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Research Article

Investigating the Effect of Ohmic Heating, Conventional Heating, and Cold Plasma on Inactivation of Raw Milk Microorganisms

Pouria Zarei[1*], Elham Shafiei[1], Ghazal Ayatolahi[1], Pedram Zarei[2], Seyed Mohammd Ali Raesosadat[1], Alireza Salehi[1]

[1]Student, Department of Food Science, Collage of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran
[2]Graduate Student, Department of Veterinary and Biomedical Science, Collage of Agriculture, Pennsylvania State University, State College, USA

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Abstract

As a complete food source, milk contains nutrients, minerals, and vitamins needed by the body. However, raw milk has many microorganisms that can cause a wide range of diseases for consumers, so sterilization is one of the important steps in the dairy industry that must be paid enough attention to obtain a healthy product that preserves its primary nutritional value. The current study is a review study with the aim of investigating different thermal methods such as conventional heating (CH) and ohmic heating (OH), and new non-thermal methods such as cold plasma (CP) on inactivation Raw milk microorganisms and the effectiveness rate of this methods are compared. This study includes a review of 13 experimental studies, of which 5 studies investigate the inactivation rate of OH and CH sterilization on *Staphylococcus thermaphilus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium* and *Lactobacillus acidophilus*, and 8 other experimental studies investigate the inactivation rate of CP method as a non-thermal alternative method which is less destructive to foods on *Staphylococcus aureus*, *Enterococcus faecalis*, and on microorganisms in general. According to the contents mentioned in this study, it can be concluded that OH has the ability to produce safe products by preserving nutrients, and at the same time, it does not harm the quality of the products. The amount of microbial load of milk using the OH method was much lower than the CH method, and also less time and energy is needed to reach the sterilized state of the milk, as the energy and time required by OH are 72% and 19% less than CH respectively. Also, CP affects the production of biofilm from microorganisms, especially *Staphylococcus aureus* and *Enterococcus faecalis*, and reduces the microbial load in foods through this way, and by increasing the time of CP irradiation on bacterial isolates, a significant decrease in the Bacteria growth rate is observed.

Keywords: *Ohmic heating, Conventional heating, Cold plasma, Microbial load*

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**** Corresponding Author***
Student, Department of Food Science, Collage of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran.
E-mail: Pouriazarei95@gmail.com, Orcid: https://orcid.org/0000-0002-9298-3512

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Introduction

As a complete food source, milk contains nutrients, minerals and vitamins needed by the body, and as one of the most important products of the agricultural sector, milk is of great importance (Górska-Warsewicz *et al.,* 2019). Since milk is a rich and complex environment of various nutritional compounds, it can be useful for other microorganisms, including fungi and bacteria. This food item can contain various compounds such as protein, carbohydrates, fat, vitamins and minerals (Ksontini *et al.,* 2011). Raw milk is an ideal growth medium for microorganisms (Gil & Ortega, 2019).

Microorganisms are organisms that cannot be seen with the naked eye (Hine & Martin, 2015). Microorganisms include bacteria, protozoa, viruses, and some fungi and are found in abundance. Microorganisms are divided into two groups, useful and harmful, and there are generally three types of microorganisms in nature, which are: 1- gradual decomposers, 2- neutrals, compromisers and opportunists, and 3- constructors or regenerators. Neutral microorganisms constitute the largest group. They join the ruling group in the environment; therefore, when there are more constructive and regenerating microorganisms in the environment, neutrals join the constructive process. Since raw milk is used in various dairy industries such as yogurt, cheese, buttermilk, etc., the composition of its microbial flora is significant. Most of our attention is on bacterial flora (Quigley *et al.,* 2013). The most important spoilage and pathogenic microorganisms that may exist in milk are: *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* (Sun *et al.,* 2008). A prevalent belief that milk possesses particularly healthy properties and attributes when it is consumed in its raw form has arisen and, as a result of some perceived health benefits, it has become especially consumed by individuals that may have lowered immunity, such as very young, very old or immune-compromised people, as well as persons with specific dietary habits. Over recent decades, a public debate has, nevertheless, emerged about the actual risks and benefits that direct human consumption of raw milk, as a drinking milk, may have. From a scientific perspective, food naturalness does not straightforwardly imply food healthiness, tastiness and safety. In fact, 27 milk-borne disease outbreaks occurred from 2007 to 2012 in the EU and an association with the consumption of raw drinking milk was claimed (EFSA Panel on Biological Hazards (BIOHAZ), 2015).

Sterilization refers to chemical and physical methods to destroy all microorganisms and their transmitting agents, such as fungi, bacteria, bacterial spores and viruses from the surface of objects. In fact, sterilization means an environment free from bacteria, fungi, viruses and other pathogenic and non-pathogenic forms. The purpose of sterilization is to prevent the transmission of infection (Ferreira-Santos *et al.,* 2020). Food sterilization is one of the most important processes in the food industry. Heating is one of the oldest methods used to sterilize food, which has been used by humans for many years. With conventional processing technologies, safe products can be obtained, but the quality of the product is damaged and the nutritional value is reduced. Conventional heating (external heating with hot water; CH) is the old common method of sterilizing raw milk. Consumers prefer products that have minimal processing and contain be the most nutrients (Stratakos & Koidis, 2015).

Today, interest has been shown in the use of electrical energy in food processing. The results of studies in this field have provided opportunities for food manufacturers to produce new, high-quality and sustainable products with alternative thermal sterilization methods. Ohmic heating (internal heating with electric current; OH) is a thermal method for sterilizing raw milk. Ohmic heating, which is considered a new method, produces heat by passing an electric current through the food, which resists the electric current (Ramaswamy *et al.,* 2014; Varghese, *et al.,* 2014; Fellows, 2017). Heat is generated rapidly and uniformly in the liquid matrix as well as in the particles, producing a higher quality sterile product suitable for aseptic processing (Fellows, 2017; Varzakas & Tzia, 2015). Ohmic heating is useful for its ability to inactivate microorganisms through thermal and non-thermal cell damage (Fellows, 2017; Ramaswamy *et al.,* 2014; Varghese *et al.,* 2014). Consumers' increasing attention to the nutritional value and sensory characteristics of food products and the tendency to use processed food materials with the highest nutritional value and the most similarity in terms of organoleptic characteristics to the untouched product (Jiménez-Sánchez *et al.,* 2017). It has attracted producers to use non-heat methods in order to increase the shelf life of food without having a negative effect on their sensory properties. Milk is also one of the foods that use non-heat methods to reduce the microbial load and increase its shelf life is expanding day by day. The important efforts of researchers are access to new non-thermal methods with the aim of producing food with minimal pathogenic load. Therefore, the optimization of the use of atmospheric pressure cold plasma (CP) ionized gases is considered as one of the new non-thermal technologies for food sterilization and processing, especially dairy products. The use of atmospheric pressure CP ionized gases is considered as one of the new non-thermal technologies for food sterilization and processing, especially dairy products. Cold plasma can be defined in such a way that when energy enters a gas, for example, it is placed in an electric field, its atoms lose one or more electrons and gain a positive charge, in other words, they are ionized. Plasma is a strong or weak ionized gas, it is the fourth state of matter after solid, liquid and gas and it forms more than 99-99% of the visible world. Plasma is a collection of charged particles, including positive and negative ions, electrons, atoms and molecules, quasi-stables, free radicals and photons. One of the types of plasma is cold or non-thermal plasma. In CP, electrons have much higher temperatures than heavy particles (ions, atoms and molecules) and these plasmas remain at ambient temperature. Basically, CP is used when there is a need for an ionized gas with a low temperature at room temperature. This type of plasma can be produced at low pressures or at atmospheric pressure (Moreau *et al.,* 2008; Moon *et al.,* 2009; Kim *et al.,* 2011; Joshi *et al.,* 2010; James *et al.,* 2008)

Recently, the European Food Safety Authority (EFSA) has been called upon to provide scientific opinion on the public health risks related to the consumption of raw drinking milk (EFSA Panel on Biological Hazards (BIOHAZ), 2015). The hazards related to raw drinking milk are also well evidenced on the websites of authoritative institutions like the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (Melini *et al.,* 2017). As a result, it is very necessary to produce high quality milk using new methods that do not damage its nutritional value. The purpose of this review study is to examine experimental studies that have been conducted in the past regarding thermal and non-thermal methods for the purpose of sterilizing raw milk. A comprehensive and complete review of the three CH methods, OH, and CP method in the effect on raw milk microorganisms, as well as the energy consumption and time of these 3 methods in comparison. This issue is very important in the field of food industry and science, because it is important for consumers and the dairy industry to have access to healthy milk that is free of any microorganisms and has the least change in terms of useful nutrients compared to its raw state. Knowing the advantages of CH method, OH and CP methods leads to the conclusion that the best method can be used in the milk industry to sterilize raw milk, the final result of which is saving energy and sterilization time and increasing the quality of milk and is an increase the health level of society.

Review of experimental studies on thermal methods

By examining the studies and research done, five different microorganisms in raw milk were selected and tested to investigate the effect of inactivation of OH and CH on them. These microorganisms include Staphylococcus thermaphilus, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, and Lactobacillus acidophilus.

1- The first experiment was done by Sun et al.:

The raw milk containing Staphylococcus thermaphilus was heated with l heat (1.5 times the set temperature, which is 70 degrees Celsius) and OH (with an alternating current of 20 kHz) for five minutes until the temperature of the center of the container reaches the set temperature. For the purpose of microbiological investigation, the samples were taken at different time intervals and at different temperatures and immediately cooled in an ice water bath. The counting of Staphylococcus thermaphilus and other living aerobics was investigated by the plate method and with the help of agar plate counting. For all the experiments, the initial number of Staphylococcus thermaphilus and other living aerobic organisms are almost the same, and when the temperature reaches the set temperature, the amount of microorganisms is greatly reduced, and the decrease in the number of microbes in OH is more than in normal heating. As a result, although both heats greatly reduce the amount of microorganisms, the effect of OH is much greater than CH. At the same time, OH plays a role in inactivating microorganisms much more effectively than CH. These results show that the lethal effect of electricity is significant compared to heat (Sun *et al.,* 2008).

2- The second experiment was done by Kim & Kang:

At first, they obtained Escherichia coli from the bacterial culture collection of Seoul National University School of Food Science, *Salmonella typhimurium* from human feces, and *Listeria monocytogenes* from poultry, humans, and rabbits. Then the obtained samples were cultured on soy tryptic agar. A solution was taken from them and then 0.2 ml of the initial microbial composition was added to 25 ml of milk. After going through these steps, the sample was placed in the chamber of the OH device. In the examination of the temperature histories, it was found that by using OH, the temperature increases much faster than normal heating. It took only 40 seconds to reach the target temperature (71.5 °C) using OH, while it took 210 seconds to reach the same temperature using CH. The results of the research showed that all three microorganisms were deactivated under the influence of OH more effectively than CH in the temperature range of 60 to 65 degrees Celsius. It is worth noting that all three pathogens under the influence of both CH and OH were not so different in reducing the number. This problem shows that the effect of OH in the inactivation of microorganisms has a much stronger role than reducing their number (Kim & Kang, 2015).

3- The third experiment was done by Silva et al.:

Listeria monocytogenes and *Lactobacillus acidophilus* were investigated as important microorganisms in milk under OH using the Weibull prediction model. In general, OH reduced the viability of Listeria monocytogenes, the appropriate number of Lactobacillus acidophilus and satisfactory results in the survival of the digestive tract. The Weibull model provided an excellent fit to the data for all conditions. Furthermore, lower δ values and increased R2 values were obtained for samples treated with OH, which underlines the best performance of OH data. In fact, considering functional and safety objectives, this method was presented as a useful technology for use in milk to produce probiotic fermented milk (Silva *et al.,* 2021).

4- The fourth experiment was done by Rocha et al.:

Processing of milk under different intensities of the electric field of OH was carried out in comparison with the sample sent to pasteurization with conventional heat to evaluate the energy consumption, processing parameters and microbiological, rheological and biological aspects. Ohmic (OH) heating provides lower energy consumption and significant microbial inactivation of lactic acid bacteria, molds and yeasts, total mesophiles and psychrophiles. In addition, OH at lower electric field strength, mainly OH8, improved the antidiabetic, antioxidant, and antihypertensive activities and rheological properties, leading to a decrease in hydroxymethylfurfural content and nitrogen index of whey protein became (Rocha *et al.,* 2022).

5- The fifth experiment was done by Balthazar et al.:

This study aimed to evaluate the energy consumption of OH and CH for the pasteurization of fresh and thawed sheep's milk and their effect on bacterial microbiota during the storage period in the refrigerator (15 days under cold storage 4 °C). OH pasteurization power consumed 72-73% less energy than CH pasteurization (515 KJ). The culture-dependent approach showed that at least a 4.2 log cycle reduction was achieved in sheep's milk submitted to CH and OH pasteurization, regardless of whether the sheep's milk was fresh or thawed. Data from amplicon sequencing showed that Staphylococcus was the most abundant genus in raw samples on day 1, while Pseudomonas became the most abundant genus after 15 days in cold storage. The relative abundance of all bacterial genera evaluated in samples pasteurized by CH and OH remained similar during refrigerated storage (Balthazar *et al.,* 2022).

Thermal methods studies final resulte

Ohmic heating is one of the emerging and nature-friendly technologies that has many applications both now and in the future. According to the contents mentioned in this study, it can be concluded that OH has the ability to produce safe products by preserving nutrients, and at the same time, it does not harm the quality of the products. With this method, fast and uniform heating can be achieved in the products. This technique has both thermal and non-thermal effects on products. The degree of success of this method in heating products is limited by factors such as the intensity of the electric field, the electrical conductivity of the product, the rate of heat generation in the product, the high rate of temperature increase, the type of electrodes, the shape of the tank and the frequency used regarding the deactivation effect of OH on milk microorganisms in this study (Norouzi *et al.,* 2021) and its comparison with CH, it can be concluded that the results show that there is a significant difference between the inactivation effect of OH and CH, and OH It has a much greater deactivation effect than CH, and also less time and energy is needed to reach the sterilized state of the milk, as the energy and time required by OH are 72% and 19% less than CH respectively However, both heating can kill microorganisms in milk, but the adverse thermal effect on milk microbial inactivation can be reduced by OH (Balthazar *et al.,* 2022).

Review of experimental studies on non-thermal methods

Previously, in most researches, vacuum systems were used to produce low pressure plasma, which required a complex vacuum environment and high cost, but due to the advantages of atmospheric plasma compared to low pressure plasma, such as the elimination of expensive vacuum systems, the ability Direct processing, high efficiency and scalability in a wide area, atmospheric plasma has been used more recently. Plasmas can be created in several ways (Domonkos *et al.,* 2021)

Usually, by applying voltage to the electrodes, a gas discharge is electrically induced. Dielectric barrier discharges 1 (DBD) is one of the CP production methods. Dielectric barrier discharge takes place between two electrodes, at least one of which is covered with a dielectric layer. This discharge is done by applying alternating high voltage between two electrodes and the existence of a dielectric layer prevents the passage of strong current between the two electrodes and the occurrence of sparks. An electric discharge, like plasma, depending on the conditions and how it is discharged, can be a good source of electrons, ions, ultraviolet radiation and free radicals. This characteristic of plasmas has revealed their ability to inactivate bacteria and other microorganisms. Therefore, in many cases, plasma is used to remove microbial contamination (Mousavi *et al.,* 2020). The effect of plasma on microbial cells is due to plasma ions and interaction with cells. Deactivation of microbes in the CP method is largely associated with the oxidative effect in the outer layer of microbial cells. The application of CP in the inactivation of microbes is based on the fact that CP is able to destroy the deoxyribose nucleic acid of the chromosome. Cold plasma works due to the interaction of energetic ions and activated species with substrate molecules. Accumulation of charge creates an electrostatic force on the outer surface of the membranes, which can cause the cell wall to rupture. During the use of CP where it is catalyzed, a complex biological response occurs and the membrane structure is compromised and enzymes are changed and complex cells are created. Plasma can be used not only in microorganisms but also in simple biological compounds such as enzymes (Moreau *et al.,* 2008; Moon *et al.,* 2009; Kim *et al.,* 2011; Joshi *et al.,* 2010; James *et al.,* 2008).

In the first study, dielectric barrier discharge was used to generate high-voltage atmospheric CP and its antibacterial and anti-biofilm potency against *Staphylococcus aureus* isolated from bovine clinical mastitis cases was investigated. According to the results obtained in the study, with the increase in the counting time of atmospheric CP on the isolates, a significant decrease in the growth rate of bacteria was observed. This result is in agreement with the results obtained from other studies that showed that atmospheric CP reduced the growth of Gram-positive and Gram-negative bacteria (Santosh *et al.,* 2017; Mai-Prochnow *et al.,* 2016).

In the second study, the antimicrobial effect of plasma on some food-borne bacteria was investigated and it was found that after treatment with plasma, the amount of microorganisms decreased a lot (Ulbin-Figlewicz *et al.,* 2015).

In the third study, it was shown that CP is a non-thermal and non-destructive method that has a high germicidal capability. The findings of this research, which is in line with the present study, showed that increasing the irradiation time increases the germicidal power of CP (Saba *et al.,* 2013).

In the fourth study, the researchers showed that the bactericidal rate of the DBD method depends on the cell density and the exposure time of the bacteria to atmospheric CP (Joshi *et al.,* 2010)

In the fifth study, *Staphylococcus aureus* was used as a model to investigate the effect of atmospheric CP. It was shown that this method has a great destructive effect on this bacterium according to the exposure time (Hamad & Mahmood, 2013).

In other studies, the antimicrobial effect of atmospheric CP produced by dielectric barrier discharge was investigated on the amount of biofilm production in *Enterococcus faecalis* bacteria and showed that the ability to produce biofilm in the isolates was greatly reduced and atmospheric CP produced by discharge Dielectric barrier has a significant ability to reduce the formation of biofilm of bacteria. However, the type of biofilm-producing bacteria affects the performance of atmospheric CP. Also, in another study, they showed that atmospheric CP technology is an effective strategy to disable biofilm production (Ziuzina *et al.,* 2015; Jiang *et al.,* 2012; Razuqi *et al.,* 2017).

Cold plasma method studies final result

Advantages of this method: 1- It is a dry process 2- It needs very little energy and time 3- Easily adapts to the food production environment 4- The reactive species of gases return to the initial gas state within a few minutes to a few hours after treatment. 5- It needs a short period of time for decontamination 6- It is not compatible with the environment (natural gases including nitrogen, oxygen, argon, hydrogen are used) But because there are not many studies on the safe application of the technology in real food systems, the effects of cold atmospheric plasma on the nutritional and chemical properties of food are not well known, and therefore more studies should be done on the disadvantages of this technique. According to the results of this study, it can be concluded that atmospheric CP has been proposed as a potential strategy for decontamination in the food industry, which with decontamination in a short period of time makes milk free from pathogenic species. Also, by affecting the production of biofilm by microorganisms, it reduces contamination in food and food production lines, surfaces and equipment; However, considering that during the research conducted so far, no exact relationship has been obtained between the application of voltage and the effect of cold atmospheric plasma on various microorganisms, more research in the field of safety seems necessary, but by increasing the time of CP irradiation on bacterial isolates, a significant decrease in the Bacteria growth rate is observed (Nikmaram & Keener 2022 ; Chizoba Ekezie *et al.,* 2017).

Conclusion

Conventional heat is an old and ineffective method for sterilizing raw milk because it requires a lot of energy and time to destroy all microorganisms and can harm the nature of its primary nutrients, but OH is a much newer method. which has the power to destroy raw milk microorganisms as well and much more than the CH method, and also less time and energy is needed to reach the sterilized state of the milk, as the energy and time required by OH are 72% and 19% less than CH respectively. Cold plasma is more innovative than the previous two methods and is more compatible with the environment, it requires very little energy and time to destroy raw milk microorganisms and its performance is generally different from thermal methods which are affecting the production of biofilm by microorganisms.

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Conflict of interest

The authors declare no conflict of interest.

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بررسی اثر گرمایش اهمی، گرمایش معمولی و پلاسمای سرد بر غیر فعال سازی میکروارگانیسم های شیر خام

پوریا زارعی[1*]، الهام شفیعی[1]، غزال آیت الهی[1]، پدرام زارعی[2]، سید محمد علی رئیس السادات[1]، علیرضا صالحی[1]

[1]دانشجو، گروه علوم غذایی، دانشکده دامپزشکی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران

[2]دانشجوی فارغ التحصیل، گروه دامپزشکی و علوم زیست پزشکی، کالج کشاورزی، دانشگاه ایالتی پنسیلوانیا، استیت کالج، ایالات متحده آمریکا

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چکیده

شیر به عنوان یک منبع غذایی کامل حاوی مواد مغذی، مواد معدنی و ویتامین های مورد نیاز بدن است. با این حال، شیر خام دارای میکروارگانیسم‌های زیادی است که می‌توانند طیف گسترده‌ای از بیماری‌ها را برای مصرف‌کنندگان ایجاد کنند، بنابراین استریلزاسیون یکی از مراحل مهم در صنعت لبنیات است که باید برای به دست آوردن یک محصول سالم که ارزش غذایی اولیه خود را حفظ می‌کند، توجه کافی به آن شود. مطالعه ی حاضر یک مطالعه مروری با هدف بررسی روش‌های حرارتی مختلف از جمله گرمایش معمولی (CH) و گرمایش اهمی (OH) و روش‌های جدید غیر حرارتی مانند پلاسمای سرد (CP) بر غیرفعال‌سازی میکروارگانیسم‌های شیر خام است و میزان اثربخشی این روش ها باهم مقایسه شده است. این مطالعه شامل مروری بر ۱۳ مطالعه تجربی است که ۵ مطالعه به بررسی میزان غیرفعال‌سازی استرلیزسیون به روش OH و CH بر روی *استافیلوکوک ترمافیلوس*، *لیستریا مونوسیتوژنز*، *اشریشیا کلی*، *سالمونلا تیفی موریوم* و *لاکتوباسیلوس اسیدوفیلوس* می پردازد و ۸ مطالعه تجربی دیگر به بررسی میزان غیرفعال‌سازی با روش CP به عنوان یک روش جایگزین غیرحرارتی که اثر تخریبی کمتری بر روی غذاها در غیر فعالسازی *استافیلوکوکوس اورئوس*، *انتروکوکوس فکالیس* و به طور کلی میکروارگانیسم ها دارد، می پردازد. با توجه به مطالب ذکر شده در این مطالعه می توان نتیجه گرفت که OH با حفظ مواد مغذی توانایی تولید محصولات ایمن را دارد و در عین حال به کیفیت محصولات آسیبی نمی رساند. مقدار بار میکروبی شیر با استفاده از روش OH بسیار کمتر از روش CH بوده و همچنین زمان و انرژی کمتری برای رسیدن به حالت استریل شده شیر مورد نیاز است زیرا انرژی و زمان مورد نیاز برای OH نسبت به CH به ترتیب ۷۲٪ و ۱۹٪ کمتر است. همچنین CP با تاثیر بر تولید بیوفیلمی که توسط میکروارگانیسم ها به ویژه در *استافیلوکوکوس اورئوس* و *انتروکوکوس فکالیس* تولید می شود، بار میکروبی غذا را کاهش می دهد و با افزایش زمان تابش CP بر روی جدایه های باکتری، کاهش قابل توجهی در سرعت رشد باکتری ها مشاهده می شود.

واژه های کلیدی: *گرمایش معمولی، گرمایش اهمیک، پلاسمای سرد، بار میکروبی*

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* *نویسنده مسئول: دانشجو، گروه علوم غذایی، دانشکده دامپزشکی، واحد کازرون، دانشگاه آزاد اسلامی، کازرون، ایران.*

Orcid: https://orcid.org/0000-0002-9298-3512 ،Email: pouriazarei95@gmail.com