

Determination of Clove Extract Anesthetic Dosage in Transportation Activities of Carp (*Cyprinus carpio*) Supply Chain

*Penentuan Dosis Anestesi Ekstrak Cengkeh dalam Aktivitas Pengangkutan pada Rantai Pasok Ikan Karper (*Cyprinus carpio*)*

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Abstract

This study aims to obtain the best dose of clove extract anesthetic in the transportation of carp (*Cyprinus carpio*) from suppliers to consumers in the supply chain of carp. Alive, healthy, and not defective carp from the Fish Seed Center with 3-5 cm size were used as objects in this study. Carp fish bag and 50 x 35 x 30 cm Styrofoam boxes were used as containers which were placed randomly during the delivery of carp. The study was conducted with four treatments of different clove extract levels, treatment A as a control (0 ppm), B (3.3 ppm), C (6.7 ppm), and D (10 ppm), with three replications each. The examination of the anesthetic condition of carp was carried out four times during transportation, at 0, 6, 12, and 24 hours. Analysis of Variance (ANOVA) was conducted to see the impact of the treatments, and then the Tukey test was carried out to see the differences between treatments. The analysis was carried out using SPSS version 21. The results showed that increasing the anesthetic dose of clove extract impacted the health condition and survival rate of the carp seedlings during transportation. The highest survival rate (85%) was achieved at a concentration of 6.7 ppm.

Keywords: anesthesia dosage, clove extract, carp, the survival rate

Abstrak

Penelitian ini bertujuan untuk mendapatkan dosis anestesi ekstrak cengkeh terbaik dalam pengangkutan ikan karper (*Cyprinus carpio*) dari pemasok ke konsumen dalam rantai pasok ikan karper. Ikan karper yang digunakan sebagai objek dalam penelitian ini adalah ikan karper dari Balai Benih Ikan dengan ukuran 3-5 cm, dalam keadaan hidup, sehat, dan tidak cacat. Kantong ikan karper dan Styrofoam berukuran 50 x 35 x 30 cm digunakan sebagai wadah selama pengiriman ikan karper dan penempatannya dilakukan secara acak. Penelitian dilakukan dengan 4 perlakuan kadar ekstrak cengkeh yang berbeda, yaitu A sebagai kontrol (0 ppm), B (3,3 ppm), C (6,7 ppm), dan D (10 ppm) dengan masing-masing 3 ulangan. Pemeriksaan kondisi anestesi pada ikan karper dilakukan 4 kali selama pengangkutan, yaitu 0, 6, 12, dan 24 jam. Analisis of Variance (ANOVA) dilakukan untuk melihat dampak dari perlakuan kemudian uji Tukey dilakukan untuk melihat perbedaan antar perlakuan. Analisis dilakukan dengan SPSS versi 21. Hasil penelitian menunjukkan bahwa penambahan dosis anestesi ekstrak cengkeh berdampak pada keadaan kesehatan dan sintasan ikan karper saat transportasi. Sintasan tertinggi (85%) dicapai pada konsentrasi 6,7 ppm.

Kata kunci: dosis anestesi, ekstrak cengkeh, ikan karper, sintasan

INTRODUCTION

The current demand for fresh carp (*Cyprinus carpio*) is relatively high. Consumers want fresh carp to be available in good quality. Fresh carp fish production involves several stakeholders in a supply chain. The supply chain members for fresh carp before it reaches the end consumers include

suppliers of seeds, cultivators, and sellers of fresh carp. The quality and quantity of fresh carp are influenced by its handling from the seeds supplier to the consumer's hands, especially during transportation. Excellent and appropriate transportation methods are needed so that the consumers receive the fish alive. Stunning is required in the transportation of the live fish. Stunning fish can reduce the

the level of oxygen consumption, excretion of CO₂ and NH₃, and residues (Boijink et al., 2016; Dominic, Inasu, & Swapna, 2016).

There are two types of products used for stunning, i.e. artificial and natural products. Artificial stunning products, for example, MS-222 and salt solutions (Yanto, 2012) and sodium bicarbonate (Pawar, Ingole, & Sreepada, 2013). Natural stunning products, for example, ice cube (Maraja, Salindeho, & Pongoh, 2017), chamomile oil (*Matricaria chamomilla*) (Canet et al., 2017), a tobacco extract, noni extract, papaya extract, *Tephrosia vogelii* bark extract (Solomon, Cheikyula, & Anju, 2014) and clove (*Syzygium aromaticum*) extracts (Palimbu & Mandiangan, 2019; Kamble, Saini, & Ojha, 2014; Hassan, Abdulrahman, & Salman, 2016; Sumahiradewi, 2014; Diyaware, Bokko, & Suleiman, 2015 Clifton, Erlina, & Astori 2014).

Clove extract is a natural stimulant, carminative, antiemetic, antiseptic, antispasmodic, and having a unique taste and aroma (Wahyulianingsih, Handayani, & Malik, 2016). Clove extract is a natural anesthetic agent, effective for the stunning process of fish (Fauziah et al., 2011). The aromatherapy content in cloves can stun and make the fish relax, which maintained fish survival. The clove extracted content is environmentally friendly, cheap, and easily obtained (Mikhsalmina, Muchlisin, & Dewiyanti, 2017).

The proper dose of clove extract used in fish anesthesia can decrease the tense effect, curb the fish metabolism and movement; hence the fish remain alive for a longer transportation duration. Therefore, research on the usefulness of cloves in the carp fish stunning process is needed because of the superiority of the clove extract content.

Several experiments had been carried out to study the efficacy of clove oil as an anesthetic for carp (Kamble, Saini, & Ojha, 2014; Hassan, Abdulrahman, & Salman, 2016). Sumahiradewi (2014) used cloves as an anesthetic agent for the transportation of tilapia. Mikhsalmina et al. (2017) used clove extract as an anesthetic product to transport milkfish (*Chanos chanos*) seedlings. The use of clove oil to transport catfish seeds was also carried out in other studies (*Clarias Gariepinus*) (Diyaware et al., 2015). Research by Clifton et al. (2014) tested the survival rate of *Tor sp.* with the most significant survival rate of 83.33% in the treatment of 0.015 ml/l clove extract concentration. Some of these studies indicate that cloves oil and extract provide natural anesthetic agents which is effective for stun fish during their

transport from suppliers to consumers in the fish supply chain.

This study aims to obtain the best dose of clove extract anesthetic in carp transport from suppliers to consumers in the carp supply chain. The benefit of the results of this study is to provide information about the best dosage and natural ingredients of anesthesia in carp transportation to maximize its survival rate so that the perfect fulfillment of consumer orders in the carp fish supply chain can be improved.

METHODS

This research was conducted at the Pangkajene Fish Seed Center (FSC), Tanete Village, Maritenggae District, Sidenreng Rappang Regency, South Sulawesi Province. Materials and tools used during the study were Styrofoam, plastic bags, aerators, oxygen tank, small fishnets, basins, buckets, rubber bands, thermometer, dissolved oxygen meter, pH meter, digital scales, stationery, and cameras. The experimental design made was four treatments with three replications for 24 hours of transportation. The dosage of adding clove extracts used was based on the results of Diansyah's (2006) research, treatment A as a control (0 ppm), B (3.3 ppm), C (6.7 ppm), and D (10 ppm). The sample used was carp fish with a 3-5 cm length, produced at the Pangkajene FSC. Plastic bags and Styrofoam boxes with 50 x 35 x 30 cm size were used as containers during the delivery of carp and randomly placed.

Research Procedure

Clove extract Preparation

The clove extract was made by mashed 100 grams of dry cloves using a blender. The clove powder was then extracted by boiling it in 1000 ml of distilled water. The clove boiling water was then filtered to separate the dregs. The extract obtained was stored in a tightly capped dark glass bottle to maintain the quality.

Preparation of Carp in a Container

This stage started by preparing 12,000 carps with a length of 3-5 cm produced at the Pangkajene Fish Seed Center (FSC). The plastics bag that had been filled with 2 liters of water was then filled with 1000 carp fish (the density was 500 fish/liter). The water used was natural freshwater that had been deposited for 24 hours. The carp bag used is made of polyethylene (PE) with a thickness of 0.06 mm and size of 50 cm x 30 cm

(the volume was 5 liters).

The clove extract was then added to each bag according to the treatment dose tested. Oxygen was added to the plastic bags at a ratio of 3: 1 (3 parts oxygen to 1 part water), so the fish still get enough oxygen. The plastic bags then tied using a rubber band. The carps packed in plastic bags were then put into a Styrofoam box and placed in a pick-up truck covered with a tarpaulin for 24 hours. Observation of fish response to anesthesia was carried out during the transportation process at specific periods, i.e. 0, 6, 12, and 24 hours.

Data Analysis

The impact of giving an anesthetic dose of clove extract was analyzed using Analysis of Variance (ANOVA) to see the effect of anesthetic dose. The Tukey test was used to see the difference in anesthetic dose results (Supardi, 2013). Data analysis was performed using the Statistical Package for Social Science (SPSS) 21 for Windows software. Presentation diagrams were made using Microsoft Excel 2007 software. The reaction of fish temperament in the stunning stage at a certain period was analyzed descriptively based on the research results of Coyle, Durborow, & Tidwell (2004), as shown in Table 1.

The survival rate of carp in each treatment was analyzed by the following formula (Muchlisin et al., 2016):

$$SR = \frac{N_t}{N_0} \times 100 \% \quad (1)$$

where,

SR = Survival rate (%)

N_t = the number of living carp fish at the end of the study

N₀ = number of carps that died at the end of the study

RESULTS AND ANALYSIS

Response of Carp during Anesthesia

Anesthesia can be done with chemical solutions, low temperature, or electricity (Coyle *et al.*, 2004). Anesthetic agents, directly and indirectly, damage certain cationic equilibrium in the brains of fish during anesthesia. The disruption of ionic equilibrium in the brain causes the fish to feel numb due to decreased nerve performance. Table 2 shows the results of observations of the reaction of carp fish during anesthesia.

Table 2 shows that the carp behaved naturally by administering an anesthetic dose of 0 ppm clove extract from the 6th hour to the 24th hour during the transportation time. The normal condition of the fish can be observed from the mobility of their balanced swimming motion and normal reactions to external stimulants. Carp did not react at a dose of 0 ppm until the end because no clove extract anesthetic was given in the fish container during transportation.

The use of an anesthetic dose of 3.3 ppm clove extract showed that carp did not react until the 6th hour, which can be seen from the consistently normal behavior of carp during anesthesia. This is because the active substance of clove extract has not impacted the balance of carp's nerve function and brain tissue. However, there is still an opportunity for the clove extract active substance to enter the carp body through the gills and membrane diffusion in carp. The reaction of carp fish to clove extract was shown at 6 to 12 hours. Carp sensitivity to external stimulants is slow, and mobility is slow. This is because the force of the anesthesia begins to affect the carp's nervous system. The anesthetic dose of clove

Table 1. Fish activity in the stunning stage at a certain period

Level	Synonym	Fish Activity/Behavior
0	Normal	Sensitive to external stimulants, operculum mobility, and regular muscle stretching.
Ia	Light fainting (light sedation)	The sensitivity to external stimulants is slightly reduced, the mobility of the operculum is slowed down, the harmony is normal.
Ib	Fainting (deep sedation)	There is no sensitivity to external stimulants, except with intense pressure, the mobility of the operculum is slowed down, normal harmony.
Iia	Loss of Partial Balance	Muscle stretching is weak, swimming irregularly, responds only to powerful vibrational stimulants and friction, tight operculum mobility.
Iib	Loss of Total Balance	The stretching of the muscles ends, the mobility of the operculum is slow but rhythmic, the spinal cord response disappears.
III	Reflex Movement Absent	Absent reactivity, reduced operculum mobility, and no rhythm, weak heart rate, no response
IV	Collapsed (medullary pillars)	The mobility of the operculum ends, respiration stops, then a few minutes after that, the heart rate stops.

Table 2. The reaction of carp during anesthesia

Treatment	Observation Duration (hours)			
	0	6	12	24
A (0 ppm)	Sensitive to external stimulants, harmony and reasonable mobility	Sensitive to external stimulants, harmony and reasonable mobility	Sensitive to external stimulants, harmony and reasonable mobility	Sensitive to external stimulants, harmony and reasonable mobility
B (3,3 ppm)	Sensitive to external stimuli, harmony and natural mobilization	Sensitive to external stimulants, harmony and natural mobilization	Sensitivity to external stimulants decreases and mobility is reduced	Sensitivity to external stimulants decreases and mobility is reduced
C (6,7 ppm)	Reactive with external stimulants, harmony and reasonable mobility	Weak mobility, sensitivity to weak stimulants, relaxed mobility, and occasionally to the surface of the water.	Sensitivity to external stimulants decreased	No sensitivity to external stimulants and slow mobility
D (10 ppm)	Reactive with external stimulants, harmony and reasonable mobility	Absent reactivity to external stimulants and slow movement.	Normal movement, high sensitivity to stimulants, and wild and restless mobility.	Very weak mobility, weak sensitivity to external stimulants, and a dazed and oblique body at the bottom of the container.

extract of 3.3 ppm is still below the threshold that affects consciousness so that the stunning process takes a long time.

The anesthetic dose of clove extract at 6.7 ppm used showed its effect on carp starting at the 6th hour, and then the carp experienced deactivation at the 24th hour. This dose causes weaken the mobility of carp. Some of the carp began to faint at the 12th hour, and then all the carp fainted at the 24th hour. A dose of 6.7 ppm is good and appropriate to stun fish because the anesthetic power is not too strong.

The carp began to react at 6 hours using an anesthetic dose of 10 ppm of clove extract. The fish began to lose balance and tilted at the bottom of the container at 12 to 24 hours. This indicates that the use of a 10 ppm dose of 10 ppm makes the fish in a fragile state, affecting the fish's resistance during transportation. According to Cahyono & Mulyani (2012), the use of a dose of 10 ppm is considered a bit excessive to apply because the strength of the anesthesia given is very high. A dose of 10 ppm can make the fish too weak when transported. The use of too high a concentration is also economically inefficient.

The induction time for the active substance of clove extract will be faster if the clove extract dose added to the carp fish container is high. The absorption of anesthetic substances into the fish body is faster if the anesthetic dose added in the

fish container is higher (Maryani, Efendi, & Utom, 2018). The carp fainted more rapidly at the higher the anesthetic dose of clove extract. This is due to the increasing doses of anesthetics absorbed into the respiratory tissue so that the nervous system does not function more quickly (Cahyono & Mulyani, 2012). Fish that enter the fainting stage are indicated by the fish stopping swimming, the operculum slowing down, and falling to the bottom (Amris, Rahim, & Yaşın, 2020). This is due to the active ingredients of clove extract entering the fish body through the gills and muscle tissue (Öğretmen, Gölbaşı, & Kutluyar, 2016). Ferreira, Schoonbee, & Smit (1984) stated that fish anesthetics could also enter the fish body through the dermis. Anesthetic substances that enter the blood tissue will be circulated throughout the body, including the nervous system, brain, and other tissues so that the fish becomes numb and fainted. The anesthetic agent concentration in the brain or nerve tissue determines the power of anesthesia in fish.

The fainting state of the fish can be divided into four stages: light sedation, deep sedation, loss of balance, zero reflex activity, and medullary collapse. The deep sedation phase is the highly recommended stage for carp stun, as fish movements tend to stop at this stage. Carp are not affected by external disturbances, and their balance is stable. The use of oxygen for each deep

sedation of carp is at the basal rate needed by the fish to stay alive.

Carp fish underwent deep sedation when using an anesthetic dose of 6.7 ppm clove extract. Fish consciousness is regained by putting fish into clean water. The water contains enough oxygen that enters through the gills into the bloodstream and cleans the residue of anesthetics on the carp's body. Gills play an essential role in cleaning the fainting material in the fish awareness process (Mikhsalmina et al., 2017).

Survival Rate

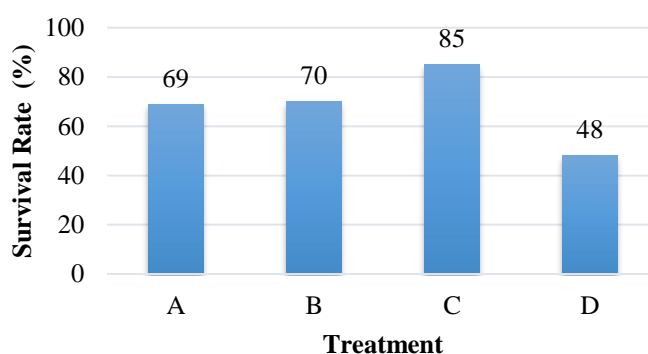
The survival rate is the percentage of the number of carp that live in one experiment duration. Figure 1 shows the survival rate of carp for each treatment. The effect of the anesthetic dose of clove extract on carp was analyzed using ANOVA. The results showed that anesthetic treatment using clove extract at different doses significantly affected carp with a significance value of 0.000 ($p < 0.05$). The Tukey test was then carried out to see the difference in the results of the anesthetic dose administration. The Tukey test results showed that treatment A was not significantly different from B with a significance value of 0.568 ($p > 0.05$). However, treatment A was significantly different from C and D with a significance value of 0.00 ($p < 0.05$).

Figure 1 shows the highest survival rate of 85% obtained at an anesthetic dose of 6.7 ppm clove extract. This is presumably because the carp experienced a gradual fainting state starting in the 6 hours of transportation. Fish in a fainted state can reduce stress during the transportation process (Mikhsalmina et al., 2017). Calm fish conditions can reduce stress, reduce metabolic speed, and reduce oxygen consumption (Maraja et al., 2017). The survival rate in the use of clove extract

anesthetic doses was 3.3 ppm lower than the survival rate in clove extract anesthetic doses of 6.7 ppm. This is due to the low anesthetic strength so that the carp metabolism is still relatively high and the carp do not faint. This condition will make oxygen consumption high enough, and carp experience stress during transportation so that many carp die from lack of oxygen. The lowest synthesis of 48% was achieved using a synthesis dose of 10 ppm of clove extract. The very high anesthetic strength at this dose makes the fish weak at the end of the transport duration. Metabolism and oxygen demand will increase in conscious fish. If the availability of oxygen is not added, the fish will be deprived of oxygen and gradually weak, so that death will occur (Abid, Masithah, & Prayogo, 2014).

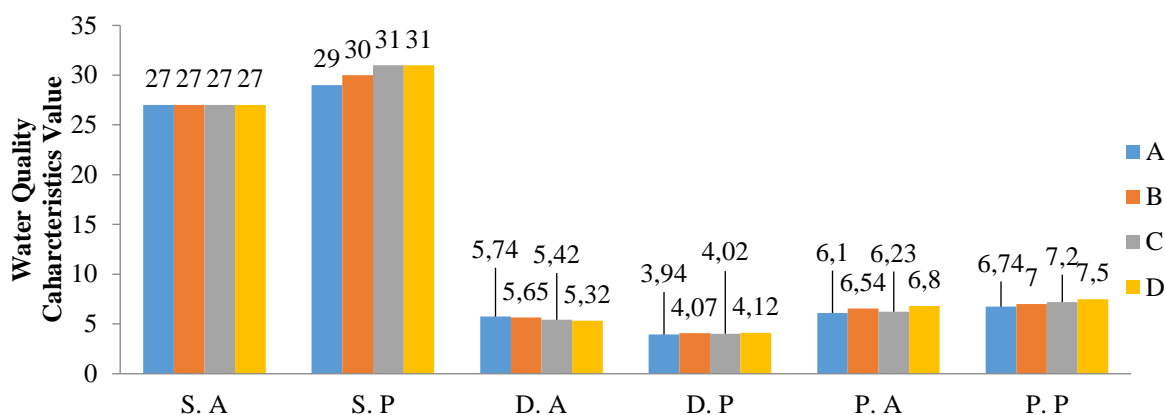
Water Quality

The quality of water used as a living medium for carp in the container during transportation also needs to be measured to identify its effect on carp fish synthesis. Water quality includes the physical, chemical, and biological conditions of water that affect the survival, growth, reproduction, and biomass production of cultivated animals (Nasir & Khalil, 2016; Pellu, Rebhung, & Eoh, 2018). Temperature, acidity, and oxygen levels in water are essential because they impact the workability of the anesthetic agent, the metabolic rate of fish, and the absorption strength of anesthetics (Öğretmen et al., 2016). Water quality measurements are carried out before anesthesia and during unloading. The results of water quality measurements before anesthesia are not much different from the water quality when unloading, and the water quality is still suitable for (Midihatama, Subandiyono, & Haditomo, 2018) (Figure 2). Water quality standards used as natural living media for carp fish are.



A: 0 ppm (control); B: 3,3 ppm; C: 6,7 ppm; D: 10 ppm

Figure 1. Carp Survival Rate for Each Treatment



Treatment A (0 ppm (control)), B (3.3 ppm), C (6.7 ppm), D (10 ppm)

S.A = Initial Temperature ($^{\circ}\text{C}$), S.P = Final Temperature ($^{\circ}\text{C}$)

D.A = Initial Dissolved Oxygen (ppm), D.P = Final Dissolved Oxygen (ppm)

P.A = Initial pH, P.P = Final pH

Figure 2. Graphic of Water Quality Measurement (Temperature, DO, and pH) Results

temperature 25°C - 30°C , pH 6-8, and dissolved oxygen 3-5 ppm (Saparinto, 2012; Prasetyo, Desrina, & Yuniarti, 2017) Carps that faint during transportation are not caused by a decrease in water quality as a medium of life, but carp are fainted due to clove extract used as an anesthetic agent (Fauziah et al., 2011).

The lowest temperature increase occurred in treatment A (increased 2°C), while the highest temperature increase occurred in treatment C and D (each increased 4°C). This different temperature increase is because in treatment A the survival rate of fish is low, the number of fish in the media is less, while in treatment C and D, the survival rate of fish is high, which results in higher oxygen consumption at the end of the study. The dose of clove extract anesthetic has no direct effect on the medium temperature. The higher the clove extract dose given to the optimal dose (6.7 ppm), the better it is to stun the fish. Fish that have been passed out have a low body metabolism. Low body metabolism results in low oxygen use during transport. Survival affects temperature changes because the more fish that survive will have an impact on oxygen consumption.

The most decrease in dissolved oxygen (DO) occurred in treatment A (decreased by 1.8 ppm), while the lowest decrease in DO occurred in treatment D (decreased by 1.2 ppm). Decreased DO is more inclined to oxygen consumption by fish for metabolism. Putra (2015) states that the level of oxygen consumption is a marker of the metabolic rate of fish. Treatment A did not add clove extract as an anesthetic so that initially, the fish were in normal metabolism and their oxygen

needs were also normal. During the transportation process, the stress effect plays a role in oxygen consumption and carbon dioxide expenditure level because the fish are conscious and require much energy to adjust (Yanto, 2012). This adjustment ultimately increases the metabolic rate of fish, especially basal metabolism; the processes of respiration, blood circulation, and intestinal peristalsis (Putra, 2015). Treatment D is a treatment with the highest addition of clove extract (10 ppm) so that during the transportation process, the fish is in a faint state. Fish in a fainted state have a low metabolic rate (Riesma, Hasan, & Raharjo, 2016) because of the slowing of the operculum movement, which reduces the rate of respiration and oxygen consumption of the fish. DO changes are also indirectly influenced by the dose of clove extract anesthetic because the higher the clove extract dose is given, the more minor DO changes. Fish that are given the highest dose of clove extract are weak due to the strong anesthetic effect, so that their oxygen consumption is also low. The high concentration of anesthetics causing the fish to faint more quickly, and the number of fainting fish is higher (Abdullah, Suwandi, & Nugraha, 2012; Midihatama et al., 2018). The low DO reduction is also influenced by the survival rate. The more fish that are alive, the greater the use of oxygen until the end of the study (Yanto, 2012; Pellu et al., 2018).

All research treatments experienced an increase in pH. The highest increase in pH occurred in treatment C (increased by 0.93) because the oxygen content decreased while the carbon dioxide content increased. Changes in pH

are influenced by the dose of clove extract anesthesia indirectly. The higher the fish metabolism, the higher the oxygen consumption and the increased amount of carbon dioxide (Putra, 2015; Yanto, 2012).

CONCLUSIONS

The supply chain performance of fresh carp can be improved by increasing the survival rate of the carp during transport from suppliers to consumers so that complete order fulfillment can be increased. Clove extract that is widely available in Indonesia can be used as a natural anesthetic agent during the transportation of the carp. The use of clove extract as an anesthetic impacts the health and survival of carp during transportation. The highest survival rate of 85% was achieved using an anesthetic dose of 6.7 ppm clove extract.

Further research can be carried out to determine the anesthetic dose of clove extract with the number of carp transported more with the consideration of increasing transportation cost efficiency if the carrying capacity of carp fish is increased. Increasing the duration of treatment can also be considered for further research to determine the optimum anesthetic dose of clove extract if the transportation time is longer or the transportation distance from suppliers to consumers is getting longer.

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