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The effect of adding fermented shrimp waste extract in the ration on the balance of protein efficiency in egg production

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Abstract

Background: The purpose of the study was to determine and obtain the level of addition of fermented shrimp waste extract in the ration as a feed additive that produces an optimal protein efficiency balance value in the egg production of purebred chickens.

Materials and Methods: The study used 40 40-week-old layer laying hens in 20 cage units. The study used a randomized design method complete with five types of treatment, consisting of R 0 (ration without the use of fermented shrimp waste extract), R1 (ration containing 0.5% fermented shrimp waste extract), R2 (ration containing 1.0% fermented shrimp waste extract), R3 (ration containing 1.5% fermented shrimp waste extract), and R4 (ration containing 2.0% fermented shrimp waste extract). Each treatment was repeated four times with the observed variables being ration consumption, protein consumption, egg weight, and protein efficiency balance. The difference in effect between treatments was tested using the Tukey Test.

Results: The results of the study found that the addition of fermented shrimp waste extract in the ration affected ration consumption and protein consumption, but did not affect egg weight and protein efficiency balance.

Conclusions: The use of fermented shrimp waste extract in the 2% ration is optimal for the balance value of protein efficiency.

Keywords: Fermented extract; Shrimp waste; Laying hens; Balance protein efficiency

1 Introduction

Eggs are one source of animal protein that has a delicious taste, easy to digest and has high nutrition. In addition, eggs are easy to obtain and reasonably priced. The nutritional content of eggs consists of protein, and fat. The nutritional content of eggs consists of: water 73.7%, protein 12.9%, fat 11.2%. Fat in egg yolks reaches 32%, while in egg whites the fat content is very little [1]. An important factor in raising the egg production of chickens is the ration given to chickens of laying breeds. Ration consumption is the amount of ration given minus the amount of ration left on the current ration [2]. Egg production will be achieved efficiently if the ration provided is sufficient for the needs of chickens in accordance with age and maintenance management [3]. The amount of ration consumption in laying hens in the starter, grower, and layer phases is different. In addition to the large number of rations consumed by laying hens, nutritional value in the content of such rations is also considered.

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Feed additives with optimal composition can increase livestock productivity through increasing ration digestibility and ration consumption which provides a balance between amino acid supply and energy to grow and produce [4]. There are two types of feed affixes, namely natural and synthetic feed affixes [5]. Natural feed affixes are feed additives obtained from nature; synthetic feed affixes made by chemical processes. Synthetic feed affixes have the disadvantage of leaving residues in the body of chickens. Residues when eaten by consumers can cause allergic reactions and poisoning as well as the development of resistant germs [6]. The shortcomings possessed by synthetic feed affixes can be overcome with fermented shrimp waste extract.

Shrimp waste is the result of shrimp stripping activities to be exported in the form of headless and skinless shrimp. Shrimp waste comes from the skin, head, and tail of shrimp. Shrimp head waste reaches 35% - 50% of the total weight of shrimp [7]. The shell part of shrimp waste contains more chitin and less protein, while the head contains less chitin but more protein.

Chitin is an anti-nutrient substance found in shrimp waste. Chitin can be overcome, one of which is by biological processing using microbes. Chitin binds protein to shrimp waste and physically, chitin limits digestive enzymes to protein, causing low digestibility when consumed by livestock [8].

The fermentation process can degrade nutrients from chitin, with the help of chitinase enzymes. Fermentation of shrimp waste using microbes including bacteria *Bacillus licheniformis, Lactobacillus sp.*, and yeast *Saccharomyces cerevisiae*. *Bacillus licheniformis* produces chitinase enzymes and protease enzymes in relatively high quantities with deproteination properties where the enzyme degrades glycosidic β (1,4) bonds in chitins and liberates some nitrogen or protein in the form of N-Acetyl-D-glucosamine monomers and acetyl amino so that proteins are released from chitin bonds [9]. *Lactobacillus sp*. functions to break down glucose, sucrose, maltose, and lactose so that mineral deposits occur [10]. *Saccharomyces cerevisiae* is a yeast that produces amylase, lipase, protease, and other enzymes that can help the process of digestion of food substances in the digestive organs [11].

After the fermentation process is carried out, then the addition of selenium as a salt bridge to bind amino acids, organic acids, and others. Selenium is also used to maximize astaxanthin performance. Sodium Alginate is added as an edible coating (coating) astaxanthin. Sodium Alginate can be applied as a suitable packaging material because alginate is safe for consumption for animals and humans. Sodium Alginate has the potential to form biopolymer film or coating components because alginate has a unique colloidal structure, as a stabilizer, binder, suspension, film former, geller, and emulsion stability [12].

Astaxanthin is a carotenoid xanthophyll present in various microalgae and yeasts that has been approved in the United States and European Union as a food colouring used as a source of pigment in rations for salmon and shrimp [13]. Astaxanthin is known as one that affects the colour of egg yolks, not only that astaxanthin can increase egg production and chicken health. Astaxanthin has strong antioxidant activity through oxygen neutralization, clearing free radicals, inhibiting lipid peroxidation, improving immune system function, and regulating gene expression [14]. Astaxanthin is a carotenoid that has antioxidant power. Astaxanthin has 10 times more antioxidant activity than other carotenoids such as zeaxanthin, lutein, canthaxanthin, and β carotene, as well as 100 times more than α-tocopherol [15]. Antioxidants are molecules that can remove free radicals from a system either by reacting with them to produce other harmless compounds or interfering with oxidation reactions [16]. Astaxanthin administration to laying hens can increase fertility, improve health status, and reduce mortality. Egg production and yolk colour also increase, while salmonella infections are drastically reduced due to the formation of stronger membranes [17].

The balance of protein efficiency is closely related to egg weight, protein consumed, and the amount of ration consumed [18]. The nutrient content contained in fermented shrimp waste extract is expected to increase the metabolic efficiency of laying hens so that the number and weight of eggs increases. Egg weight is closely related to the balance of protein efficiency, so it is expected to increase the balance of protein efficiency in laying hens.

Fermented shrimp waste extract contains several amino acids, namely non-essential amino acids (serine, glycine, glutamine acid, aspartic acid, arginine, alanine, histidine, cystine, tyrosine, proline) and essential amino acids (threonine, methionine, lysine, leucine, isoleucine, phenylalanine, valine). The bioactive content in fermented shrimp waste extract is astaxanthin 26.75%, methyl ester 31.28%, nickel protoporphyrin disodium 26.36%, 4.9-Epoxy-1H-benz 14.85%, propanamine 0.76% (Test results of the Jakarta Police Forensic Laboratory, 2019). The astaxanthin content is 26.75% in the liquid extract of fermented products, then added a compactor with a ratio of 16.30%: 83.70%. Based on this comparison, the content of astaxanthin in shrimp waste fermentation extract-based feed affixes amounted to 436 ppm.

Vitamin A supplementation in rations has been done by [19]. Hy-line laying hens aged 56 weeks experienced an increase in egg weight with the addition of vitamin A (6,000 and 9,000 IU/kg), but ration intake and weight loss had no effect [19]. Vitamin A needs in laying hens are 8,000 – 12,000 IU / kg (DSM vitamin supplementation guidelines, 2006). The results of the calculation of vitamin A needs for laying hens based on these studies (6,000 and 9,000 IU / kg) were converted in the form of β carotene needs to 3.6 mg / kg and 5.4 mg / kg and equivalent to astaxanthin contained in feed affixes based on fermented shrimp waste extract, the use of feed affixes was obtained by 1%.

Based on the description above, it can be hypothesized that the addition of fermented shrimp waste extract in the ration at a rate of 1% can produce an optimal balance of protein efficiency in laying hens.

2 Material and methods

2.1 Experimental livestock

The study used 40 chickens laying hens of the 40-week-old layer phase Hy-line strain. Chickens are divided into 5 types of treatment and repeated 4 times.

2.2 Trial enclosure

The cages used in this study were individual cages of 20 experimental cages. Laying hens are randomly divided into 20 cages, each cage containing two chickens. The cage uses a cage system with a size of 40 cm x 30 cm x 35 cm, made of iron wire. Each cage is equipped with a feed bin and a place for drinking water.

2.3 Feed ingredients constituent of rations

The ration is made based on the standard protein and energy requirements for laying hens are 17.5% and 2700 kcal / kg (Hy-line International, 2016). The feed ingredients used in this study included yellow corn, soybean meal, coconut oil, MBM, fermented shrimp waste extract, bone meal, $CaCO_3$, and grit. The feed ingredients are obtained from Poultry Shop Missouri Bandung. Rationing is carried out 2 times a day in the morning and evening with the amount of each feeding 57.5 grams / head. The nutrient content of ration constituent materials can be seen in Table 1.

Table 1 Metabolizable energy and nutrient content of experimental feed ingredients

Feed ingredients	**ME**	**CP**	**EE**	**CF**	**Ca**	**P**	**Lys**	**Meth**
(Kcal/kg)		**…………………………(%)……………………………**						
Yellow corn	3,350	8.60	3.80	2.20	0.02	0.08	0.26	0.36
Soybean meal	2,230	44.00	0.90	6.00	0.32	0.29	2.90	0.65
Coconut oil	8,600	-	-	-	-	-	-	-
MBM	2,375	43.00	10.93	2.46	9.80	4.50	2.08	0.54
FSWE*	3,033	25.15	0.96	-	6.81	0.85	0.28	-
Bone meal	-	-	-	-	24.00	12.00	-	-
$CaCO_3$	-	-	-	-	40.00	-	-	-
Grit	-	-	-	-	30.87	1.11	-	-

* FSWE= Fermented Shrimp Waste Extract

2.4 The composition of the research ration

The ration given to laying hens aged 40 weeks is a ration from a mixture or researcher formulation in the form of mash. Rations are prepared based on standard protein content and metabolic energy requirements, as in Table 2.

Table 2 Trial ration formulation

No	Feed ingredients	R0	R1	R2	R3	R4
		……………………………….(%)……………………………				
1.	Yellow corn	58.10	57.70	57.50	57.30	57.30
2.	Soybean meal	21.00	20.90	20.60	20.30	20.30
3.	Coconut oil	1.00	1.00	1.00	1.00	1.00
4.	FSWE*	0.00	0.50	1.00	1.50	2.00
5.	MBM	8.50	8.50	8.50	8.50	8.50
6.	Bone meal	3.15	3.15	3.15	3.15	3.15
7.	$CaCO_3$	4.50	4.50	4.50	4.50	4.50
8.	Grit	3.75	3.75	3.75	3.75	3.75

* FSWE= Fermented Shrimp Waste Extract

Table 3 Metabolizable energy content and nutrients of experimental rations

Nutrient content	R0	R1	R2	R3	R4	Necessity
ME (kcal/kg)	2,702	2,703	2,704	2,706	2,707	2700-2750
CP (%)	17.54	17.59	17.56	17.54	17.52	17.5
EE (%)	3.93	3.92	3.91	3.89	3.88	3-4
CF (%)	2.87	2.89	2.92	2.94	2.96	≥ 2.6
Ca (%)	4.62	4.65	4.69	4.72	4.75	≥ 4.3
P (%)	0.91	0.92	0.93	0.95	0.96	≥ 0.80
Lysine (%)	0.89	0.89	0.89	0.88	0.88	≥ 0.88
Methionine (%)	0.52	0.52	0.52	0.52	0.51	≥ 0.48

2.5 Research procedure

Chickens are weighed first to find out the initial weight, after that the chickens are put into the cage with the number of two chickens per experimental unit, each cage is coded on the front of the cage by attaching a label that has been coded.

The implementation of the study was carried out for 60 days. Habituation of rations is carried out in the first 3 days. Day 4 to day 60 observations were made.

The provision of drinking water is carried out ad *libitum.*

2.6 Observed modifiers

The modifiers observed in this study are as follows: The modifiers observed in this study are protein efficiency balances with formulas successively as follows:

2.6.1 Ration consumption

Ration consumption (g) is the amount of ration given minus the residual ration.

2.6.2 Protein consumption

Protein consumption (g) is the amount of protein consumed by chickens

2.6.3 Egg weight

Balance Protein efficiency is the ratio between egg weight and protein consumption.

$$\text{IEP} = \frac{Berat\ telur\ total}{Konsumsi\ Protein\ total}$$

2.7 Experiment design and statistical analysis

The research method used is the experimental method. The experimental design used in this study was a randomized design complete with 5 treatments. Each treatment was repeated 4 times, bringing the total to 20 experimental units. The differences between treatments are tested using the following Tukey test.

The treatment carried out, namely:

- R0 = Ration without fermented shrimp waste extract.
- R1 = Ration contains 0.5% fermented shrimp waste extract
- R2 = Ration contains 1.0% fermented shrimp waste extract
- R3 = Ration contains 1.5% fermented shrimp waste extract
- R4 = Ration contains 2.0% fermented shrimp waste extract

3 Results and discussion

3.1 The effect of treatment on ration consumption

The average ration consumption of laying hens during rearing ranged from 101.43 – 111.11 grams, the average ration consumption sequentially from the lowest was R4 treatment (101.43 grams), R2 treatment (105.05 grams), R0 treatment (108.88 grams), R3 treatment (109.05 grams), and R1 treatment (111.11 grams). Statistical analysis through fingerprints was carried out to determine the effect of treatment on the consumption of rations of laying hens during the study. The results of the fingerprint analysis showed that the provision of fermented shrimp waste extract in the ration had a real effect ($P<0.05$) on the ration consumption of laying hens. Furthermore, the Tukey Test is carried out to determine the best treatment, the results of which are listed in Table 4.

Table 4 Tukey test results effect of treatment on ration consumption

Treatment	Average Ration Consumption (g/t/d)
R_0	108.88 ^{bc}
R_1	111.11 ^{c}
R_2	105.78 ^{ab}
R_3	109.05 ^{bc}
R_4	101.43 ^{a}

Description: Different letters show real different results ($P<0.05$)

Based on Table 4, there are differences in influence between treatments. Ration consumption with the addition of 0.5% fermented shrimp waste extract increased but not significantly compared to rations that were not given fermented shrimp waste extract. The use of fermented shrimp waste extract at the rate of 2%, the consumption of real rations is lower than the ration feeding without fermented shrimp waste extract. The use of shrimp waste extract as much as 2% in the ration, effective against ration consumption.

Fermented shrimp waste extract contains several amino acids, namely non-essential amino acids (serine, glycine, glutamic acid, aspartic acid, arginine, alanine, histidine, cystine, tyrosine, proline) and essential amino acids (threonine, methionine, lysine, leucine, isoleucine, phenylalanine, valine) (Analysis results of PT Saraswanti Indo Genetech Bogor-Indonesia, 2019) and contains several acids organic (Cyclobutane,1,2,2,3,3,4-Hexadeutero-1,4-BIS(1,2,2), Butanoic acid, 3-methyl-,methyl ester (CAS) Methyl isovalerate, Pentanoic acid, Pentanoic acid, 4-methyl-, methyl ester (CAS) Methyl isohe, Hexanoic acid, Methyl caproate, Benzeneacetic acid, Methyl phenylacetate, Methyl hydrocinna, Hexadecanoic acid, Methyl palmitate, Hexadecahydro (6a.alpha.,6b), Cholest-5-en-3-ol (3.beta.), Cholesteryl acetate) (Test results of UPI Bandung-Indonesia Instrument Chemistry Laboratory, 2019).

Bacteria Bacillus licheniformis, Lactobacillus sp., and yeast *Saccharomyces cerevisiae* used during the fermentation process have other uses, namely being able to produce single-cell proteins. Single cell proteins are products of high protein biomass and are derived from microorganisms [20]. The advantages of single-cell protein are of high nutritional value because they have high levels of protein, vitamins, and fats and have a complete amino acid content [21].

The complete content in the fermented shrimp waste extract can meet the needs of laying hens. The higher the level of fermented shrimp waste extract added to the ration, causing the ration nutrient to increase, so that ration consumption decreases because it has met its needs. This is in accordance with the opinion of [22] which states that the nutrient content of rations can affect ration consumption.

3.2 The effect of treatment on protein consumption

The average protein consumption of laying hens ranged from 17.77 – 19.54 grams, the average protein consumption was sequentially from the lowest, namely R4 treatment (17.77 grams), R2 treatment (18.58 grams), R0 treatment (19.10 grams), R3 treatment (19.13 grams), and R1 treatment (19.54 grams). Statistical analysis through fingerprinting was carried out to determine the effect of treatment on protein consumption of laying hens. The results of the fingerprint analysis showed that the provision of fermented shrimp waste extract products had a real effect (P<0.05) on the protein consumption of laying hens. Furthermore, the Tukey Test is carried out to determine the best treatment, the results of which are listed in Table 5.

Table 5 Tukey test results effect of treatment on protein consumption

Treatment	Average Ration Consumption (g/t/d)
R_0	19,10 ^{bc}
R_1	19,54 ^{c}
R_2	18,58 ^{ab}
R_3	19,13 ^{bc}
R_4	17,77 ^{a}

Description: Different letters show real different results (P<0.05)

Based on Table 5, there are differences in influence between treatments. Protein consumption in rations with the addition of 0.5% fermented shrimp waste extract increased but not significantly compared to rations that were not given fermented shrimp waste extract. Protein consumption in rations with a 2% fermented shrimp waste extract rate was significantly lower than rations that were not given fermented shrimp waste extract.

Protein consumption also decreases along with the increase in the dose of adding fermented shrimp waste extract because protein consumption is strongly influenced by ration consumption. This is in accordance with the opinion of [23], which states that protein consumption is influenced by the amount of ration consumption. Consumption of large amounts of rations will be followed by large consumption of protein and vice versa. High ration consumption can affect protein consumption into meat and amino acids are fulfilled in the body so that the metabolism of cells in the body takes place normally [24]. Excess consumption of protein from the ration can be stored in the form of energy, while lack of protein can lead to impaired maintenance of body tissues, impaired growth, and decreased meat hoarding.

Fermented shrimp waste extract contains a wide range of amino acids and organic acids. Organic acids have good benefits for the digestibility of livestock. Organic acids function as acidifiers. The mechanism of action of the acidifier is to improve digestion by improving the quality of enzymes, as well as lowering stomach pH and reducing pathogenic bacteria in the digestive tract [25]. The addition of organic acids can maintain the balance of microbes in the digestive tract by maintaining the pH of the digestive tract, so that protein absorption increases [26].

The function of protein is as a source of amino acids for the formation of body tissues, therefore good protein must contain enough amino acids for livestock. This is in accordance with [7] statement that good protein for livestock is one that contains amino acids in sufficient and balanced quantities. Based on the results of the study, the needs of amino acids (lysine and methionine) are fulfilled because according to the provisions, namely (≥ 0.88; ≥ 0.48), namely R0 (0.89%; 0.52%), R1 (0.89%; 0.52%), R2 (0.89%; 0.52%), R3 (0.88%; 0.52%), and R4 (0.88%; 0.51%). Low ration consumption indicates that amino acid needs are fulfilled. This is in accordance with the statement [22] that if the number of amino acids in the ration is low, animals will consume more rations to achieve maximum growth and vice versa.

3.3 The effect of treatment on egg weight

The average weight of chicken eggs ranges from 59.32 – 62.31 grams with the average egg weight sequentially from the lowest, namely R4 treatment (59.32 grams), R1 treatment (59.53 grams), R2 treatment (60.47 grams), R3 treatment (60.69 grams), and R0 treatment (62.31 grams). Statistical analysis through fingerprinting was carried out to determine the effect of treatment on the egg weight of laying hens. The results of the fingerprint analysis showed that the administration of fermented shrimp waste extract products had no real effect (P>0.05) on the egg weight of laying hens.

Table 6 Average egg weight of laying hens during the study

Deuteronomy	**Egg weight**				
	R0	**R1**	**R2**	**R3**	**R4**
	……………………… gram/grain…………………………………				
1	58.66	60.73	58.19	59.42	57.91
2	64.08	59.04	60.38	61.54	59.28
3	67.75	60.51	62.96	59.71	59.30
4	58.75	57.83	60.34	62.10	60.78
Total	249.25	238.11	241.87	242.77	237.27
Average	62.31	59.53	60.47	60.69	59.32

The unreal egg weight (P>0.05) in the treatment ration with the addition of fermented shrimp waste extract was caused by the adequacy of nutrients needed by livestock, as evidenced by the results of statistical analysis that the addition of fermented shrimp waste extract did not reduce egg weight, although ration consumption decreased. Fermented shrimp waste extract contains several kinds of essential amino acids, including methionine and lysine. Methionine and lysine are very influential on egg weight. The content of methionine and lysine respectively in the ration ranges from 0.51-0.52% and 0.880.89%. This is in accordance with the opinions of [27] and [28]. Methionine is a critical essential amino acid that greatly affects egg weight. According to [18] the amino acid methionine is superior to other amino acid sources in affecting egg weight, methionine acts as a methyl donor that plays a role in helping metabolism in the body such as the metabolism of choline, protein, and carbohydrates. In rations containing low protein (12%), but balanced with supplementation of the amino acid's lysine and methionine, it was found to provide normal production and normal egg weight [29]. According to [30] when viewed in quantity, with the right dose the average weight value of native chicken eggs treated with lysine or methionine supplementation there was an increase in egg weight.

Protein greatly affects the weight of eggs. This is in accordance with the opinion of [31] which states that egg weight is determined by protein intake during egg laying. In connection that egg weight is influenced by the weight of albumin and yolk, which consist mostly of protein, therefore high protein intake causes high egg weight [32].

The mineral content (Ca and P) in the ration with the addition of fermented shrimp waste extract was better than the control ration. However, ration consumption is low so that the amount of minerals (Ca and P) that enter the chicken's body is relatively the same. The content of protein and minerals (Ca and P) in the ration plays a role in the process of egg formation. This is in accordance with the opinion of [33] which states that egg weight is influenced by the content of calcium, phosphorus, protein, and energy contained in the ration and the age of chickens.

3.4 The effect of treatment on protein efficiency balance

Table 7 Average balance of protein efficiency in laying hens during research

Deuteronomy	Balance Protein Efficiency				
	R0	**R1**	**R2**	**R3**	**R4**
1	3.01	3.11	3.14	3.08	3.42
2	3.29	3.05	3.29	3.14	3.30
3	3.58	3.10	3.29	3.15	3.27
4	3.17	2.92	3.30	3.33	3.37
Total	13.06	12.19	13.02	12.70	13.36
Average	3.26	3.05	3.26	3.17	3.34

The average balance of protein efficiency in laying hens ranges from 3.05 – 3.34 with the average balance of protein efficiency sequentially from the lowest, namely R1 treatment (3.05), R3 treatment (3.17), R0 treatment (3.26), R2 treatment (3.26), and R4 treatment (3.34).

Statistical analysis through fingerprinting was carried out to determine the effect of treatment on the balance of protein efficiency in laying hens. The results of the fingerprint analysis showed that the provision of fermented shrimp waste extract products had no real effect (P>0.05) on the balance of protein efficiency in laying hens.

The balance value of protein efficiency in laying hens has not decreased. This is because the nutrients needed by livestock are sufficient, adequate nutrition cannot be separated from amino acids. Fermented shrimp waste extract contains a wide range of essential and non-essential amino acids. Amino acids are needed by livestock. According to [34] that amino acids stimulate protein synthesis in the liver, pancreas, spleen, and lungs which further act as mediators in metabolic pathways for body protein synthesis.

The balance value of protein efficiency in laying hens given fermented shrimp waste extract ranged from 3.26 - 3.34. The balance value of protein efficiency is higher than [35] research. The decrease in the balance value of protein efficiency in laying hens is due to the help of *Bacillus licheniformis, Saccharomyces cerevisiae* and *Lactobacillus sp.*, which helps the process of nutrient absorption. This is in accordance with the opinion of [35] which states that the increase in the balance value of protein efficiency is caused by the addition of probiotics containing *Bacillus subtilis, Bacillus cereus, Bacillus thuringites, Lactobacillus bulgaricus,* and *Streptococcus thermophilus which* can compete with pathogenic microbes in the digestive tract to prevent the onset of gastrointestinal infections and have an impact positive for increased nutrient absorption.

4 Conclusion

Based on the results and discussion, it can be concluded that the addition of fermented shrimp waste extract in the ration affects ration consumption and protein consumption, but does not affect egg weight and protein efficiency balance. The use of fermented shrimp waste extract in the ration of 2% is optimal for the balance value of protein efficiency.

Fermented shrimp waste extract can be used as *a feed supplement* in the ration of laying hens and its use is recommended as much as 2%. In order to find out more about the benefits of adding fermented shrimp waste extract, further research is recommended on egg quality testing.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans' subjects by any of the authors.

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