The effect of differences in altitude location of an aquaculture on fish’s hematocrit and fish’s haemoglobin of Carp fish and resistance to bacterial attack

Rosidah, A Rizal, I Rustikawati and F Octavia

Faculty of Fishery and Marine Science, Universitas Padjadjaran
Bandung-Sumedang, Km 21, Jatinangor Campus 40600 Sumedang 453 63 West Java

E-mail: ros_ahdi@yahoo.com

Abstract. The aim for this research is to analyze the relation between the height place of aquaculture and life directness towards Aeromonas hydrophila attack for hematocrit and hemoglobin degrees on Cyprinus carpio L. This research have been doing on February - March 2017 at UPT PTBP Kepanjen (as Group 1), Malang (332 amsl) and UPT PBAT Umbulan IBAT as Group 2 (955 amsl). The treatment that be used is Cyprinus carpio infected by Aeromonas hydrophila. Parameters was observed, are hematocrit, hemoglobin degrees, clinical symptom of fish and the quality of water. The result of this investigation, hematocrit and hemoglobin degrees of Cyprinus carpio in level low land (Group 1, 332 amsl) around 12.8 % and 9.58 g/dL, meanwhile hematocrit and hemoglobin degrees in level up land (Group 2, 955 amsl) around 24.19 % and 12.64 g/dL. Cyprinus carpio L. after infected by Aeromonas hydrophila, it shows the clinical symptoms : its mucus be left, bleeding, its scale get peeled off and dropsy. The life directness of that Cyprinus carpio L. maintaining in 15 days 0 %. The differences between the height level of aquaculture for hematocrit and hemoglobin degrees also life directness towards Aeromonas hydrophila attack on Cyprinus carpio L.

1. Introduction
Goldfish or Carp fish (Cyprinus carpio L.) is an important commodity in freshwater aquaculture that is relatively easy to cultivate, so it is popular in almost every region of Indonesia [1]. Cultivation of carp can be done in various altitude places, both in the lowlands and in the highlands. Differences in altitude of a place result in differences in local environmental conditions.

Increased or decreased air pressure and oxygen content in the blood will affect the content of hematocrit and haemoglobin in fish. Examinations of hematocrit and haemoglobin can be used as one of the benchmarks to determine the state of fish health or as an early diagnosis in determining fish health. The hematocrit value in fish of less than 22 % indicates anaemia [2].

According to Amanda [3], most fish that are in stress condition will get sick and die. One of the causes of stress in fish is inappropriate oxygen content of the water. Stress in fish will affect the resistance of the fish to disease. Disease that can easily attack the fish is haemorrhagic septicaemia caused by the bacteria Aeromonas hydrophila [4].

According to Kabata [5], Aeromonas hydrophila bacteria cause a high mortality rate (80-100 %) within 1-2 weeks. Fish infected by the bacteria will experience bleeding on several body parts such as the chest, the abdomen, and the base of the fins [6]. So far, there has been no research on the effect of
differences in height of the cultivation area on hematocrit and haemoglobin of carp fish associated with the resistance to bacterial attack from Aeromonas hydrophila; therefore, it needs to be researched

2. Methodology
This research was conducted in February until March 2017, located at UPT PTBP Kepanjen and UPT PBAT Umbulan IBAT Punten, Malang. This research was a descriptive study conducted to determine the relationship between the altitude of fish pond with hematocrit and haemoglobin levels in carp fish and endurance against bacteria attack from Aeromonas hydrophila. At each location, fish were taken as many as 65 heads weighting of 200 g/tail, 5 tails were used for blood sampling and 60 tails were for test challenge with the infecting bacteria Aeromonas hydrophila.

3. Results and Discussion
3.1. Hematocrit
Hematocrit is the volume of blood cells compared with blood plasma that is expressed as a percentage. Based on observations of the hematocrit, carp fish that have been maintained at different altitudes had different hematocrit levels (table 1).

| Altitude (amsl) | Average Hematocrit Level (%) |
|-----------------|-----------------------------|
| 332 (Group 1)   | 12.8                        |
| 955 (Group 2)   | 24.19                       |

Table 1 shows that carp fish that was maintained at 332 amsl (Group 1) had an average hematocrit level of 12.8 %; while in carp fish cultivated at 955 amsl (Group 2), the average hematocrit level was 24.19 %. The hematocrit of carp fish cultured at 332 amsl (Group 1) was below normal; while the hematocrit of carp fish cultured at 955 amsl (Group 2) was within normal limits. From the results, it can be concluded that carp fish from higher than 332 amsl (Group 1) had anaemia. This is in accordance with the statement of Gallaugher [2] that a hematocrit value lower than 22 % indicated the fish is anaemic. According to Handayani [7], anaemia is a circumstance in which circulating erythrocytes do not fulfil their function to provide O₂ for body tissues. Anaemia can be affected by several factors including age, sex, and altitude.

Higher hematocrit values of carp were found in Group 2 (955 amsl) compared with the hematocrit of carp fish at 332 amsl (Group 1) that was due to dissolved oxygen content (DO) in setting media below normal range of 2-3 mg/L (Table 2), thus causing a lack of oxygen (O₂) supply in the blood. Blood is divided into three parts: red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes).

White blood cells (leukocytes) are one component of the blood that serves as a non-specific defence that inhibits the attack of pathogenic bacteria through phagocytosis. Leukocytes can also be used as an indicator of infection in the body. The body will produce more leukocytes when there is a foreign matter that enters the body [8]. Based on the observation, the fish leukocytes were different between those cultured at the altitude of 322 amsl (Group 1) and at 955 amsl (Group 2) (table 2).

| Altitude (amsl) | Average Leukocytes Level (cell/mm³) |
|-----------------|-------------------------------------|
| 332 (Group 1)   | 26,896                              |
| 955 (Group 2)   | 28,832                              |

In Table 2, it can be observed that carp fish kept at 332 amsl (Group 1) had a lower average leukocyte value (26,896 cells/mm³) compared with carp fish cultured at 955 amsl (Group 2) (28,832
The levels of carp fish leucocytes from both locations were within the normal range. According to Lagler [9], the number of white blood cells in fish ranged from 20,000 to 150,000 cells/mm$^3$. Increasing number of leucocytes can be used as a sign that the fish is in stress condition. The stress can be caused by deterioration in water quality [10].

### 3.2. Hemoglobin

Haemoglobin (Hb) plays an important role in the transport of gases, especially oxygen ($O_2$) from the gills pumped by the heart to all cells and organs, transporting nutrients into cells, disposing metabolic waste, and so on. Based on the observed haemoglobin concentration of carp fish cultured at 332 amsl (Group 1) and 955 amsl (Group 2) had different haemoglobin levels (table 3).

| Altitude (amsl) | Average Hemoglobin Level (g/dL) |
|-----------------|-------------------------------|
| 332 (Group 1)   | 9.58                          |
| 955 (Group 2)   | 12.6                          |

Table 3 shows that carp fish cultivated at 332 amsl (Group 1) had lower average haemoglobin level (9.58 g/dL) compared to carp fish cultivated at 955 amsl (Group 2) which had an average haemoglobin level of 12.64 g/dL. Both groups were classified as above normal, since normal haemoglobin level for carp is 6.22 ± 0.91 g/dl [11]. The value of haemoglobin may be affected by the age of the animal, species, environment, feed, presence or absence of erythrocyte damage, and blood handling at the time of examination [12].

The average value of haemoglobin level of carp fish from low (Group 1, 332 amsl) and high (Group 2, 955 amsl) altitudes had considerable differences compared to normal carp fish.

The higher the haemoglobin level, the oxygen ($O_2$) transport process to all parts of the fish body tissue will be more optimal, thus affecting the maximum consumption of $O_2$; whereas the lower the haemoglobin level or the more severe the anaemia, the decrease in maximum $O_2$ consumption is also greater [13,14].

From the description above, it shows that hematocrit, leukocyte and haemoglobin levels in carp fish are affected by the difference in altitude, as seen from the examination of hematocrit (table 1), leukocytes (table 2) and haemoglobin levels (table 3) of fish culture at the altitudes 332 amsl (Group 1) and 955 amsl (Group 2).

### 3.3. Clinical symptoms of fish infected with Aeromonas hydrophila

Observation of clinical symptoms in fish from Group 1 (332 amsl) and Group 2 (955 amsl) was performed for 15 days after the fish got bacterial infection of *Aeromonas hydrophila* by injection. The Observation included changes in morphology and behaviour. Morphological changes were seen with damage to the surface of the body, such as excess mucus, bleeding, peeling of scales, prominent eyes (exophthalmia), and dropsy (table 4), whereas behavioural changes were seen from reduced response to feed (table 5) and to the given shock (table 6).
Table 4. Clinical symptoms of carp fish during the challenge test.

| Day of Observation | Altitude (amsl)                |
|--------------------|--------------------------------|
|                    | 332 (Group 1) | 955 (Group 2) |
| 1                  | -              | -              |
| 2                  | -              | -              |
| 3                  | A              | -              |
| 4                  | A              | A              |
| 5                  | a and b        | A              |
| 6                  | a and b        | A              |
| 7                  | a, b and c     | a and b        |
| 8                  | a, b, c and e  | a and b        |
| 9                  | a, b, c and e  | a and b        |
| 10                 | a, b, c and e  | a, b, c and e  |
| 11                 | 0              | a, b, c and e  |
| 12                 | 0              | a, b, c and e  |
| 13                 | 0              | a, b, c and e  |
| 14                 | 0              | 0              |
| 15                 | 0              | 0              |

Information of Clinical Symptoms:
- = There are no Clinical Symptoms
0 = Death
a = Excessive Mucus
b = Bleeding
c = scales peel off
d = The fish eyes stand out (eksophthalmia)
e = abdominal bloating (dropsy)

Table 4 shows that on Day 1 and 2 after bacterial infection of *Aeromonas hydrophila*, carp fish from the altitude 332 amsl (Group 1) and 955 amsl (Group 2) did not show any damage to the body and the fish were still in healthy condition (figure 1). Bacteria *Aeromonas hydrophila* has an optimal growth in the exponential phase occurring at the 4th hour to the 12th hour [15]. But on the basis of observation, the clinical symptoms began to appear on the 3rd day. It is suspected that the bacteria *Aeromonas hydrophila* that entered the body of carp did not directly become virulent, because they were still in sticking stage that takes time to enter the stage of infection or virulence [16].

![Figure 1. Healthy carp fish.](https://example.com/figure1.jpg)

The third day after the infection, the fish cultured at 332 amsl (Group 1) began to show symptoms of excess mucus on the body surface (figure 2), while those at 955 amsl (Group 2) started to show similar symptom on Day- 4 (figure 2). The mucus produced by the carp fish from Group 1 was more than the mucus produced by the carp in Group 2 (figure 2). Once *Aeromonas hydrophila* starts to infect a fish, the fish will release more mucus as a defence.
Another clinical symptoms began to appear on the 5th day in carp fish cultured at 332 amsl (Group 1), which was bleeding (figure 3), followed by peeling of the scales and inflation of the abdomen on the next day (figure 4). For Carp fish cultured at 955 amsl (Group 2) similar symptoms started to appear on Day 7 and Day 10 respectively (figure 4).

The carp fish from Group 1 suffered severe bleeding causing the cultivating water media to turn red, while the bleeding in carp fish from Group 2 was not too severe so that it did not change the colour of the water. This condition is associated with the attack of bacteria, so that fish experience disruption or physiological changes. Fish infected with *Aeromonas hydrophila* bacteria will show clinical symptoms of haemorrhage and abdominal bloating due to fluid accumulation in the abdominal cavity [15]. The physiological changes in carp that were infected by *Aeromonas hydrophila* bacteria were a defence mechanism of fish itself. According Syawal [17] in Nurjannah[18], physiological changes in fish, such as inflammation due to infection, is a nonspecific immune response characteristic.

In addition to morphological changes, carp infected by *Aeromonas hydrophila* bacteria also experienced behavioural changes such as decreased appetite (table 5) and reduced reflexes to shock (table 6).
Table 5. Feeding response of carp fish during the challenge test.

| Day of Observation | 322 (Group 1) | 955 (Group 2) |
|---------------------|---------------|---------------|
| 1                   | +             | +             |
| 2                   | +             | +             |
| 3                   | -             | +             |
| 4                   | -             | +             |
| 5                   | -             | -             |
| 6                   | -             | -             |
| 7                   | -             | -             |
| 8                   | -             | -             |
| 9                   | -             | -             |
| 10                  | 0             | -             |
| 11                  | 0             | -             |
| 12                  | 0             | -             |
| 13                  | 0             | 0             |
| 14                  | 0             | 0             |
| 15                  | 0             | 0             |

Information of Feeding Response:
( + ) = Normal Feeding Response
( - ) = Less Feeding Response
( 0 ) = No Feeding Response

Table 5 shows that the carp fish response to feeding at different altitudes was still normal until Day 2. This shows that the fish was still in good condition, so they could respond to the feeding properly. Fish was still in good health because *Aeromonas hydrophila* that infected the fish did not directly become virulent, but it still in the adhesive stage on the fish body and it took time to enter the infection stage [16].

The less of feeding response (-) appetite was seen on Day 3 to Day 9 in carp fish cultured at 332 amsl (Group 1) while the fish from e 955 amsl (Group 2) showed similar symptom on Day 5 to Day 12. Less feeding response appetite was seen with the rest of the feed into a precipitate to the bottom of aquarium. Decreased feeding response was due to the metabolic process that is disrupted. Decrease in response to feeding in fish is caused by metabolic disorders in the body post bacterial infection of *Aeromonas hydrophila* [19]. The absence of feeding response or no feeding response (0) in goldfish from the altitude of 332 amsl (Group 1) occurred on the 10th to 15th day of observation, whereas in carp fish from the altitude of 955 amsl (Group 2) occurred on the 13th day of observation until to-15.

In addition to decreasing response to feeding, carp fish that was infected by bacterium *Aeromonas hydrophila* also showed decreased response to shock. Reflex tests were performed by tapping the aquarium walls at each treatment and fish infected with *Aeromonas hydrophila* tended not to respond (table 6).
Table 6. Fish response to shock during the challenge test.

| Day of Observation | 332 (Group 1) | 955 (Group 2) |
|--------------------|---------------|---------------|
| 1                  | +             | +             |
| 2                  | +             | +             |
| 3                  | +             | +             |
| 4                  | -             | +             |
| 5                  | -             | -             |
| 6                  | -             | -             |
| 7                  | -             | -             |
| 8                  | -             | -             |
| 9                  | 0             | -             |
| 10                 | 0             | -             |
| 11                 | 0             | -             |
| 12                 | 0             | 0             |
| 13                 | 0             | 0             |
| 14                 | 0             | 0             |
| 15                 | 0             | 0             |

Information of Feeding Response:
(+) = Normal Feeding Response
(-) = Less Feeding Response
(0) = No Feeding Response

Table 6 shows that response to the shock in carp fish cultured at 332 amsl (Group 1) was still normal until the 3rd day, while those kept at 955 amsl (Group 2) still showed normal response until the 4th day of observation. This indicated that the fish were still fairly healthy and the immune system was good, so that bacteria did not cause damage yet.

In carp fish cultured 332 amsl (Group 1); there was a lack of response to the shock on the 4th day until the 8th day, while in those from 955 amsl (Group 2) similar symptom was seen on Day 5 to Day 11. This shows that the immunity of carp fish began to decline due to bacterial attack of *Aeromonas hydrophila*.

In carp fish kept at 332 amsl (Group 1), the disappearance of response to shock occurred on the 9th day until the 15th day, whereas in carp fish from 955 amsl (Group 2) similar symptom occurred on the 12th day until the 15th day. The fish immune system had been greatly compromised so they were not able to resist attacks against the bacteria *Aeromonas hydrophila*.

3.4. Survival rate of carp fish

Observation of the survival rate of carp fish was conducted after the challenge test by infecting *Aeromonas hydrophila* bacteria for 15 (fifteen) days. Fish culture at different altitudes produced different survival rate (figure 5).
Based on figure 5, carp fish cultured at 332 amsl (Group 1) had a lower survival rate compared to those kept at 955 amsl (Group 2). Carp fish originated from Group 1 only lasted for 10 (ten) days from the total cultivation period of 15 (fifteen) days after challenge test. The death of carp fish originating from Group 1 started from the 3rd day with 3.34 % of the population died after the injection of Aeromonas hydrophila and each day the death rate was different. In carp fish originating from Group 2, the mortality started from Day 4 with 1.67 % and the number of fish that died each day was different.

Deaths in carp fish from both altitudes were due to hematocrit, leukocyte, haemoglobin, and dissolved oxygen in water (DO). In carp fish cultured at 322 amsl (Group 1) the death rate was more rapid due to lower hematocrit, leukocyte and haemoglobin levels. Reduction of all those factors can affect the immune system. Moreover, fish resistance decreased due to lack of drug delivery as prevention or treatment.

3.5. Water quality

Water quality analysis was performed three times during the challenge test. Water quality observations during the study are shown in table 7.

| Altitude (amsl) | Temperature (°C) | pH    | DO (mg/L) |
|-----------------|------------------|-------|-----------|
| 332 (Group 1)   | 25 – 26          | 7 – 7.5 | 4 – 6   |
| 955 (Group 2)   | 20 – 23          | 6 – 7  | 2 – 3    |
| Normal          | 25 – 30          | 6.5 – 9 | >3       |

In fish culture at 332 amsl (Group 1), the temperature range, pH, and DO lower normal conditions were 25-26 °C, pH 7-7.5 and DO 4-6 mg/L, whereas at 955 amsl (Group 2), the temperature range and DO were under normal conditions and the pH was within the normal range. The temperature values at the time of observation were in the range of 20 - 23°C, pH 6 - 7 and DO 2 - 3 mg/L. According to Supriyatna [1], the ideal temperature for fish life is 25 - 30 °C and according to Paulo [20] the normal pH value for fish life ranges from 6.5 to 9, while the normal dissolved oxygen (DO) value for fish life should be higher than 3 mg/L.

![Figure 5. Survival rate of carp fish.](image-url)
If the condition of water quality is abnormal then it affects the survival rate of the fish. Water temperature affects growth rate, metabolism rate, and appetite [21]. Abnormal acidity levels (pH) can decrease reproductive capacity and fish growth [20]. The DO concentration is influential in the metabolic process, which can affect growth rate and feed conversion [21].

4. Conclusion
1. Differences in altitude affect the differences in hematocrit and haemoglobin levels of carp fish.
2. Carp fish cultured at 332 amsl (Group 1) had hematocrit and haemoglobin values lower than those kept at 955 amsl (Group 2).

5. Reference
[1] Supriatna Y 2013 Fish cultivation in water-saving pool (Jakarta : AgroMedia Pustaka) 78 (In Indonesia)
[2] Gallaugher P H, H Thorarensen and A P Ferrel 1995 Resp. Phys. 102 279-292
[3] Amanda 2012 Observation of hematocrit value of tombro fish blood (Cyprinus carpio l.) preserved at elevation place (Yogyakarta) 76
[4] Mandiri T K T 2009 Guidelines for farm and cultivation of carp fish (Bandung : CV. Nuansa Aulia) p 194
[5] Kabata Z 1985 Parasites and diseases of fish cultured in the tropics (Taylor and Francis) 318
[6] Hidayat R, Patana and Lesmana I 2015 J Agrobioi.
[7] Handayani W and Haribowo A S 2008 Salemma Medika 150 (In Indonesia)
[8] Dianti L, Prayitno S B, Arijati R W 2013 J.Aquac. Man. Tech. 2 63-71.
[9] Lagler K F, Bardach J E, Miller R R, and Passino D R M 1977 Ichthiology p 506
[10] Paulo C F C, Pedro H S K, Elaine A, Correia S, and Bernardo B 2009 Neotrop. Ichthyol. 7 283-288
[11] Yanto H, Hasan H and Sunarto 2015 J. Aquac. ISSN 0853-2532 11-20 (In Indonesia)
[12] Wardhana A H, Kencanawati E, Nurmawati, Rahmaweni, and Jatmiko C B 2000 Influence of provision of buffal stock (Eurhorbia hirta l.) on eretrocytes, hemoglobin and hematocrit value in infected chickens with eimera tenella (Surabaya : University of Airlangga)
[13] Anderson D P and Siwicki A K 1993 Injection or immersion delivery of selected immunostimulant to trout demonstrate enhancement of non spesific defence mechnanism and protective immunity pp 185-426
[14] Huldani 2010 J. Vet. Sci. Med.
[15] Wahjuningrum D 2013 J. Aquac. Ind. 12 94-104 (In Indonesia)
[16] Lestari F N 2015 Effectiveness of addition of cambodian leaf extract on feed to prevent infection of aeromonas hydrophila in carp fish juvenile (Cyprinus carpio) (Bandung : University of Padjadjaran)
[17] Syawal H and Siregar Y I 2010 Immunization of siam jambal fish with ichthhyophthirius multifilis vaccine (Pekanbaru : University of Riau) 163-167
[18] Nurjannah R D D, Prayitno S B, Sarjitro and Lusiastriti A M 2013 J. Aquac.Management Tech. 2 72-83
[19] Muslim M P, Hotly and Widjajanti H 2009 Use of garlic extract (Allium sativum) to treat seeds of patin siam (Pangasius hypophthalmus) infected bacteria aeromonas hydrophila 8 91-100
[20] Boyd C E 1982 Water quality in warm fish ponds for aquaculture (Auburn University) 359
[21] Agustininingtyas N 2014 Utilization of heterotrophic bacteria on dumbo catfish cultivation (Clarias sp.) with water conversion system against FCR (Food Conversion Rate) and protein retention (Surabaya : Universitas Airlangga)