



Comparison of the effectiveness of *Campylobacter jejuni* growth ability in culture media added supplements lysed sheep's blood with lysed cow's blood in chicken meat samples

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Abstract

Blood is a good growth medium for bacteria by preventing the accumulation of toxic oxygen (peroxide and superoxide) and increasing the growth ability of these bacteria. The purpose of this study was to evaluate the ability of lysed sheep blood and lysed cow blood as a substitute for lysed horse blood on *Campylobacter jejuni* growth media, as well as evaluate the growth of *C. jejuni* from chicken meat samples on culture media supplemented with lysed sheep blood and lysed cow blood compared to the media cultures using lysed horse blood supplements. The concentration of lysed sheep blood, lysed cow blood, and lysed horse blood used was 5% each. The chicken meat samples used in this study were 30 samples. Based on the results, 14 samples of *C. jejuni* bacteria showed the ability to grow up to 10^7 and 16 samples up to 10^6 . In the media added with lysed cow blood supplement only one sample was able to grow up to 10^6 , 17 samples grew up to 10^5 , and 12 samples grew up to 10^4 . These results statistically used the one-way analysis of variance (ANOVA) test indicating a significant difference significant ($P < 0.05$). Further tests using post hoc least significance different (LSD) showed a comparison of the average growth in lysed horse blood, lysed sheep blood, and lysed cow blood significantly different ($P < 0.05$), while the average value of each treatment was the addition of lysed horse blood supplements, lysed sheep blood, and cow blood consecutive lysis was 8.551 ± 0.340 , 7.117 ± 0.544 , and 5.265 ± 0.548 . The addition of lysed sheep blood and lysed cow blood to bolton broth media in this study can be used for *C. jejuni* isolation. However, the blood of lysed sheep had a higher effectiveness in the ability to grow *C. jejuni*, which was close to the ability to grow with the addition of lysed horse blood as a control, compared to the addition of lysed cow blood.

Keywords: *Campylobacter jejuni*, chicken meat, lysed sheep blood, and lysed cow blood

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Background

Campylobacter sp. is one of the bacteria that causes foodborne disease of animal origin (Schlundt et al., 2004; Silva et al., 2011). These bacteria can contaminate food products of animal origin such as chicken, beef, duck meat, milk, and other food products of animal origin. *Campylobacter* species that are known to contaminate food products of animal origin are *Campylobacter jejuni* and *Campylobacter coli* (Stern et al., 1992).

Incidence of *Campylobacter* sp. infection in animals varies greatly, but *C. jejuni* and *C. coli* infections in chicken farms play an important role, this is based on data which states that almost 98% of *C.*

jejuni bacteria are found in chicken carcasses with bacterial counts exceeding 10^3 CFU per 100 g carcass samples (Altekruse et al., 1999). Efforts to reduce the incidence of infection in chickens are important efforts to improve production systems and to reduce *C. jejuni* and *C. coli* contamination and have an important role in the field of veterinary public health.

The development of methods that are fast and accurate as well as materials that are relatively inexpensive and easy to obtain are an important unit in a test to detect *C. jejuni* and *C. coli* in food products of animal origin. Many methods can be used to isolate *C. jejuni* from samples. These methods were designed taking into account the conditions

and prerequisites for the growth of *Campylobacter*. Several isolation methods for *C. jejuni* include the initial isolation method developed by Skirrow, the Doyle method, the BAM method in 2001, and Methods for testing for microbial contamination in meat, eggs and milk, as well as their processed products SNI 2897:2008. Currently testing based on homologous DNA and the use of polymerase chain reaction (PCR) have also been developed (McClure and Blackburn, 2003).

One method that is often used in the laboratory is the isolation of bacteria in culture media. In the standard method based on SNI 2897:2008, the *Campylobacter* isolation process begins with sample preparation (rinsing), pre-enrichment, and enrichment. In the pre-enrichment stage, there is a process of adding 5% lysed horse blood supplement, and antibiotics into bolton broth.

In North American laboratories, wool sheep lysis blood is used as the most efficient blood supplement for the manufacture of blood agar media and is used as a standard for defining hemolytic reactions, and can inhibit the growth of non-pathogenic bacteria (Egwuatu et al., 2014). In Europe, horse blood is often used to manufacture 5% lysed blood media and blood agar media. Horse blood contains a lot of factor V (Nicotinamide Adenine Dinucleotide) as a coenzyme needed by anaerobic bacteria such as *Stapylococcus* sp., *Streptococcus* sp., *Haemophilus haemolyticus*, and *C. jejuni* (Nurhidayanti, 2019).

Providing 5% lysed horse blood supplements needs to be considered and studied further due to the difficulty of obtaining 5% lysed horse blood in commercial products. Based on this, it is necessary to find the best solution to find a substitute for 5% lysed horse blood supplements with a relatively cheaper cost and easy to obtain such as 5% lysed sheep blood or 5% lysed cow blood which has the same ratio as 5% lysed horse blood supplements in terms of growth of *Campylobacter* sp.

Materials and Method

This research was conducted at the Laboratory of Veterinary Public Health, Medan Veterinary Center from June to August 2022. The sample used in this study was chicken meat found in the Laboratory of Veterinary Public Health, Medan Veterinary Center. The selected chicken meat is fresh chicken meat by looking at the entry date of the sample no more than 3 weeks of storage at -20 °C. The number of samples used was 30 samples of chicken meat.

This research is an experimental study by comparing two types of supplements (5% lysed sheep blood and 5% lysed cow blood) on the growth capacity of *Campylobacter* in modified charcoal-cefoperazone-deoxycholate agar (mCCDA) culture media. *Campylobacter* bacteria were obtained from the control bacteria *C. jejuni* ATCC® 33560.

Preparation of Lysed Blood Sample Collection

As many as 5% lysis blood in this study used sheep blood, cow blood, and horse blood. The sheep and cattle used were male thin-tailed sheep and male Simmental cross cattle aged of 1-3 years, while commercial horse blood was used for lysis. Making 5% lysis blood is by taking sheep and cattle blood through the jugular vein. The volume of blood taken was 10 ml and Alserver's solution was added with a solution ratio of 1:1 and the date of manufacture was recorded. Next step, a blood hematology examination is performed to count red blood cells and packed cell volume (PCV). Then, the solution was washed with the addition of 1:4 phosphate buffer saline (PBS) and centrifuged at 1500 rpm for 15 minutes until an erythrocyte precipitate formed and the supernatant was discarded. The precipitate was washed again with PBS 1:4, then centrifuged at 1500 rpm for 15 minutes. This washing was carried out three times. After the 3rd washing, the supernatant was discarded, and the erythrocyte precipitate suspension was stored. The blood of lysed sheep and cattle used in this study was 5%. The blood of 5% lysed sheep and cattle was obtained by suspending the 1 ml deposit obtained by

finding the percentage of the hematocrit, then calculating it with the formula $M1.V1 = M2.V2$, then adding the solution according to the calculation.

***Campylobacter jejuni* Culture Preparation**

Culture preparation was carried out by taking one ose of *C. jejuni* culture into 10 ml of brain heart infusion (BHI) broth medium. Then incubated for 48 hours at 42 °C. After incubation, the quantity of *C. jejuni* cells was counted before being inoculated into the sample.

Meat Sample Preparation

Chicken meat samples were prepared 25 g each and washed with sterile distilled water until clean. After that, the chicken meat was pasteurized at 80 °C for 15 minutes. Chicken meat that has been weighed weighing 25 g each is labeled for the total plate count (TPC) test and for inoculating *Campylobacter* bacteria. Before inoculating *Campylobacter* cultures, the samples were cooled down to room temperature.

Supplement Preparation

Supplements of 5% lysed sheep blood and 5% lysed cow blood each using a different sterile tube were put into bolton broth with a ratio of 1:20 and added 1 vial of antibiotic supplement (Cefoperazone 10 mg; Trimethoprim lactate 10 mg; Amphotericin B 5 mg) for 500 ml medium. Control of lysis blood supplement using 5% lysed horse blood.

Preparation for Isolation and Enumeration of *Campylobacter jejuni*

At this stage, *C. jejuni* was inoculated by rinsing 25 g of chicken meat sample with 100 ml of 0.1% BPW solution containing 1 ml of 10^9 CFU/ml *C. jejuni*. Furthermore, the sample was crushed with a stomacher for 2 minutes at a speed of 260 rpm to obtain a dilution of 10^{-1} . From this dilution, it was then made up to a dilution of 10^{-7} .

Dilutions of 10^{-1} to 10^{-7} were put into a sterile centrifuge tube and centrifuged for 15 minutes at 16,000 rpm at 3-4 °C. The supernatant was discarded and 3 ml of

sediment was taken and put into a sterile plastic which had been added 100 ml of bolton broth with different lysed blood supplements was labeled. Then, it was incubated for 24 hours at 42 °C, under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂). Next was done by transferring 0.1 ml of sample solution to a cup that already contains mCCDA selective media. The sample is leveled with a hockey stick. After that, the plates were incubated upside down for 48 hours at 42 °C under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂). After incubation, the number of colonies formed on the plates was counted using the BAM method.

Identification of *Campylobacter jejuni*

Catalase test

A catalase test was performed on colonies suspected of being *C. jejuni*. In the catalase test, as many as 1-2 loops of colonies suspected of *C. jejuni* were transferred to the glass slide. Then into the glass preparation, drops of H₂O₂ solution just above the colony. After dropping the H₂O₂ solution, colonies that are positive for *C. jejuni* will appear as gas bubbles (O₂), indicating positive bacteria to the catalase test.

Bacterial stain

Bacterial staining was started by transferring 1-2 loops of a colony suspected to be *C. jejuni* into a glass slide that had previously been dripped with 1-2 loops of sterile distilled water. Colonies were then flattened and dripped with Ziehl's fuchsin stain. After that, the excess dye was washed in the preparation glass using sterile distilled water. Then fixation was carried out, and the preparations were ready to be observed under a light microscope at a magnification of 1000 x. To see the motility of bacteria, it can be done by eliminating the fixation stage and covering the preparation glass with a cover glass. *C. jejuni* bacteria will appear red with Ziehl fuchsin staining, have a spiral shape, wavy rods, and are motile.

Data Analysis

Descriptive data analysis in this study was used to describe the growth comparison of *Campylobacter* on mCCDA after the addition of lysed sheep or lysed bovine blood supplements to bolton broth media. The data obtained in this study were first tested for normality and homogeneity. After the data were declared to be normally distributed and homogeneous, statistical analysis of the data was performed using the one-way analysis of variance (ANOVA) method using the statistical program for social science (SPSS) with a confidence level of 95% or $\alpha = 0.05$ (Avaro et al., 2010). The data obtained was further tested (post hoc test) with the least significance different (LSD) or least significant difference (BNT) test. LSD value measurement formula (BNT): (Trihendradi, 2010).

$$BNT_{\alpha} = (t_{\alpha, df_e}) \cdot \sqrt{\frac{2(MS_E)}{r}}$$

Results and Discussion

This study used 30 samples of chicken meat. Samples were obtained from sample stocks in the Laboratory of Veterinary Public Health of Medan Veterinary Center which were taken randomly by looking at the date of entry of the sample no more than three weeks.

All chicken meat samples were sterilized by pasteurization at 80 °C for 15 minutes. To prove that the sample is sterile, a total plate count (TPC) test is performed. The results of the TPC test for all samples showed the number 0 CFU/g, which means that the sterilized sample had no bacteria. All samples that had been sterilized were rinsed with 1 ml of *C. jejuni* isolate 1.5 x 10⁹ CFU/ml which was obtained from the calculation of 1/2 mcFarland.

All samples that had been cultured for *C. jejuni* in this study showed colonies as shown in Figure 1. Colonies were round and small, grayish white in color, with smooth colony edges and convex surfaces. This is in accordance with the results of research by Nachamkin and Blaser (2000), which states that The *C. jejuni* colonies that formed

tended to be colorless or gray. This colony looks expanding or round and convex. Both types of colonies can appear on a Petri dish.



Figure 1. Morphological colonies of *C. jejuni* growth on mCCDA media

On mCCDA media, there were differences in the ability to grow *C. jejuni* in each treatment given the blood supplement of 5% lysed horses, 5% lysed sheep, and 5% lysed cows, which can be seen in Table 1.

In this study, isolated colonies on mCCDA media were taken for confirmation tests using the Gram stain method and the catalase test. The results of the Gram stain and catalase test for each sample can be seen in Table 2 and Figure 2. There are several bacterial staining techniques, but the most commonly used is the Gram stain. Gram staining is the most commonly used method for staining bacterial cells (BAM, 2001).

Based on the results of observations under a microscope, the morphology of *C. jejuni* bacteria can be seen in the form of a curved or spiral rod, like the letter S and is a Gram-negative bacterium. According to Stern et al. (1992), observation with wet preparations under a microscope will show *C. jejuni* cells which are highly motile, in the form of wavy rods, S-shaped or spiral-like, very small in size and thin. Gillespie et al. (2005) added that *C. jejuni* is a very small Gram-negative bacterium (0.2 - 0.5 wideµm, length 0.5 - 5µm), corrugated, thin, some zigzag or spiral in shape. These bacteria do not form spores, are catalase positive, can reduce nitrate and are highly motile with flagella present at one or both ends of their bodies but 20% of these bacteria are non-motile. In this study, *C. jejuni* underwent a change in shape from curved rods to cocci.

This can happen due to long observation, cell stress, and oxygen contamination for a long time. According to Doyle (1989), the cell shape of *C. jejuni* will change to a non-motile and non-culturable cocci if it is old enough (long lived). *C. jejuni* can also change from cultured to uncultured form very quickly after its log growth phase ends. Apart from being affected by age, *C. jejuni*

is prone to changes in morphological form due to changes in environmental conditions that stress the bacteria. Stress on the bacteria is indicated by a change in morphology from a wavy rod shape to a cocci shape. One of the changes in environmental conditions that can make *C. jejuni* is the increase in oxygen levels.

Table 1. The ability to grow *Campylobacter jejuni* in each medium containing the blood of lysed horses (control), sheep, and cattle

Sample code	Total <i>C. jejuni</i> cells (CFU/ml)		
	Lysed horse blood 5%	Lysed sheep blood 5%	Lysed cow blood 5%
1	4.17x10 ⁸	5.57 x 10 ⁷	4.00 x 10 ⁵
2	6.10 x 10 ⁷	4.10 x 10 ⁶	3.00 x 10 ⁴
3	3.77 x 10 ⁸	2.57 x 10 ⁷	4.20 x 10 ⁵
4	5.83 x 10 ⁸	5.60 x 10 ⁶	5.00 x 10 ⁵
5	2.73 x 10 ⁸	5.50 x 10 ⁶	3.30 x 10 ⁵
6	6.50 x 10 ⁸	3.33 x 10 ⁷	3.90 x 10 ⁵
7	5.33 x 10 ⁸	1.50 x 10 ⁶	7.40 x 10 ⁵
8	5.30 x 10 ⁸	3.47 x 10 ⁷	4.50 x 10 ⁵
9	4.13 x 10 ⁸	3.71 x 10 ⁶	5.00 x 10 ⁴
10	3.63 x 10 ⁸	5.50 x 10 ⁶	5.40 x 10 ⁵
11	3.60 x 10 ⁸	2.87 x 10 ⁷	2.00 x 10 ⁴
12	3.47 x 10 ⁸	3.82 x 10 ⁶	2.70 x 10 ⁵
13	6.33 x 10 ⁸	3.60 x 10 ⁷	5.00 x 10 ⁴
14	4.50 x 10 ⁷	4.87 x 10 ⁷	5.30 x 10 ⁵
15	5.73 x 10 ⁸	5.40 x 10 ⁶	7.00 x 10 ⁴
16	5.00 x 10 ⁸	4.27 x 10 ⁷	4.70 x 10 ⁵
17	7.00 x 10 ⁸	4.60 x 10 ⁶	4.10 x 10 ⁵
18	4.40 x 10 ⁸	9.40 x 10 ⁷	4.00 x 10 ⁴
19	4.17 x 10 ⁸	4.61 x 10 ⁶	4.30 x 10 ⁵
20	6.40 x 10 ⁷	6.47 x 10 ⁷	1.73 x 10 ⁶
21	3.57 x 10 ⁸	7.53 x 10 ⁷	5.00 x 10 ⁴
22	4.73 x 10 ⁸	6.67 x 10 ⁷	6.60 x 10 ⁵
23	6.70 x 10 ⁸	3.63 x 10 ⁶	5.00 x 10 ⁴
24	5.20 x 10 ⁷	3.80 x 10 ⁶	6.00 x 10 ⁴
25	4.77 x 10 ⁸	6.50 x 10 ⁶	6.00 x 10 ⁴
26	5.00 x 10 ⁸	4.30 x 10 ⁶	3.60 x 10 ⁵
27	3.57 x 10 ⁸	3.40 x 10 ⁷	2.00 x 10 ⁴
28	6.10 x 10 ⁸	4.60 x 10 ⁶	4.80 x 10 ⁵
29	6.37 x 10 ⁸	4.90 x 10 ⁶	5.00 x 10 ⁴
30	5.10 x 10 ⁸	5.73 x 10 ⁷	4.60 x 10 ⁵

The catalase test was carried out by dropping H₂O₂ on a colony suspected of being *C. jejuni* and the results shown in all samples in this study were the formation of gas bubbles (foam) when H₂O₂ was dropped (Figure 3). This is because *C. jejuni* is a positive catalase bacterium, meaning that this bacterium is capable of producing the

catalase enzyme which can catalyze the reaction of breaking down H₂O₂ into oxygen gas and water. Hydrogen peroxide (H₂O₂) and superoxide are usually produced by some bacteria from the reduction reaction of oxygen compounds. Both of these molecules are toxic to *C. jejuni* (Khoiruddin, 2008).

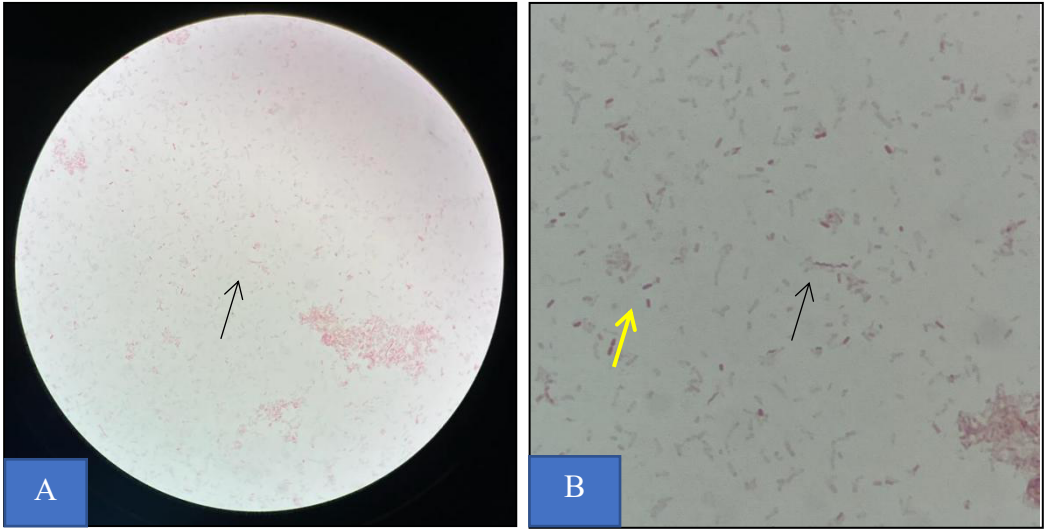


Figure 2. Morphology of *C. jejuni* at 100x magnification (A) and morphology of *C. jejuni* in the form of cocci (yellow arrows) and curved rods (black arrows) at 1000x magnification (camera zoom) (B)

Table 2. The results of Gram stain and catalase test

Gram stain	Catalase test	Sample code		
		Horse blood lysis	Lysed sheep blood	Lysed cow's blood
Stem curved (spiral) red color	Positive	1,2,3,4,5,6,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30	1,3,4,5,6,7,8,9,10,11,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30	2,23,4,5,6,7,8,9,11,12,13,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29
		7	2,12	1,10,14,30
Stem curved (spiral) and coccus, red color	Positive			

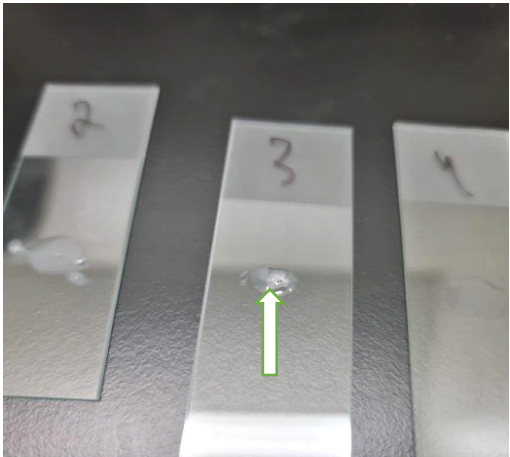


Figure 3. The results of positive catalase test indicated by the presence of gas bubbles (foam)

The addition of 5% lysed sheep blood and 5% lysed cow blood to bolton broth media in this study showed the following successive results 14 samples tested showed the ability to grow *C. jejuni*

bacteria up to 10^7 out of a total of 30 samples and had the lowest growth ability, up to 10^6 ; whereas with the addition of 5% lysed cow blood there was only one sample that was able to grow up to 10^6 , 17 samples

grew up to 10^5 , and had the lowest growth rate of up to 10^4 . This was not much different from the results of Alvaro et al. (2010) namely by adding 5% lysed sheep blood to the culture medium, the highest growth of *C. jejuni* was up to 10^8 and the lowest was 10^6 , while by adding lysed cow blood to the culture medium, the highest growth of *C. jejuni* was up to 10^6 and the lowest was 10^5 .

Blood is a good growth medium for bacteria by preventing the accumulation of toxic oxygen (peroxide and superoxide) and increasing the growth of organisms because substances present in blood act as detoxification (Bolton et al., 1984; Corry et al., 2001). According to Cappuccino (2014) the conditions for a good media to support bacterial growth must contain the right nutrients for the microorganisms to be cultured and are sterile and do not contain substances that can inhibit the growth of the bacteria themselves (Cappucino and James, 2014). The addition of 5% lysis blood aims to neutralize toxic products that may be formed due to media exposure to light or air. According to Bolton and Robertson (1982) *C. jejuni* is very sensitive to the presence of toxic peroxide and superoxide compounds which are products formed from the media due to chemical reactions catalyzed by light.

When compared to controls, lysed horse blood has a *C. jejuni* growth rate of up to 10^8 , higher than lysis sheep blood with *C. jejuni* growth only able to 10^7 . According to Oxoid (2023) this can happen because when horse blood is lysed, Erythrocytes release the enzyme thymidine phosphorylase which can convert thymidine into thymine compounds. This enzyme is not owned by sheep erythrocytes. The lytic agents used for lysed horse blood are saponins. Baumann et al. (2000) and Ayuningtyas et al. (2015) suggested that saponins can cause an increase in the permeability of the lipid bilayer of red blood cells to macromolecules which will later cause hemolysis. Scientificlabs (2023) adds that Saponins can interfere with red blood cell membranes by binding to complexes with cholesterol in the lipid layer and causing the process of lysis of red blood cells to move faster. This process can produce an excellent

supplement of lysed blood and can be used in media products for the growth of organisms such as *C. jejuni*.

Low growth *C. jejuni* using lysis of bovine blood supplements may be caused by several factors, namely enzymatic activity, the high content of phospholipids in bovine blood, copper deficiency, and not having enough NAD. According to Chauhan et al. (1982) erythrocytes have superoxide dismutase and catalase enzymes, which can increase enzymatic activity. The increase and decrease in the activity of these two enzymes can affect the detoxification capacity of the blood. In addition, according to Florin-Christensen et al. (2001) ruminant erythrocytes have a lot of choline-phospholipids which can affect the physiological activity of blood cells.

On the other hand, copper deficiency in cattle can cause changes in the capacity of the antioxidant mechanisms in cells (Sukalsky et al., 1997). Other factors that can affect the growth *C. jejuni* is the lack of factor V or Nicotinamide Adenine Dinucleotide (NAD) during the process of making blood cell lysis.

The data analysis used in this study is the one-way ANOVA test. Before calculations are carried out using one way ANOVA, it is necessary to fulfill one of the mandatory requirements, namely the data normality test. The purpose of doing a normality test is to find out whether data is normally distributed or not. The normality test used in this study is the Shapiro-Wilk test (Table 3). The number of samples is less than 50, then the data is said to have a normal distribution and the one-way ANOVA test can be carried out if $p > 0.05$.

Data criteria residuals are normally distributed or not with the Normal P-Plot approach can be done by looking at the distribution of the points in the image. If the distribution of these points is close to a straight line (diagonal), then it is said that the residuals are normally distributed. On the other hand, when the distribution of these points is away from the straight line, the data is not normally distributed. Based on the results of the normality test, the residuals are normally distributed (Figure 4).

Table 3. Normality test

Test	Stats
Shapiro-Wilk's	0.944 *

The next step is to carry out homogeneity test of variance to analyse whether the samples taken have the same variance. Table 4 shows that the

homogeneity test has a significance value variance or probability $p > 0.05$, so it can be concluded that the data comes from populations that have the same variance. Then, all of the data was analyzed using the one-way ANOVA hypothesis test which is presented in Table 5.

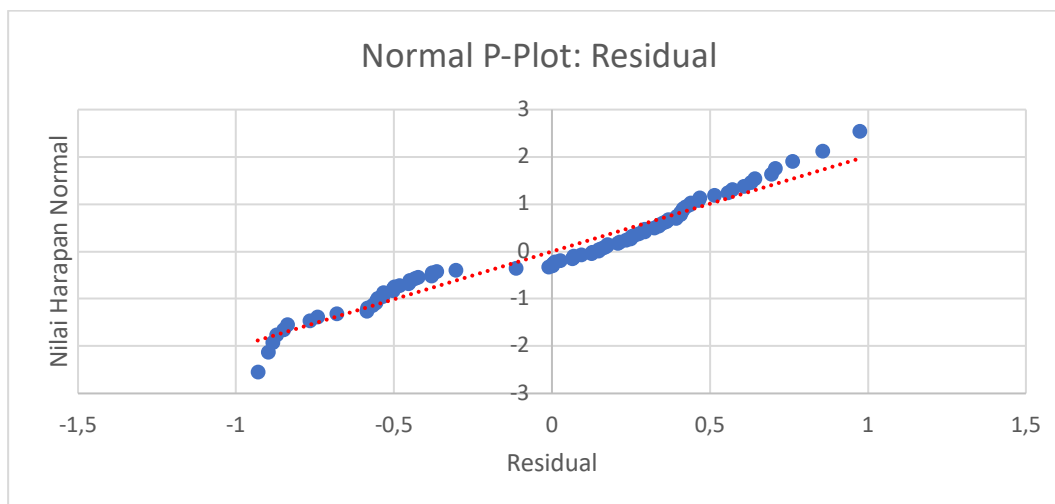


Figure 4. The results of normal P-Plot test

Table 4. Levene's test for homogeneity of variance

		Levene statistics	df1	df2	Sig.
lg10_h	Based on means	19.26	2	87	.000

Table 5. One way ANOVA test

Variety source	df	Sum of squares (Ssq)	Mean of squares (Msq)	F-stat.	Sig.	F. 05	F. 01
Treatment (P)	2	162.8917	81.4459	343,339**	0.000	3.101	4,858
Error	87	20.6379	0.2372				
Total	89	183.5296					

Based on the analysis using one way ANOVA, the results show $p < 0.05$, so accept H_0 and reject H_1 . The LSD post hoc Test is used to determine whether a group has a significant difference from other groups. The results of the LSD post hoc test analysis in this study showed an asterisk (*), which means that all groups had significant differences from other groups.

In Table 6 it can be seen that from the two treatments (P2 and P3) the 5% lysed blood supplement given, it can be seen that P2 (lysed sheep blood supplement 5%) has the best average growth value of 7.117 ± 0.544 , where the value is close to P1 (5% lysed horse blood supplement) as a control

which is equal to 8.551 ± 0.340 . Then followed by P3 (5% lysed cow blood supplement) where the average value of growth was 5.265 ± 0.548 .

Table 6. Treatment average value

Treatment (P)	Average
1	8.551 ± 0.340
2	7.117 ± 0.544
3	5.265 ± 0.548

Note:

P1 = 5% Lysed horse blood, P2 = 5% Lysed sheep blood

P3 = Lysed cow blood 5%

Conclusion

The addition of lysed sheep blood and cow blood to bolton broth media in this study can be used for *C. jejuni* isolation. It was indicated by positive results in mCCDA media in both treatments. Lysed horse blood as a control had a higher growth ability of *C. jejuni*, followed by lysed sheep blood and cow blood. Comparison of the effectiveness of the growth ability of *C. jejuni* has the average value of growth for each successive treatment as follows 8.551 ± 0.340 , 7.117 ± 0.544 , and 5.265 ± 0.548 , with the average value of growth ability was different significantly ($P < 0.05$).

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