องท้องของปลาจำนวน 2.8 x 10^{3} - 1.4 x 10^{9} โคโลนี/มล. สุ่มปลาดุกที่ติดเชื้อ *A. hydrophila* และไม่ติดเชื้อ มาเจาะเลือดเพื่อตรวจหาค่าของ serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), creatinine (Cr), alkaline phosphatase (ALP) และค่าเม็ดเลือดแดงอัดแน่น (Hct) จากผลการทดลองพบว่า ปลาที่ติดเชื้อแบคทีเรีย *A. hydrophila* มีค่าทางเคมีของเลือดแตกต่างจากปลาที่ไม่ติดเชื้อแบคทีเรีย ซึ่งหมายถึง ปลาที่ติดเชื้อแบคทีเรียได้เกิดความเสียหายของอวัยวะที่สำคัญ คือตับ (SGOT, SGPT และALP) ไตส่วนหน้า (Hct) และไตส่วนท้าย (BUN, Cr และ ALP) ยิ่งไปกว่านั้น ค่าที่เปลี่ยนไปของ ALP และ Hct เป็นผลที่เนื่องมาจากปลาที่ติดเชื้อแบคทีเรียเกิดความเครียดโดยเฉพาะค่าของ Hct ที่ต่ำลงนั้นแสดงให้เห็นว่าปลาที่ติดเชื้อแบคทีเรีย *A. hydrophila* เป็นโรคเลือดจางและมีออกซิเจนในเลือดต่ำ ค่าทางเคมีของเลือดที่ได้รับจากงานวิจัยนี้ นับได้ว่ามีประโยชน์และสามารถนำไปใช้ในการวินิจฉัยโรคเบื้องต้นได้

คำสำคัญ: *Aeromonas hydrophila* การติดเชื้อแบคทีเรีย ค่าทางเคมีของเลือด สุขภาพปลา ปลาดุกบิ๊กอุย อุณหภูมิน้ำ

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Comparative Blood Chemistry of Hybrid Catfish *(Clarias gariepinus x C. macrocephalus)* Infected with *Aeromonas hydrophila* to those Non-infected with *A. hydrophila*

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Abstract: Hybrid catfish *(Clarias gariepinus x C. macrocephalus)* was purchased from the private farm at 12.2 ± 1.1 cm total length and 11.2 ± 3.1 g body weight. All catfish were divided into 4 groups as following: low water temperature (19 - 20^{O}C) for 1 day experiment (Lt1d), low water temperature for 4 days experiment (Lt4d), high water temperature (29 - 30 ^{O}C) for 1 day experiment (Ht1d) and high water temperature for 4 days experiment (Ht4d). Each group composed of infected and non-infected catfish. Non-infected catfish was intra-peritoneal injection with 0.1 ml normal saline. All infected catfish were 0.1 ml *Aeromonas hydrophila* intra-peritoneal injection which lethal doses were 30, 50 and 90% fished killed: LD30, LD50 and LD90. The concentrations of bacteria were 2.5 x 10^3 - 5.1 x 10^6 colony forming unit (cfu)/ml for 2 groups of low water temperature (Lt1d and Lt4d) but 2.8 x 10^3 - 1.4 x 10^9 cfu/ml for 2 groups of high water temperature (Ht1d and Ht4d). Blood was randomly taken from sedated fish to investigation and interpretation of catfish health. Blood chemistry parameters were serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), creatinine (Cr), alkaline phosphatase (ALP) and hematocrit (Hct). Blood chemistry values revealed that infected fish causes blood changes owing to *A. hydrophila* infection. These results interpret to the damages of liver (SGOT, SGPT and ALP), anterior kidney (Hct) and posterior kidney (BUN, Cr and ALP). Furthermore, changes of ALP and Hct showed that infected fish were in stress situation. Catfish from bacterial infection groups will experience to anemia and lead to hypoxia because of low Hct. The obtained data are very useful to provide blood parameters which enable early detection of mass mortality caused by bacterial infection.

Keywords: *Aeromonas hydrophila,* Bacterial infection, Blood chemistry, Fish health, Hybrid catfish, Water temperature

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Introduction

It is known that hybrid catfish *(Clarias gariepinus x C. macrocephalus)* grows fast (Koeypudsa et al., 2007), is important food-producing fish and is resistant to diseases (Koeypudsa et al., 2006). Even though catfish is disease tolerant, the advancement of intensive culture is often leading to bacterial infection. *Aeromonas hydrophila* is an opportunistic gram-negative bacteria inducing ulcerative hemorrhagic septicemia (Ibrahem et al., 2010; Das et al., 2011; Carraschi et al., 2012) and up to 80% mortality (Silva et al., 2012). The mass mortality of *A. hydrophila* infection in catfish is following to severe economic loss (Angka et al., 1995; Harikrishnan et al., 2012).

In veterinary fields, it is recognized that prevention of infected fish caused by bacteria is more desirable goal than treatment (Falco et al., 2012; Harikrishnan et al., 2012). Bacterial diseases cause hematological changes (Carraschi et al., 2012; Falco et at., 2012) and then serve as reliable indicators of fish health (Hossain et al. 2018). Hematological profiles are essential for assessment of physiological and pathological status (Al-Dohali et al., 2011). Hence, hematology is applied to be a disease diagnostic tools for prognosis and therapeutic when fish exposed to diseases (Yu et al., 2010). Furthermore, blood chemistry parameters are also applied to monitor fish status of well being when exposed to biotic and abiotic stressors (Chen et al., 2003; Tavares-Dias and Moraes, 2007; Koeypudsa and Jongjaraenjai, 2011; Peres et al., 2012).

Virulence of *A. hydrophila* were studied in walking catfish *C. gariepinus* (Anka et al, 1995), *Ophicephalus striatus* and *C. batrachus* (Lio-Po et al., 1998), *Channa punctatus* (Rajendiran et al., 2008), and *Danio rerio* (Rodriguez et al., 2008). Those fishes were both artificial and natural infection with *A. hydrophila.* Histological changes were also studied in *C. gariepinus* x *C. macrocephalus* (Koeypudsa and Jongjareanjai, 2010), *Piaractus mesopotamicus* (Carraschi et al., 2012), and *Pseudoplatystoma corruscans* x *P. fasciatum* (Silva et al., 2012). The artificial infection of *A. hydrophila* on those fishes were presented in liver and kidney.

Blood parameters of *A. hydrophila* infection were studied in variety of fish by many researchers such as follows. Harikrishnan et al. (2003) found that *A. hydrophila* infection in *Cyprinus carpio* caused hemoglobin and hematocrit counts decreased when compare to non-infection. As well as *A. hydrophila* infection in *Channa punctatus* (Rajendiran et al., 2008), white blood cell, red blood cell, hemoglobin, and hematocrit were declined when compared to control group. Awad and Austin (2010) fed

Oncorhynchus mykiss with 1% lupin, *Lupinus perennis*, to prevent *A. hydrophila* infection. They found that the number of red blood cells and white blood cells in recipient fish were higher than those in the control group. Harikrishnan et al. (2010) fed *Carassius auratus* with mixed herbal leaf in ratio 1:1:1 (*Curcuma longa: Oscimum sanctum: Azadirachta indica*) to *A. hydrophila* infection group. They found that white blood cell level of infected untreated groups were significantly increased when those were compared to infected treated groups. Das et al. (2011) were intraperitoneal challenged *Puntius sarana* with *A. hydrophila*. They found a significant decrease in plasma glucose level, erythrocyte counts and hematocrit level were found after challenge 2 days. Silva et al. (2012) found that *A. hydrophila* infection in hybrid surubim (*Pseudoplatystoma corruscans* x *P. fasciatum*) was led to decrease in haematocrit percentage and erythrocyte number, but to increase in monocytes and neutrophils.

As literature reviewed, there is no report related hematological parameters to hybrid catfish (*C. gariepinus* x *C. macrocephalus*) induced to *A. hydrophila* infection. Moreover, Peres et al. (2012) suggested that it is not appropriate to apply reference values for one species to be extrapolated to another species. Thus, this research was conducted to compare the changes of blood chemistry between healthy and infectious hybrid catfish after exposure to *A. hydrophila*, both acute (1-day) and chronic (4-day) infection. The obtained blood chemistry profiles will lead to reasonable predict bacterial infection and to indicate hybrid catfish health status.

Materials and Methods

This research was approved by the Ethics Committee for Animal Experimentation, Faculty of Veterinary Science, Chulalongkorn University (number 1031074).

Hybrid catfish (C. gariepinus* x *C. macrocephalus): Purchased fish were taken from Suphanburi province. Fish weight and total length were 11.2 ± 3.1 g and 12.2 ± 1.1 cm respectively in number. Afternoon daily, fish were commercial pellets feeding with 1% body weight. Before the experiment began, all fish were acclimated for 2 weeks in laboratory. The experiment fish were not fed 1 day prior to blood collection and were not reused. Water was aerated, was 10% changed every morning and was analyzed for quality monitoring (Table 1).

Bacteria: *A. hydrophila* was isolated from natural infection of hybrid catfish in Thailand (AH64-kidney_Roi-ed). Bacteria was identified

Table 1 Water quality throughout experiment among 4 hybrid catfish groups

Parameter (Unit)	Lt1d	Lt4d	Ht1d	Ht4d
Air temperature (°C)	20	19	29	30
Alkalinity (mg/l)	108	108	106	109
Ammonia (mg/l)	0.1	0.1	0.1	0.2
Dissolved oxygen (mg/l)	5.7	5.6	5.5	5.4
Hardness (mg/l)	98	102	95	101
Nitrate (mg/l)	0.4	0.5	0.4	0.6
Nitrite (mg/l)	0.2	0.2	0.3	0.2
Osmolarity (mosmol/l)	7	9	8	6
pH	7.4	7.4	7.3	7.3
Water temperature (°C)	19	19	29	29

Lt1d = low water temperature (19 - 20°C) 1-day experiment

Lt4d = low water temperature (19 - 20°C) 4-day experiment

Ht1d = high water temperature (29 - 30°C) 1-day experiment

Ht4d = high water temperature (29 - 30°C) 4-day experiment

and was kept on agar slopes until application. Bacteria was tested for pathogenesis by inoculation into hybrid catfish before experiment is started. Bacterial re-isolation and identification were done from infected fish.

Experimental procedure: The 800 catfish were divided into 4 groups. Two groups of catfish were installed in air-condition room (19 - 20 °C, Lt) and another 2 groups were placed in open-air laboratory (29 - 30°C, Ht). Hybrid catfish from Lt and Ht groups were composed of 1-day and 4-day experiment as following: Lt1d, Lt4d, Ht1d and Ht4d. Each experiment obtained 1 control and 3 treatments of lethal dose (LD): LD30, LD50 and LD90. Every treatment had 5 replicates and each replicate got 10 fish in 60 l glass aquarium. All aquaria were aerated with air stone throughout experiment. Control fish was intraperitoneal injection with 0.1 ml sterile saline water. Bacterial concentrations as following: 8.6×10^4, 5.5×10^5 and 5.1×10^6 colony forming unit (cfu)/ml were used for Lt1d treatment which bacterial concentration at 30, 50 and 90 percentage fish killed, LD30, LD50 and LD90, within 1day. Whereas fish from Lt4d treatment were intracelomic inoculation with 2.5×10^3, 9.5×10^4 and 5.1×10^5 cfu/ml as using for LD30, LD50 and LD90

at 4 days. Ht1d treatment fish was *A. hydrophila* intraperitonially inoculated with the doses of LD30, LD50 and LD90 at 1 day as following: 8.8×10^3, 1.8×10^5 and 1.4×10^9 cfu/ml. The dosage for Ht4d treatment of LD30, LD50 and LD90 at 4 days were 2.8×10^3, 1.7×10^4 and 2.3×10^6 cfu/ml. The procedures have been approved by Chulalongkorn University Animal Care and use committee.

Blood collection: Catfish from Lt1d and Ht1d group was randomly selected from every replicate at 0, 3, 6 and 24-hr whereas fish from Lt4d and Ht4d group was taken on 1, 2, 3 and 4-day. The elected fish was withdrawn blood by tuberculin syringe (Terumo, 26Gx1") from caudal vein under clove oil sedated (5 ppm).

Analytical procedures: Hematocrit (Hct) was measured by centrifugation of heparinized microhematocrit capillary tubes (Hematology centrifuge, SR10000, Thailand). All blood samples were analyzed on the same day using an automated analyzer (BT 1000/2000 Plus, Biotecnica instrument, Italy). The analysis includes serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), creatinine (Cr) and alkaline phosphatase (ALP).

Statistical analysis: All data were expressed as mean of 5 replicates ± standard deviation. One-way analysis of variance (ANOVA) was performed to compare the means of differences. Significant difference among means were determined by Duncan's multiple ranges test. For all of the analysis, $p \leq 0.05$ was considered statistically significant.

Results

Liver indices: From control group, the values of SGOT (7.6 ± 4.1 - 12.2 ± 8.6, Figure 2), SGPT (102.6 ± 17.6 - 150.8 ± 20.7, Figure 2) and ALP (3.2 ± 2.7 - 6.2 ± 1.6, Figure 1) were not statistically significant different (Figure 1). The levels of SGOT, SGPT and ALP from infected groups: LD30, LD50 and LD90 were statistic significantly increased. The SGOT from LD30 were elevated from 15.8 ± 7.8 to 125.4 ± 38.9 unit (Table 2) and were raised from 13.8 ± 2.3 to 36.6 ± 14.2 unit (Figure 2). The SGPT from LD30 were lifted from 208.6 ± to 392.2 ± 171.7 unit (Figure 2). The amounts of ALP from LD50 were up from 2.6 ± 3.9 to 8.4 ± 0.8 mg% (Figure 1) and accelerated from 3.0 ± 1.8 to 21.0 ± 13.7 mg% (Figure 2). The numbers of ALP from LD90 were increased from 1.0 ± 0.7 to 7.6 ± 1.9 mg% (Figure 1).

Anterior kidney indices: The Hct values from control groups were disturbed and were statistical significance throughout the experimental period. The levels of Hct from infected groups: LD30, LD50 and LD90 were statistic significantly decreased. The Hct from LD30 were fall from 48.0 ± 3.2 to 22.6 ± 7.6%

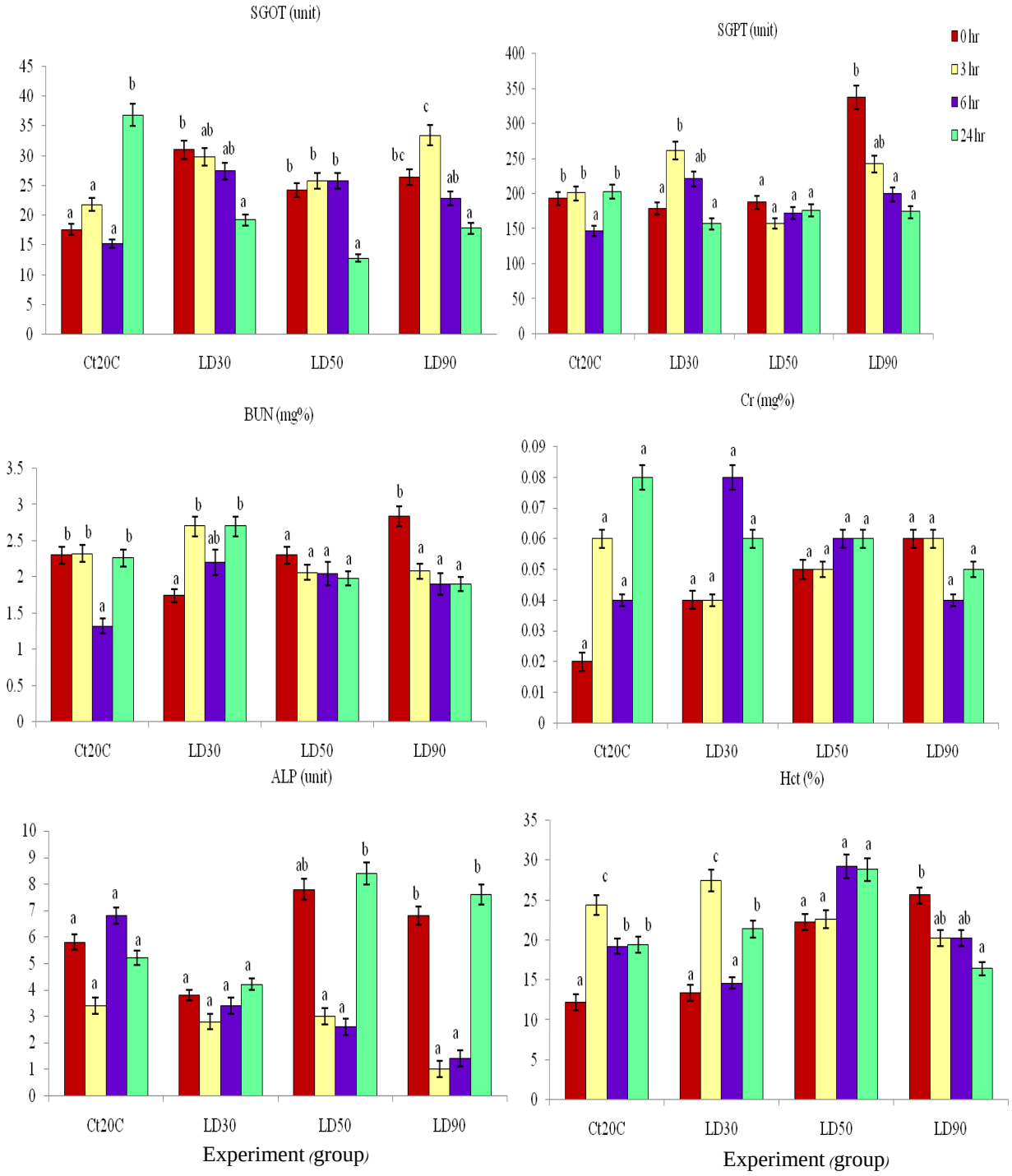

Figure 1 Fish blood chemistry values (mean of 5 replications) of low temperature (19-20^{O}C) 1-day experiment (Lt1d). Different lower case letters at the same group indicate statistical significant ($p \leq 0.05$, ANOVA, Duncan). SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase, BUN: blood urea nitrogen, Cr: creatinine, ALP: Alkaline phosphatase, Hct: Hematocrit, Ct20C: control group of Lt1d experiment, LD30: 30% fish killed at 1-day observation, LD50: 50% fish killed at 1-day observation, LD90: 90% fish killed at 1-day observation caused by *A. hydrophila* inoculation.

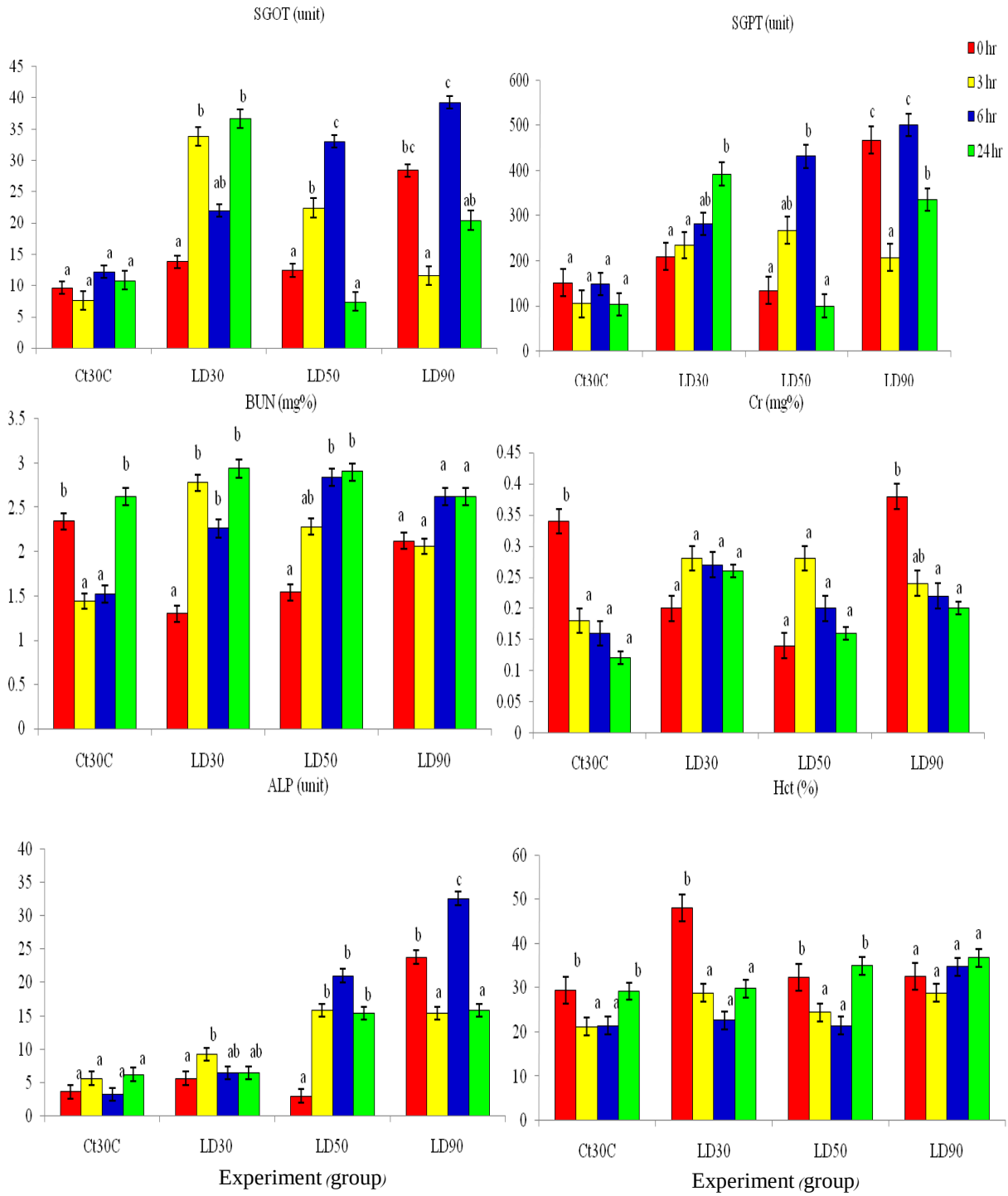

Figure 2 Fish blood chemistry values *(*mean of 5 replications*)* of high temperature *(*29-30^{O}C*)* 1-day experiment *(*Ht1d*)*. Different lower case letters at the same group indicate statistical significant *(p* ≤ 0.05, ANOVA, Duncan*)*. SGOT*:* serum glutamic oxaloacetic transaminase, SGPT*:* serum glutamic pyruvic transaminase, BUN*:* blood urea nitrogen, Cr*:* creatinine, ALP*:* alkaline phosphatase, Hct*:* Hematocrit, Ct30C*:* control group of Ht1d experiment, LD30*:* 30% fish killed at 1-day observation, LD50*:* 50% fish killed at 1-day observation, LD90*:* 90% fish killed within 1-day observation caused by *A. hydrophila* inoculation.

Table 2 Fish blood chemistry values *(*means ± s.d*.)* of low temperature *(*19-20^{O}C*)* 4-day experiment *(*Lt4d*)* after exposure *A. hydrophila* at 3 different concentrations.

Day	Ct20C	LD30	LD50	LD90
serum glutamic oxaloacetic transaminase, SGOT (unit)				
1	43.60 ± 8.00^{B}	15.80 ± 7.82^{A}	68.4 ± 38.57^{B}	75.8 ± 22.55^{A}
2	24.00 ± 9.30^{A}	26.40 ± 8.20^{A}	111.4 ± 40.27^{C}	79.6 ± 12.54^{A}
3	63.80 ± 8.70^{C}	125.40 ± 38.93^{C}	8.6 ± 0.89^{A}	74.6 ± 19.5^{A}
4	58.40 ± 24.30^{BC}	67.40 ± 40.42^{B}	5.8 ± 0.83^{A}	79.8 ± 13.66^{A}
serum glutamic pyruvic transaminase, SGPT (unit)				
1	533.00 ± 153.60^{B}	190.00 ± 65.39^{A}	357.60 ± 136.67^{B}	1136.40 ± 228.33^{B}
2	302.60 ± 45.20^{A}	273.60 ± 116.80^{A}	1093.20 ± 199.5^{C}	600.20 ± 213.00^{A}
3	570.80 ± 240.20^{B}	1215.20 ± 235.59^{C}	130.40 ± 29.63^{A}	601.20 ± 219.44^{A}
4	549.00 ± 125.80^{B}	752.80 ± 559.48^{B}	218.60 ± 63.93^{AB}	760.20 ± 188.07^{A}
blood urea nitrogen, BUN (mg%)				
1	4.00 ± 0.27^{C}	2.36 ± 1.15^{AB}	14.24 ± 10.94^{B}	3.56 ± 0.74^{A}
2	2.42 ± 0.60^{AB}	2.66 ± 0.20^{AB}	3.24 ± 0.08^{A}	3.96 ± 1.39^{A}
3	1.90 ± 0.50^{A}	3.12 ± 0.58^{B}	1.54 ± 0.25^{A}	4.06 ± 1.91^{A}
4	2.72 ± 0.50^{B}	1.94 ± 0.82^{A}	1.38 ± 0.67^{A}	4.78 ± 2.80^{A}
creatinine, Cr (mg%)				
1	0.22 ± 0.08^{B}	0.06 ± 0.05^{A}	0.44 ± 0.27^{B}	0.20 ± 0.10^{A}
2	0.05 ± 0.04^{A}	0.05 ± 0.04^{A}	0.14 ± 0.05^{A}	0.22 ± 0.10^{A}
3	0.12 ± 0.08^{A}	0.16 ± 0.05^{B}	0.08 ± 0.07^{A}	0.24 ± 0.05^{A}
4	0.07 ± 0.04^{A}	0.08 ± 0.04^{A}	0.12 ± 0.08^{A}	0.16 ± 0.08^{A}
alkaline phosphatase, ALP (unit)				
1	8.20 ± 2.58^{A}	6.20 ± 1.64^{A}	13.60 ± 5.94^{B}	19.40 ± 0.89^{B}
2	4.60 ± 2.0 ^{A}	10.60 ± 7.89^{A}	8.80 ± 1.78$^{A\ B}$	13.00 ± 1.87^{A}
3	8.40 ± 4.03^{A}	8.20 ± 2.04^{A}	8.20 ± 3.11^{A}	14.60 ± 2.5^{A}
4	4.40 ± 2.19^{A}	8.00 ± 1.87^{A}	10.80 ± 1.3^{AB}	13.00 ± 3.46^{A}
hematocrit, Hct (%)				
1	20.80 ± 2.77^{A}	24.60 ± 0.54^{A}	22.60 ± 4.03^{A}	28.40 ± 2.07^{D}
2	26.40 ± 2.07^{B}	25.00 ± 2.44^{A}	18.60 ± 2.6^{A}	19.00 ± 1.00^{B}
3	21.00 ± 4.52^{A}	26.00 ± 3.74^{A}	23.60 ± 1.34^{A}	22.60 ± 1.67^{C}
4	23.80 ± 1.92$^{A\ B}$	24.00 ± 5.43^{A}	20.60 ± 5.45^{A}	15.80 ± 3.03^{A}

Different upper case letters at the same column indicate statistical significant *(*$p \leq 0.05$, ANOVA, Duncan*)*. Ct20C*:* control group of Lt4d experiment, LD30*:* 30% fish killed at 4-day observation, LD50*:* 50% fish killed at 4-day observation, LD90*:* 90% fish killed at 4-day observation caused by *A. hydrophila* inoculation.

(Figure 2). From LD50 experiment, Hct amounts were decelerated from 27.8 ± 1.6 to 24.6 ± 1.6% (Table 3). The Hct from LD90 experiment were decreased from 25.6 ± 5.1 to 16.4 ± 3.9% (Figure 1) and were fall from 28.4 ± to 15.8 ± 3.0% (Table 2).

Posterior kidney indices: The Cr from all groups (Figure 1), infectious group: LD30, LD50 (Figure 2), and LD90 (Table 2) were not statistically significant different. The levels of BUN from control group were variable and were statistical significances. The values of BUN from infected groups: LD30, LD50 and LD90 were statistic significantly increased. The numbers of BUN from LD30 were elevated from 1.7 ± 0.6 to 2.7 ± 0.9 mg% (Figure 1) and were increased from 1.3 ± 0.4 to 2.9 ± 0.2 mg% (Figure 2). The levels of BUN from LD50 were elevated from 1.5 ± 0.6 to 2.9 ± 0.8 mg% (Figure 2). The amounts of BUN from LD90 were accelerated from 1.9 ± 0.1 to 3.2 ± 0.4 mg% (Table 3).

Discussion

Pathogenic bacteria: *A. hydrophila* produces extracellular toxin on the host which causes cytotoxicity, ulcerative syndrome, hemorrhagic septicemia and inflammation (Peatman et al., 2007; Sahoo et al., 2011). The toxins are hemolysins, proteases, enterotoxins, endotoxins and cholinesterases (Silva et al., 2007). Rogdriguez et al. (2008) addressed that kidney cell viability was reduced to 30% when incubate with viable *A. hydrophila* and decreased to 10% when exposed to its toxin. Bacteria were found in fish lesion, liver and kidney, after intramuscularly injected but not after 5 days post-injection (Angka et al., 1995) because fish are resilient against bacterial infection (Hur et al., 2019). Silva et al. (2012) emphasized that low bacterial concentrations were not shown clinical signs but were shown moderate histological changes in liver and kidney (Chen et al., 2004). Peracute form of diseases might destroy fish before any changes are presented (Rodriguez et al., 2008). Therefore, those agreed with this study because the present study hardly found any gross lesions on dead catfish, both acute (Lt1d and Lt4d) and chronic infection (Lt4d and Ht4d). Moreover, Koeypudsa and Jongjareanjai (2011) described that low water temperature (19.5 ± 0.5 ^{O}C.) caused stress in catfish. Catfish were easily susceptible to *A. hydrophila* infection and were elevated from weakly virulent level to virulent level (Koeypudsa and Jongjareanjai, 2010).

Liver indices: SGOT, SGPT and ALP are non-functional enzymes which are normally localized within the cells of liver, heart, gills, kidney and muscle (Koeypudsa et al, 2007; Osman et al. 2010). These enzymes are recognized to be important to assessment

Table 3 Fish blood chemistry values *(*means ± s.d.*)* of high temperature *(*29-30^{O}C*)* 4-day experiment *(*Ht4d*)* after exposure *A. hydrophila* at 3 different concentrations.

Day	Ct30C	LD30	LD50	LD90
serum glutamic oxaloacetic transaminase, SGOT (unit)				
1	11.60 ± 3.78^{B}	10.20 ± 3.56 ^{B}	28.20 ± 4.20 ^{C}	18.20 ± 2.28^{C}
2	20.20 ± 3.11^{C}	13.60 ± 2.07 ^{C}	21.20 ± 4.43 ^{B}	10.80 ± 2.28^{B}
3	7.60 ± 1.34^{A}	8.20 ± 1.30 ^{B}	9.60 ± 1.14 ^{A}	4.60 ± 1.67^{A}
4	11.60 ± 1.51^{B}	2.40 ± 0.54^{A}	10.00 ± 1.87^{A}	20.80 ± 7.08^{C}
serum glutamic pyruvic transaminase, SGPT (unit)				
1	130.20 ± 25.15^{A}	105.00 ± 18.85^{BC}	286.60 ± 106.22^{B}	189.20 ± 27.45^{A}
2	290.80 ± 56.35^{B}	135.20 ± 15.35^{C}	241.60 ± 76.4^{B}	131.80 ± 38.91^{A}
3	90.20 ± 14.49^{A}	83.60 ± 10.59^{B}	60.20 ± 26.66^{A}	124.00 ± 41.92^{A}
4	100.20 ± 10.63^{A}	49.80 ± 38.49^{A}	103.80 ± 16.22^{A}	180.80 ± 82.73^{A}
blood urea nitrogen, BUN (mg%)				
1	1.72 ± 0.31^{A}	2.04 ± 0.23^{A}	2.78 ± 0.16^{B}	1.96 ± 0.11^{B}
2	2.52 ± 0.58^{B}	3.02 ± 0.44^{B}	2.44 ± 0.74^{AB}	2.48 ± 0.31^{C}
3	2.76 ± 0.42^{B}	2.06 ± 0.48^{A}	1.92 ± 0.70^{A}	0.76 ± 0.16^{A}
4	2.18 ± 0.34^{AB}	1.92 ± 0.54^{A}	2.24 ± 0.45^{AB}	3.20 ± 0.47^{D}
creatinine, Cr (mg%)				
1	0.14 ± 0.05^{A}	0.14 ± 0.05^{A}	0.30 ± 0.10^{B}	0.16 ± 0.05^{A}
2	0.16 ± 0.05^{AB}	0.28 ± 0.08^{B}	0.22 ± 0.08^{AB}	0.32 ± 0.10^{B}
3	0.24 ± 0.05^{B}	0.34 ± 0.05^{B}	0.14 ± 0.05^{A}	0.12 ± 0.04^{A}
4	0.46 ± 0.08^{C}	0.16 ± 0.08^{A}	0.18 ± 0.08^{A}	0.28 ± 0.09^{B}
alkaline phosphatase, ALP (unit)				
1	8.00 ± 4.84^{AB}	9.40 ± 2.19^{A}	8.60 ± 0.67^{A}	15.40 ± 4.61^{A}
2	3.80 ± 1.30^{A}	8.60 ± 2.7^{A}	3.60 ± 0.54^{A}	8.20 ± 1.30^{A}
3	8.00 ± 2.73^{AB}	8.80 ± 3.03^{A}	16.60 ± 7.79^{B}	10.80 ± 2.58^{A}
4	11.20 ± 4.65^{B}	10.00 ± 0.70^{A}	7.00 ± 1.41^{A}	12.80 ± 8.95^{A}
hematocrit, Hct (%)				
1	22.40 ± 2.30^{AB}	21.80 ± 2.77^{A}	27.80 ± 1.64^{B}	19.80 ± 3.11^{A}
2	17.60 ± 2.07^{A}	26.00 ± 5.83^{A}	24.60 ± 1.67^{A}	21.20 ± 1.64^{A}
3	22.00 ± 5.78^{AB}	24.40 ± 1.34^{A}	26.20 ± 1.09^{AB}	23.40 ± 2.88^{AB}
4	22.80 ± 1.92^{B}	26.40 ± 2.70^{A}	25.20 ± 2.04^{A}	27.20 ± 4.71^{B}

Different upper case letters at the same column indicate statistical significant *($p \leq 0.05$, ANOVA, Duncan).* Ct30C*:* control group of Ht4d experiment, LD30*:* 30% fish killed within 4-day observation, LD50*:* 50% fish killed within 4-day observation, LD90*:* 90% fish killed within 4-day observation caused by *A. hydrophila* inoculation.

vital organs function (Wells et al., 1986). The rise in SGOT, SGPT and ALP give the information on the increment of organ metabolic activities (Koeypudsa et al, 2007), organ dysfunctions (Osman et al., 2010), impaired control of fluid balance (Noor et al., 2019) and tissue injuries (Osman et al., 2010; Yu et al., 2010; Segvic-bubic et al., 2013). An elevation of values may indicate the leakage of enzymes across damaged cell membranes or increment of enzymes synthesis because of positive correlation with physiological growth (Koeypudsa et al., 2007; Koeypudsa and Jongjareanjai, 2010; Segvic-Bubic et al., 2013).

The SGOT, SGPT and ALP values of control group in this study were not significant different but were statistically significant increased from the infectious fish group: LD30, LD50 and LD90. Changes in blood chemistry parameters during bacterial infection have been reported. The elevation of SGOT and SGPT were observed in *Oreochromis niloticus* infected with *Streptococcus iniae* (Chen et al., 2004), *Salvelinus fontinalis* affected by *Flavobacterium columnare* (Rehulka and Minarik, 2007) and *Silurus asotus* infected with *Edwardsiella tarda* (Yu et al., 2010). In contrast, ALP was decreased in *Oreochromis niloticus* infected with *Streptococcus iniae* (Chen et al., 2004) and *Salvelinus fontinalis* affected by *Flavobacterium columnare* (Rehulka and Minarik, 2007). Not only low ALP, but high ALP was also presented in stress catfish. Ellsaesser and Clem (1987) reported that channel catfish showed stress situation after subjected to transportation. Moreover, stress hybrid catfish had ALP increment after exposed to high and low water temperature (Koeypudsa and Jongjareanjai, 2010).

Anterior kidney indices: Hemopoietic tissue is presented in head kidney of fish (Ziskowki et al., 2008; Koeypudsa and Jongjareanjai, 2010). As shown in this study, the reduction of Hct from infected groups: LD30, LD50 and LD90 were significantly differences because of the head kidney damages regarding to bacterial infection (Sahoo et al., 2011). Low Hct has been reported in *A. hydrophila* infection of goldfish (Harikrishnan et al., 2010) and Olive barb (Sahoo et al., 2011). Gollock et al. (2005) reported that *Anguilla anguilla* infected with parasitic *Anguillicola crassus* has been shown to decrease Hct as well. Olsen et al. (1997) presented that Hct levels of Atlantic salmon affected with rickettsia, *Piscirickettsia salmonis*, is lower than Hct values of healthy salmon. Al-Dohali et al. (2009) recorded that fish fed with general diet had lower Hct levels than healthy fish fed with probiotic supplemented diet. Yildiz and Pulatsu (1999) addressed that *Oreochromis niloticus* had low Hct because of stress effect

after treatment with formalin, malachite green and methylene blue. For fin rot diseases caused by toxic chemistry contamination in winter flounder, Hct reduction was discussed as stress response (Ziskowki et al., 2008). The findings in this study, low Hct in bacterial infection groups, suggested that care must be taken. Because, low Hct results in a shortage of oxygen in bacterial infection fish (Meyer et al. 2002; Silva et al., 2007).

Posterior kidney indices: Trunk kidney of teleosts is composed of endocrine and excretory tissues (Meyer et al., 2002). Freshwater fish produces high volumes of urine by glomerular filtration to compensate influx of water about 50% of body weight per hour, hyper-osmotic to the environment. Meyer et al. (2002) reported that whenever fish is subjected to viral hemorrhagic septicemia, hemoflagellate infection and hypoxia, reduction urine flow rate will be presented. Therefore, increasing the blood levels of nitrogenous waste products (Roche and Boge, 2000).

The infection groups of this research: LD30, LD50 and LD90, were shown high BUN concentrations. This could be caused by kidney inflammation (Al-Dohail et al., 2011) owing to bacterial infected resulting decreased renal clearance (Adams et al., 2010) and may be related to slow down of ammonia excretion (LeaMaster et al., 1990) then was an indicator of compromised health (Yang et al., 2019). Then, hybrid catfish in bacterial infection groups were approached to hyperproteinemia. These physiological changes could lead to stress and mortality in experimental fish (Honryo et al, 2019).

In conclusion, blood chemistry parameters are used as reliable indicators of fish health status to detect physiological changes and stress condition. From the results of this trial, it is logical to address that both acute (Lt1d and Lt4d) and chronic bacterial infection (Lt4d and Ht4d) causes blood chemistry variable. Infectious catfish were in stress situation and hypoxic condition. The functions of vital organ, liver and kidney, were negative impacts because of organ damages and *A. hydrophila* infected.

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