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Controlling Food Poisoning Bacteria in Fermented Chicken Sausage Using *Lactobacillus plantarum*

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ABSTRACT

Lactobacillus plantarum (*L. plantarum*) bacteria is generally recognized as safe and widely used in the food industry. The current study aimed to study the antimicrobial effects of *L. plantarum* against some food poisoning microorganisms, such as *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), and *Escherichia coli* (*E. coli*) in oriental fermented chicken sausage for 18 days of storage at 4°C. The *L. plantarum* has broad-spectrum antimicrobial effects that enhance the quality and safety of food products. *L. plantarum* reduced the count of *S. aureus*, *B. cerus*, and *E. coli* to 1.54, 4.26, and 3.03 Log_{10}, respectively, after 18 days of refrigerated storage. Moreover, there were significant effects of *L. plantarum* on pH, thiobarbituric acid, total volatile basic nitrogen, and sensory attributes of fermented sausage samples during storage time. It was revealed that *L. plantarum* enhanced the physic-chemical, sensory attributes, and shelf life of fermented chicken sausage. Moreover, *L. plantarum* inhibited the inoculated food poisoning bacteria in fermented chicken sausage. In conclusion, it is recommended to use *L. plantarum* in fermented meat products as a starter and a bio-preservative to enhance the quality of the fermented chicken sausage.

Keywords: Chicken sausage, Food safety, *Lactobacillus plantarum*, Probiotic

INTRODUCTION

Food safety is a key concern in the food industry since it has serious and long-term consequences for public health, particularly when people consume food contaminated with harmful bacteria (FDA, 2020). Moreover, meat products with functional ingredients are considered a demand in the meat industry to reduce the risk of food-borne diseases and enhance health conditions (Sirini et al., 2020). In this regard, fermented sausages fortified with probiotic bacteria are considered a functional food with several health-promoting benefits (Lafarga and Hayes, 2017).

Probiotics, such as lactic acid bacteria are generally recognized as safe and are widely used in the food industry (Oleksy and Klewicka, 2018). It has been applied in human medicine for treatment or alternative in the treatment of chronic inflammation, cancer, cardiovascular diseases, and Alzheimer's disease (Woo et al., 2014; Kurhan and Çakir, 2016), as it can enhance individuals' health, physiology, and immunity (Arasu et al., 2016). Moreover, it is used for food fermentation, improving the texture and flavor of sausages, suppressing the spoilage bacteria of food, and prolonging the shelf-life (Lin and Pan, 2017).

Lactobacillus plantarum is one of the Lactic acid bacteria, that has promising characteristics to be applied in the commercial fermented meat industry as well as it has a great antioxidant effect in the fermentation of camel sausages (Ayyash et al., 2019).

Although the effect of *L. plantarum* inoculation on the quality of fermented meat products was evaluated before (Sun et al., 2016), the current study aimed to focus on the evaluation of not only the antimicrobial effect of *L. plantarum* against some food poisoning microorganisms, such as *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cerus*), and *Escherchia coli* (*E. coli*) but also changes in physicochemical and sensory attributes of inoculated oriental fermented chicken sausage stored at refrigerated temperature for 18 days.

MATERIALS AND METHODS

Ethical approval

The present study did not involve either humans or animals as an experimental setup. The experiment was conducted at Animal Health Research Institute, Egypt.

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Bacterial strains

Referenced pathogenic bacterial strains, including *E. coli* (Lot No: 020090, Des: NCTC: 12241 and ATCC: 25922), *S. aureus* (Lot No: 460074, Des: NCTC: 10788 and ATCC: 6538), *B. cereus* (Lot No: 02900402, Des: NCTC: 10400 and ATCC: 6633), and *L. plantarum* local strain used in the current study were obtained from Media Unit, Food Hygiene Department, Animal Health Research Institute, Dokki, Giza, Egypt.

Pathogenic strains were adjusted to obtain a count of 6 $\log_{10}$ CFU mL^{-1} and *L. plantarum* was adjusted at 8 $\log_{10}$ CFU mL^{-1}.

Assessment of *in vitro* antimicrobial activity of *L. plantarum* against different food poisoning bacteria

Disc diffusion method was used following Toure et al. (2003) to assess the inhibitory range of *L. plantarum* (10^6, 10^7, and 10^8 CFU ml^{-1}) against *E. coli*, *S. aureus*, and *B. cereus* at 10^6 CFU ml^{-1} concentration.

Preparation of chicken sausage

Minced chicken breast meat was purchased from markets in Elminofia governorate, Egypt. The sausage formulations were prepared according to the formulae detailed by Mejri et al. (2017) with minor modifications using chicken meat instead of camel meat. Briefly, sausage batters were prepared for each experiment based on chicken meat content, mincing it with a meat mincer (MK-G20NR-W, Panasonic, Osaka, Japan). The added ingredients to the minced meats included 25 g kg^{-1} NaCl, 10 g kg^{-1} garlic, 4 g kg^{-1} sucrose, and mixed spices 30 g kg^{-1}.

Before the experiment, the meat was surface treated with ultraviolet light (UV) (wavelength 385 nm) for 15 minutes to minimize background micro-flora according to Morsy et al. (2018).

Challenge study

The prepared mixture was divided into seven groups, then inoculated with cultured bacteria adjusted at 10^6 for pathogenic bacteria and 10^8 for *L. Plantarum.* The first group named control entailed UV treated but not inoculated, the second group included *E. coli* inoculated group, the third group had *E. coli* + *L. plantarum*, the fourth group composed of *S. aureus* included group, the fifth group was *S. aureus* + *L. plantarum*, the sixth group contained *B. cereus* infected group, and the seventh group included *B. cereus* + *L. plantarum* inoculated group. After inoculation, samples were kept at room temperature (22°C) for 15 minutes for cell attachment and then stuffed into a sterile polyethylene sausage casing using a handheld sausage filling machine. Samples were kept at 4 ± 1°C for 18 days, and they were analyzed at the beginning of the study as well as days 3, 6, 9, 12, 15, and 18 for remaining microbial populations. The current experiment was repeated three times for each group to obtain mean values for statistical analysis (n = 3).

Microbiological assay

At each sampling day, samples were opened and then 10 g from each one of them was aseptically transferred into 90 mL of 0.1% buffered peptone water (BPW, Biolife) and stomached (model G-560E, Bohemia) for 1 minute. Ten-fold serial dilutions were made in BPW (Biolife) and 1 ml was poured on Eosin methylene blue (EMB, Biolife) for *E. coli* (ISO 21150, 2006), Baird parker (LO, Biolife) for *S. aureus* (ISO 6888-1, 2003) and *B. Cereus*, agar base-MYP (BC-MYP, Biolife) with polymyxin B sulphate supplement (Code 4240001) and egg yolk emulsion (Code 42111601) for *B. cereus* (ISO 7932, 2004). Colonies were counted after 24 hours of incubation at 37°C and expressed as log10 CFU gm^{-1}.

Physico-chemical evaluation

The measured parameters were included pH value using a digital pH-meter (model P107, Consort, Belgium), total volatile base nitrogen (TVB-N, N/100 g of sample), and Thiobarbituric acid reactive substances (TBARS, MDA kg-1) using spectrophotometric (CE 599Universal, USA, AOAC, 2005).

Sensory evaluation

Sensory evaluation of fermented sausage (control and inoculated groups) was performed under the controlled conditions of temperature 22 °C and humidity 55% by seven well-trained panelists who were working in Food Hygiene and Control Department, Animal health research institute, Egypt. The criteria used as the basis of the descriptive organoleptic assessment (color, odor, and texture) with triangle test and the hedonic rating system to score on numerical and continuous scales from 0 (the lowest score for each attribute, very bad) to 9 (the highest score for each attribute, very good). The scale points were used according to ISO 13299 (2003).

Statistical analysis

Results of physicochemical properties and sensory attributes were tested for normality and homogeneity. Then, a one-way analysis of variance was applied to evaluate the statistical significance of differences between groups followed by an LSD test as post hoc for making multiple comparisons by the Statistical Package for Social science Software

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(Version 25, SPSS Inc.; Chicago, IL, USA). The values were expressed as the mean ± standard error. A significant difference was used at the $p \leq 0.05$ probability level. Statistical analysis of concerning results of the effects of *L. plantarum* on food poisoning bacteria was carried out using student's T-test according to Steel and Torrie (1980). Significant differences were calculated at degree of freedom at p values 0.05.

RESULTS AND DISCUSSION

Natural bio-control demand has raised with varying efficacy and impacts on food quality and consumer health (Al-Juhaimi et al., 2018). Minimum inhibitory concentration of *L. plantarum* was evaluated *in vitro*, results in Table 1 showed that zones of inhibition differed according to the use of *L. plantarum* concentration. It was found 10^8 CFU ml^{-1} concentration had the widest inhibitory zone against *S. aureus*, *B. cereus*, and *E. coli.*

Challenge study

Based on the results in Table 2 *L. plantarum* showed antimicrobial effect against *S. aureus*, *B. cerus*, and *E. coli* selected for the challenge study, there was a significant difference between groups inoculated with pathogenic bacteria only and those treated with *L. plantarum* ($p \leq 0.05$). It was found the pathogenic bacteria of inoculated groups reached a count of 8.61, 7.94, and 8.61 $\log_{10}$ for *S. aureus*, *B. cereus*, and *E. coli* over the course of the experiment, respectively. *L. plantarum* decreased count of *S. aureus*, *B. cereus*, and *E. coli* in treated groups to reach 1.54, 4.26, and 3.03 $\log_{10}$ respectively, after day 18 of refrigerated storage. The *L. plantarum* reduced the microbial load of inoculated pathogens mainly against *S. aureus*, followed by *E. coli*, and *B. cereus*. The *L. plantarum* showed antimicrobial properties in fermented chicken sausage (Yadav and Pipaliya, 2017). This might be due to the low pH level of the product that affects directly inoculated bacteria or the metabolites secreted as organic acids, fatty acids, exopolysaccharides, and bacteriocins (Oleksy and Klewicka, 2018).

Table 1. Antimicrobial activity assessment of *Lactobacillus plantarum* against *Staphylococcus aureus*, *Bacillus cerus*, and *Escherichia coli* using disc diffusion method

Pathogenic bacteria	*Lactobacillus plantarum*			
	10^6	10^7	10^8	
Staphylococcus aureus (10^6)	8 ± 0.22	12 ± 0.10	18 ± 0.15	mm
Bacillus cereus (10^6)	ND*	9 ± 0.23	13 ± 0.11	mm
Escherichia coli (10^6)	ND	10 ± 0.10	15 ± 0.14	mm

ND*: Not detected

Table 2. Effect of *Lactobacillus plantarum* on different food poisoning bacteria (log CFU/gm) inoculated in chicken sausage stored at 4°C

Groups	Storage period (day)						
	1	3	6	9	12	15	18
Staphylococcus aureus (10^6)	6.38 ± 0.09	6.89 ± 0.02	7.12 ± 0.05	7.56 ± 0.06	7.88 ± 0.07	8.20 ± 0.04	8.61 ± 0.06
Staphylococcus aureus (10^6) + *Lactobacillus plantarum*	6.35 ± 0.05	5.60 ± 0.09 ***	5.12 ± 0.03 ***	4.81 ± 0.03 ***	4.00 ± 0.08 ***	3.73 ± 0.09 ***	1.54 ± 0.13 ***
Bacillus cereus (10^6)	6.50 ± 0.08	6.79 ± 0.02	7.07 ± 0.03	7.36 ± 0.04	7.71 ± 0.04	7.81 ± 0.02	7.94 ± 0.03
Bacillus cereus (10^6) + *Lactobacillus plantarum*	6.40 ± 0.05	6.20 ± 0.00***	5.99 ± 0.02***	5.55 ± 0.03***	5.13 ± 0.03***	4.99 ± 0.07***	4.26 ± 0.02***
Escherichia coli (10^6)	6.38 ± 0.09	6.89 ± 0.02	7.12 ± 0.05	7.56 ± 0.06	7.88 ± 0.07	8.20 ± 0.04	8.61 ± 0.06
Escherichia coli (10^6) + *Lactobacillus plantarum*	6.28 ± 0.15	5.83 ± 0.08***	5.37 ± 0.04***	4.86 ± 0.03***	4.92 ± 0.03***	3.54 ± 0.08***	3.03 ± 0.12***

Data are presented as (Mean ± S.E). S. E: Standard error. *** Represents statistical significance at $p \leq 0.05$

Physico-chemical evaluation

In this phase of the study, the effect of *L. plantarum* on freshness, shelf life time, and chemical quality of chicken sausage was evaluated. Results in Table 3 revealed that pH values in groups that inoculated with *L. plantarum* were significantly different ($p \leq 0.05$) than the control one. Moreover, there was a significant difference ($p \leq 0.05$) in the pH value over the storage period (18 days) in the same group. The *L. plantarum* decreases pH values in sausage due to the growth of lactic acid bacteria (Slima et al., 2017). This acidification is a straightforward metric for assessing a starter's effectiveness. Organic acids formation during fermentation reduces pH and prevents pathogens' growth (Mataragas et al., 2015). Moreover, it has positive effects on the flavor, as it strengthens the perception of aroma (Bonomo et al., 2009).

Concerning TVB-N results during challenge study in Table 4 revealed that TVB-N increased gradually during chilling storage at 4°C with significant difference ($p \leq 0.05$) between groups inoculated with pathogenic bacteria and

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those treated with *L. plantarum* due to bacterial or enzymatic actions on protein degradation. There was also a significant difference ($p \leq 0.05$) in TVB-N values over the storage period (18 days), with prolonged storage time the TVB-N values increased in groups inoculated with pathogenic bacteria, whereas in those treated with *L. plantarum* decreased. *L. plantarum* maintains lipid oxidation in fermented sausage (Slima et al., 2017).

Regarding Thiobarbituric acid (TBA), a typical indicator of lipid rancidity in meat products provides useful information on lipid oxidation (Tornuk et al., 2015). Results in Table 5 revealed that TBA increased gradually during extended chilling storage at 4 °C with significant difference ($p \leq 0.05$) between groups inoculated with pathogenic bacteria and those treated with *L. plantarum* due to oxidative action effect on fatty acids. There was a significant difference ($p \leq 0.05$) in TBA values over the refrigerated storage period, with prolonged storage time TBA values increased in groups inoculated with pathogenic bacteria, compared to *L. plantarum* treated groups. The *L. plantarum* in camel sausages (Ayyash et al., 2019) and chicken sausages (Yadav and Pipaliya, 2017) has a significant antioxidant effect.

Physico-chemical properties, including pH, TBA, and TVN, in ~~all~~ pathogenic bacteria inoculated groups were evaluated until nine days of storage While, those groups treated with *L. plantarum* were evaluated till 18 days of refrigerated storage (4°C) when samples undergo spoilage.

Table 3. Effect of *Lactobacillus plantarum* on pH of chicken sausage stored at refrigerator temperature 4°C

Groups	Storage period (day)						
	1	3	6	9	12	15	18
Control	5.90 ± 0.04	6.34 ± 0.04	6.97 ± 0.07	S	S	S	S
Staphylococcus aureus (10^6)	5.86 ± 0.02	6.51 ± 0.05[a]	7.01 ± 0.16	S	S	S	S
Staphylococcus aureus (10^6) + *Lactobacillus plantarum*	5.74 ± 0.01[ab]	4.87 ± 0.03[ab]	4.50 ± 0.04[ab]	4.47 ± 0.04	4.23 ± 0.06	5.00 ± 0.06	5.35 ± 0.07
Bacillus cereus (10^6)	5.90 ± 0.03	6.51 ± 0.05[a]	7.01 ± 0.16	S	S	S	S
Bacillus cereus (10^6) + *Lactobacillus plantarum*	5.79 ± 0.02[ab]	4.67 ± 0.03[ab]	4.55 ± 0.04[ab]	4.48 ± 0.04	4.26 ± 0.06	5.00 ± 0.06	5.32 ± 0.16
Escherichia coli (10^6)	5.97 ± 0.03	6.60 ± 0.05[a]	7.22 ± 0.08	S	S	S	S
Escherichiacoli (10^6) + *Lactobacillus plantarum*	5.84 ± 0.03[b]	4.80 ± 0.03[ab]	4.76 ± 0.03[ab]	4.46 ± 0.04	4.20 ± 0.05	5.51 ± 0.07	5.80 ± 0.08

Data are presented as (Mean ± S.E). S.E: Standard error, S: Spoilage depending on sensory evaluation. Significance at $p \leq 0.05$. [a] Significant in the control group, [b] Significant within corresponding bacterial groups

Table 4. Effect of *Lactobacillus plantarum* on TVB-N of chicken sausage stored at 4°C

Groups	Storage period (day)						
	1	3	6	9	12	15	18
Control	1.65 ± 0.03	18.33 ± 0.33	27.72 ± 0.67	S	S	S	S
Staphylococcus aureus (10^6)	1.71 ± 0.05	19.60 ± 0.32[a]	30.26 ± 0.65[a]	S	S	S	S
Staphylococcus aureus (10^6) + *Lactobacillus plantarum*	1.57 ± 0.03	4.80 ± 0.20[ab]	9.47 ± 0.25[ab]	13.66 ± 0.26	16.01 ± 0.51	19.19 ± 0.34	27.72 ± 0.67
Bacillus cereus (10^6)	1.79 ± 0.05[a]	21.00 ± 0.55[a]	32.06 ± 0.63[a]	S	S	S	S
Bacillus cereus (10^6) + *Lactobacillus plantarum*	1.63 ± 0.03[b]	5.54 ± 0.28[ab]	10.25 ± 0.36[ab]	14.39 ± 0.33	17.26 ± 0.36	19.93 ± 0.47	29.67 ± 1.41
Escherichia. coli (10^6)	2.09 ± 0.07[a]	21.66 ± 0.66[a]	32.95 ± 0.53[a]	S	S	S	S
Escherichiacoli (10^6) + *Lactobacillus plantarum*	1.78 ± 0.04[b]	6.10 ± 0.10[ab]	10.93 ± 0.33[ab]	15.06 ± 0.59	18.20 ± 0.39	20.88 ± 0.61	32.95 ± 0.53

Data are presented as (Mean ± S.E). S.E: Standard error, S: Spoilage depending on sensory evaluation. Significance at $p \leq 0.05$. [a] Significant in the control group, [b] Significant within corresponding bacterial groups

Table 5. Effect of *Lactobacillus plantarum* on Thiobarbituric acid of chicken sausage stored at refrigerated temperature 4°C.

Groups	Storage period (day)						
	1	3	6	9	12	15	18
Control	0.05 ± 0.00	0.81 ± 0.03	1.07 ± 0.06	S	S	S	S
Staphylococcus aureus (10^6)	0.05 ± 0.00	0.90 ± 0.02	1.23 ± 0.07	S	S	S	S
Staphylococcus aureus (10^6) + *Lactobacillus plantarum*	0.04 ± 0.01	0.14 ± 0.02[ab]	0.32 ± 0.04[ab]	0.49 ± 0.04	0.66 ± 0.04	0.81 ± 0.03	1.07 ± 0.06
Bacillus cereus (10^6)	0.06 ± 0.01	0.97 ± 0.04	1.37 ± 0.06[a]	S	S	S	S
Bacillus cereus (10^6) + *Lactobacillus plantarum*	0.05 ± 0.01	0.19 ± 0.03[ab]	0.40 ± 0.06[ab]	0.57 ± 0.06	0.74 ± 0.04	0.88 ± 0.04	1.37 ± 0.06
Escherichia coli (10^6)	0.06 ± 0.01	1.05 ± 0.07[a]	1.34 ± 0.09[a]	S	S	S	S
Escherichia coli (10^6) + *Lactobacillus plantarum*	0.05 ± 0.00	0.35 ± 0.14[ab]	0.53 ± 0.05[ab]	0.64 ± 0.07	0.79 ± 0.05	1.00 ± 0.07	1.49 ± 0.07

Data are presented as (Mean ± S.E). S.E: Standard error, S: Spoilage depending on sensory evaluation. Significance at $p \leq 0.05$. [a] Significant in the control group, [b] Significant within corresponding bacterial groups

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Sensory evaluation

Sensory evaluation performed to assess the overall acceptability (odor, texture, and color) of chicken sausage during storage at 4°C is shown in Table 6. Results showed that there was a significant difference ($p \leq 0.05$) between samples inoculated with pathogenic bacteria only and treated groups with *L. plantarum* on overall acceptability (odor, texture, and color). Inoculated pathogenic bacteria groups in the current study spoiled at day nine.

Over the refrigerated storage period, there was a significant difference ($p \leq 0.05$) in overall acceptability values between the first group (control one) and groups inoculated with pathogenic bacteria and that inoculated with pathogenic bacteria and *L. Plantarum*. During storage, fermented chicken sausage became unaccepted or rejected in groups without *L. plantarum* at day 9 of storage and still acceptable in those treated with *L. plantarum* until day 18. Generally, all organoleptic data were in agreement with microbiological, physical, and chemical quality indices present in Tables 1, 2, 3, and 4. Changes in color, odor, and texture during refrigerated storage occurred due to lipid oxidation and protein degradation (Sirocchi et al., 2017). Changes can also be attributed to the ability of lactic acid bacteria to produce small organic substances prevent oxidation mechanism, so enhance the aroma and give specific organoleptic attributes of the products (Tagg et al., 1976). Consumers' demand for foods depends mainly on sensory attributes (Fernández-López et al., 2005). The current study results were in agreement with Slima et al. (2017) who used *L. plantarum* to evaluate the enhanced quality and safety of beef sausages stored at 4℃ for 10 days.

Table 6. Effect of *Lactobacillus plantarum* on sensory attributes of chicken sausage stored at 4°C

Groups	Storage period (day)						
	1	3	6	9	12	15	18
Control	9.11 ± 0.14	6.44 ± 0.13	3.80 ± 0.21	R	R	R	R
Staphylococcus aureus (10^6)	8.44 ± 0.21 [a]	6.44 ± 0.13	3.80 ± 0.21	R	R	R	R
Staphylococcus aureus (10^6) + *Lactobacillus plantarum*	9.22 ± 0.18 [b]	8.70 ± 0.10 [ab]	7.23 ± 0.07 [ab]	6.91 ± 0.02 [ab]	6.14 ± 0.08 [ab]	5.30 ± 0.14 [ab]	4.93 ± 0.18 [ab]
Bacillus cereus (10^6)	9.01 ± 0.09	6.44 ± 0.13	3.47 ± 0.13	R	R	R	R
Bacillus cereus (10^6) + *Lactobacillus plantarum*	9.06 ± 0.06	8.81 ± 0.04 [ab]	7.74 ± 0.10 [ab]	7.31 ± 0.12 [ab]	6.84 ± 0.06 [ab]	6.06 ± 0.05 [ab]	5.07 ± 0.09 [ab]
Escherichia coli (10^6)	9.47 ± 0.20	6.10 ± 0.46	2.80 ± 0.39 [a]	R	R	R	R
Escherichia coli (10^6) + *Lactobacillus plantarum*	9.22 ± 0.18	8.70 ± 0.10 [ab]	7.73 ± 0.13 [ab]	6.91 ± 0.02 [ab]	6.14 ± 0.08 [ab]	5.30 ± 0.14 [ab]	4.62 ± 0.14 [ab]

Data are presented as (Mean ± S.E). S.E: Standard error, R: Rejected depending on sensory evaluation. Significance at $p \leq 0.05$. [a] Significant in the control group, [b] Significant within corresponding bacterial groups

CONCLUSION

Results of the present study revealed that *L. plantarum* has broad *in vitro* and *in vivo* antimicrobial effects against *S. aureus, B. cereus*, and *E. coli*. The use of *L. plantarum* in chicken sausage enhances shelf lifetime, physicochemical properties, sensory attributes, and safety until day 18, whereas infected groups inadmissible at day 9 of storage 4°C. It is recommended to use *L. plantarum* in fermented meat products as a starter and a bio-preservative to enhance the quality.

DECLARATION

Authors' contributions

Rasha Elsabagh designed the plan of study, revised the research article. Shaimaa M. Nada and Elsayed M. Abd-Elaaty analyzed the data, performed laboratory experiments, and drafted the manuscript. Rasha Elsabagh provided the experimental tools. All authors checked the statistical results and approved the final version of the article.

Competing interests

The authors declare no conflicts of interest.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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