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Influence of in-ovo Feeding Bovine Serum Albumin or L-Glutamine to Japanese Quails on Hatchability, Performance of Hatched Chicks, Antioxidant Activity, Lymphoid Organs and Some Blood Biochemical Parameters

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ABSTRACT

This study was designed to investigate the effects of in-ovo feeding (IOF) of L-glutamine and bovine serum albumin (BSA) on hatchability of Japanese quail breeder eggs, and the performance, antioxidant status, relative weight of lymphoid organs and some blood biochemical parameters in the new hatched chicks reared for 6 weeks. A total of 480 fertile eggs of Japanese quails (10 weeks old) were separated into four groups (3 replicates with 40 eggs each). The group T1 consisted of non-injected negative control eggs. Just before incubation, eggs of T2 group were subjected to IOF through injections in the air cell with 30ppm BSA. Eggs of T3 and T4 groups were injected with 15 and 30 ppm/egg L-glutamine (Gln), respectively. The results revealed that the highest hatchability (%) was recorded for eggs injected with Gln 30ppm. After six weeks of rearing, birds treated with IOF of BSA, Gln 15 or Gln 30ppm showed significantly ($P<0.05$) higher body weight, weight gain and better feed conversion ratio in comparison to the control group. Similarly, all treatments showed significantly higher ($P<0.05$) carcass yield than the control group, while none of the treatments had any negative effect on the bone, liver and kidney functions of Japanese quails. All treatments improved significantly ($P<0.05$) total antioxidants capacity (TAC), while in RBC lysates, all experimental groups had significantly higher SOD and GPx activities ($P<0.05$), than those in the control group. Moreover, all experimental treatments exhibited significantly ($P<0.05$) higher SOD and GPx activities in the breast muscles compared with the control group. Birds produced from eggs injected with Gln 15 or 30ppm showed higher relative weights of spleen, bursa and thymus compared to the control group. Treatment of BSA significantly ($P<0.05$) increased thymus relative weight, while the increase in both spleen and bursa relative weights was non-significant compared to control. In conclusion, Gln 30ppm appears to be the best IOF treatment in Japanese quails in terms of productive performance, antioxidants status and relative weights of spleen, bursa and thymus.

Key words: In-ovo feeding, Japanese quail, Performance, Antioxidants, Lymphoid organs, Blood parameters.

INTRODUCTION

The increasing requirements for animal protein sources led to the development of alternative genetic and dietary strategies to obtain the maximum potential yield (Hassan et al. 2016 and 2018; Mohamed et al. 2016; Elsherif et al. 2021; Samy et al. 2022). In-ovo feeding (IOF) is a relatively recent technology for chickens applied during the pre-hatching period. In this technique, the amnion of an avian embryo can be supplemented with exogenous chemicals or nutrients (Uni and Ferket 2003; Asipa et al. 2022). The activity of digestive system and digestive enzymes may be improved by the absorption of substances by an embryo during the incubation period (Uni et al. 2005).

Previous studies have investigated the use of carbohydrates (Salmanzadeh et al. 2011), vitamins (Nowaczewski et al. 2012), amino acids (Salmanzadeh et al. 2011) and minerals (Tako et al. 2004). During the final stage of incubation, it was shown that certain quantities of amino acids, such as glycine and proline, were ineffective in improving embryonic development. Chick weight at hatch was improved following the increased availability of amino acids to hatching chicks through IOF with amino acids (Ohta et al. 2001).

Glutamine (Gln) is the primary source of energy for the gastrointestinal system. It also stimulates immune response of cells (Gruzat et al. 2018), improving the absorptive capacity of the mucosa and hence the ability of

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gastrointestinal tract to absorb nutrients (Burrin and Stoll 2009; Jazideh et al. 2014). Adding Gln to the hatchery diet increased the weight of the chicks, which suggests that it can have a positive impact on the growth rate of broilers (Jazideh et al. 2014). An in-ovo experiment with Japanese quail breeder eggs did not show any adverse effect on the hatchability; rather, it improved post-hatched performance of Japanese quails (Sallam et al. 2019).

Furthermore, investigations on the role of the amino acid, glutamine, in the synthesis of antioxidant enzymes (SOD and GPx), as well as its effects on the immune response, some blood profiles, hatchability traits and performance of newly hatched chicks deserve attention from both the scientific and technical communities. Therefore, this study was planned to investigate the influence of IOF bovine serum albumin and L-glutamine on hatchability traits, performance parameters, antioxidant status, relative weight of lymphoid organs and some blood parameters in hatched Japanese quail breeder chicks.

MATERIALS AND METHODS

The authors confirm that the ethical policies of the journal were followed and approval of the appropriate ethical review committee was obtained. The authors followed EU standards for the protection of animals used for scientific purposes.

The experiment was conducted at the Agricultural Research and Production Station of National Research Centre, Al-Nubaria, Al-Beheira Governorate, Egypt. A total of 480 fertile eggs were taken from 10-week old Japanese quail breeder hens. Individually numbered and weighed eggs were fumigated with formaldehyde gas. These eggs were divided into four experimental groups (T1-T4) of 120 fertile eggs each, with three replicates in each group (40 eggs each). The group T1 consisted of non-injected negative control eggs. Just before incubation, fertile eggs of T2 group were subjected to IOF through injections in the air cell with 30ppm bovine serum albumin (BSA). Eggs of T3 and T4 groups were injected with 15 and 30 ppm/egg L-glutamine (Gln), respectively. The preparation of IOF solutions was as achieved by dissolving the BSA or Gln in the desired amount of sterile distilled water.

Eggs Injection and Holes Coverage

The egg injection was carried out five hours before the eggs were placed in the incubator for hatching. Briefly, eggs were cleansed and ethyl alcohol was used to sterilize the wide top of the egg (where the air cell is located). A stylus was penetrated into the air cell of the egg, using an extremely firm and thin stylus, and the required amount of experimental ingredients were injected using a 1ml graded syringe. Then, the punctured site was sealed with non-toxic glue (Bhanja et al. 2004). Eggs from all four experimental groups were incubated in an automatic incubator at 37.6°C and 65% relative humidity and rotated through 45° six times daily. On the 15thday of incubation, all eggs were transferred to the hatcher and kept at 37.4°C and 70% relative humidity until hatching.

Birds and Management

After the eggs were hatched and the feathers of chicks had dried completely, 45 birds were randomly selected and assigned to three replicates (15/replicate) for each treatment. Chicks of all treatment groups were reared in galvanized cages until they were 6 weeks old. Water and feed were available for the duration of the experiment. The NRC (1994) recommended diet was used to ensure that all birds received their complete nutrients requirements. Birds of all four groups were reared under the same conditions and fed the same diet.

Measurements

At 6 weeks of age, 28 quails (7 quails/treatment) were randomly selected, slaughtered, and defeathered to obtain tissue samples. During slaughtering, heparinized tubes were used to collect blood samples. Plasma was collected after centrifugation (4000 rpm for 15minutes) and stored at -20^{o}C until analysis for some blood biochemical parameters.

Blood and Tissue Biochemical Parameters

Commercially available kits were used to analyze plasma samples for levels of certain biochemical blood parameters, such as Total Antioxidant Capacity (TAC), Creatinine, Urea, and Alkaline Phosphatase. Activities of antioxidant enzymes (SOD and GPx) were evaluated in the lysates of red blood cells by using commercial kits. A commercial kit was used to assess the activities of antioxidant enzymes (SOD and GPx) in breast muscles of each bird.

Statistical Analysis:

Data were analyzed using General Linear Model (GLM) of statistics (SAS 2006), with in-ovo injection (i) treatments as the major effect, as shown in following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Y_{ij} = The j observation of the i^{th} in-ovo treatments; μ = Overall mean; T_i = The fixed effect of the i^{th} *in-ovo* treatments and e_{ij} = The random error.

Duncan's multiple range test (Duncan 1955) was applied to determine if there were any differences between the group means.

RESULTS

Hatchability and Hatchling Weight of Quails

Table 1 shows the effects of in-ovo feeding (IOF) Japanese quail with bovine serum albumin (BSA), Gln 15ppm, or Gln 30ppm on hatchability percentage and productive performance of hatched birds reared for 6 weeks. The highest hatchability ($P<0.05$) was recorded for eggs injected with Gln 30ppm, whereas other treatments negatively affected ($P<0.05$) the hatchability compared to the control group (non-injected eggs). On the contrary, non-significant differences were observed in the hatched bird weight among all treatments. Also it was found that after six weeks of rearing, all treatments significantly ($P<0.05$) improved body weight and weight gain of birds in comparison to the control group. The best ($P<0.05$) feed consumption and feed conversion ratio were recorded for birds treated by IOF with Gln 30 ppm. In addition, birds treated with IOF of BSA or Gln 15ppm significantly ($P<0.05$) improved FCR compared to the control group; however, no significant differences were recorded for feed intake in birds of these two groups compared to the control.

Table 1: Influence of in-ovo treatments of Japanese quail eggs on hatchability and productive performance parameters of hatched birds reared for 6 weeks

Parameters	T1 (Control)	T2 (BSA[1])	T3 (Gln[2] 15ppm)	T4 (Gln[2] 30ppm)	SE	Significance
Hatchability (%)	70.54[b]	61.12[d]	67.02[c]	84.50[a]	±0.70	***
Hatchling weight (gm)	8.53	8.67	8.77	8.81	±0.07	NS
Final weight (gm)	198.23[b]	226.70[a]	240.06[a]	235.17[a]	±2.72	***
Weight gain (gm)	189.70[b]	218.03[a]	231.29[a]	226.36[a]	±3.46	***
Feed intake (gm)	717.15[a]	691.75[a]	735.67[a]	624.63[b]	±4.42	***
Feed conversion ratio	3.78[a]	3.17[b]	3.18[b]	2.76[c]	±0.05	***

Means with different superscripts within the same row are significantly different (P<0.05); ***P<0.001, NS: Not significant (P>0.05). [1]Bovine serum albumin (BSA), [2]Glutamine (Gln).

Table 2: Influence of in-ovo treatments of Japanese quail eggs on carcass characteristics of hatched birds

Parameters	T1 (Control)	T2 (BSA[1])	T3 (Gln[2] 15ppm)	T4 (Gln[2] 30ppm)	SE	Significance
Carcass yield (%)	63.84[b]	71.64[a]	74.46[a]	73.54[a]	±1.48	***
Heart weight (%)	0.83	0.88	0.85	0.81	±0.02	NS
Liver weight (%)	2.48	2.57	2.47	2.52	±0.07	NS
Gizzard weight (%)	1.93	2.02	1.87	1.99	±0.06	NS

Means with different superscripts within the same row are significantly different (P<0.05); ***P<0.001, NS: Not significant (P>0.05). [1]Bovine serum albumin (BSA), [2]Glutamine (Gln)

Table 3: Influence of in-ovo treatments of Japanese quail eggs on some biochemical blood parameters of hatched birds

Parameters	Units	T1 Control	T2 (BSA[1])	T3 (Gln[2] 15ppm)	T4 (Gln[2] 30ppm)	SE	Significance
Creatinine	mg/dL	0.68[a]	0.58[ab]	0.51[b]	0.50[b]	±0.03	***
Urea	g/dL	0.75[b]	0.89[a]	0.67[c]	0.59[bc]	±0.04	***
ALP	IU/L	196.21[a]	192.41[b]	186.71[c]	194.85[ab]	±1.19	***

Means with different superscripts within the same row are significantly different (P<0.05); ***P<0.001, NS: Not significant (P>0.05). [1]Bovine serum albumin (BSA), [2]Glutamine (Gln).

Carcass Characteristics

At 42 days of age, the carcass characteristics of Japanese quails hatched from eggs injected with BSA, Gln 15ppm, or Gln 30ppm are shown in Table 2. The result of carcass yield showed the same trend as was seen for body weight and weight gain in four treatment groups. All treatments significantly increased (P<0.05) the carcass yield than the control group.

Blood Biochemical Parameters

Table 3 shows the effects of in-ovo BSA, Gln 15ppm and Gln 30ppm treatments on blood creatinine and urea levels as kidney function parameters, and alkaline phosphatase (AlP) activity as an indicator for liver and bone disorders of hatched birds at 42 days of age. For renal function, all treatments showed significant (P<0.05) with slight decrease in blood urea and creatinine levels, these values were within the normal range, indicating that none of the treatments had a negative effect on the kidney function of Japanese quails. Similarly, the results of blood ALP activity for all treatments revealed slight decrease (P<0.05) compared to control, but these values were within the normal range, indicating no adverse effects of treatments on bone and liver functions.

Antioxidant Activities

Fig. 1 presents the effects of in-ovo feeding BSA, Gln 15ppm and Gln 30ppm on total antioxidant capacity (TAC) of Japanese quails. All treatments improved significantly (P<0.05) TAC compared to the control group, but the highest TAC was recorded for birds subjected to IOF with Gln 30ppm.

The effects of in-ovo feeding of BSA, Gln 15ppm and Gln 30ppm on superoxide dismutase (SOD) and glutathione peroxidase (GPx) in Japanese quails red blood cells lysates are shown in Fig. 2. In RBC lysates, the three treatment groups showed significantly higher SOD and GPx (P<0.05) activities compared to the control group, with the highest SOD activity was recorded for birds treated by IOF with Gln at 15 or 30ppm. Furthermore, the highest GPx activity was detected in birds treated by IOF with Gln 30ppm, while the results of Gln 15ppm were comparable to those of BSA.

Fig. 3 shows the effects of in-ovo feeding of BSA, Gln 15ppm and Gln 30ppm on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in Japanese quail muscles. All treatment groups resulted in significantly (P<0.05) higher SOD and GPx activities compared to control, moreover the highest SOD activity was recorded for birds treated by IOF with Gln 30ppm, while the highest GPx activity was recorded for birds treated by IOF with BSA or Gln 30ppm.

Relative Weight of Lymphoid Organs

The effects of in-ovo feeding of BSA, Gln 15ppm, and Gln 30ppm on relative weight of lymphoid organs in Japanese quails are shown in Fig. 4. The results indicated that birds produced from eggs injected with Gln 15 or 30 ppm showed significantly higher (P<0.05) relative weight of spleen, bursa and thymus compared to those of the control group. However, BSA significantly (P<0.05) increased thymus relative weight, while no significant with slightly increase was found in both spleen and bursa relative weight compared to control group.

DISCUSSION

Hatchability and Hatchling Weight of Chicks

The current findings regarding hatchability of IOF treated eggs are consistent with those of Uni et al. (2005), who reported that Gln in-ovo feeding had favorable effect on increasing hatchability percentage throughout the late stages of embryogenesis. As described by Shafey et al. (2010), Gln in - ovo injection improves embryo energy

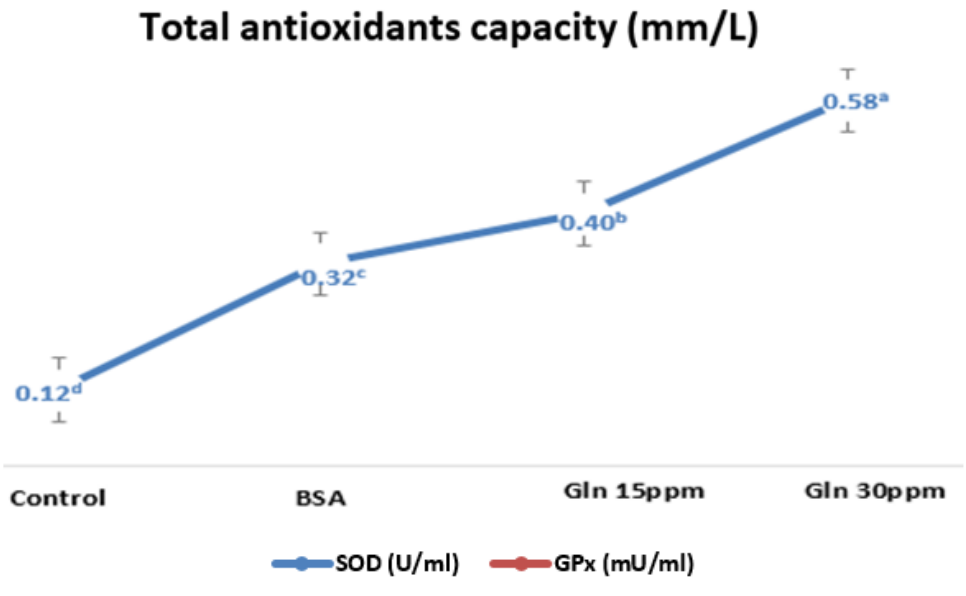

Fig. 1: Influence of in-ovo feeding bovine serum albumin (BSA), glutamine (Gln) Japanese quail on total antioxidants capacity.

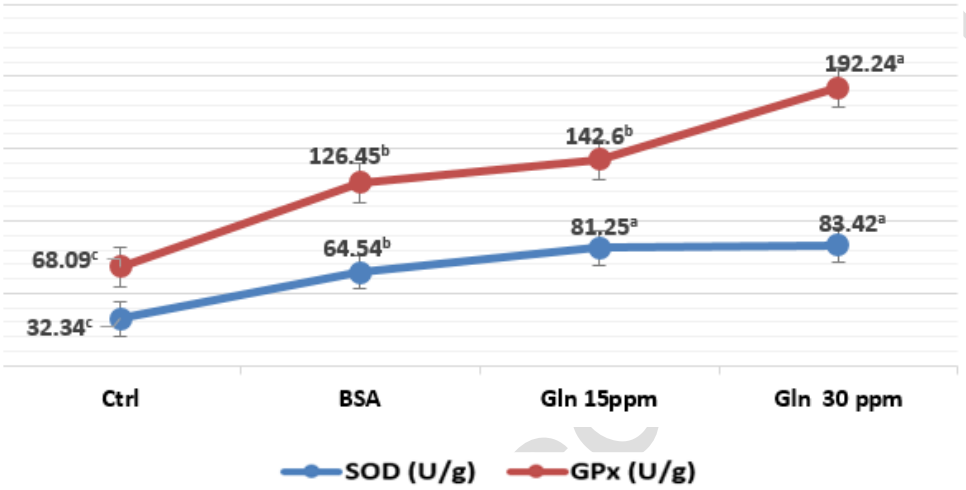

Fig. 2: Influence of in-ovo feeding bovine serum albumin (BSA) and glutamine (Gln) to Japanese quail on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in RBCs lysates.

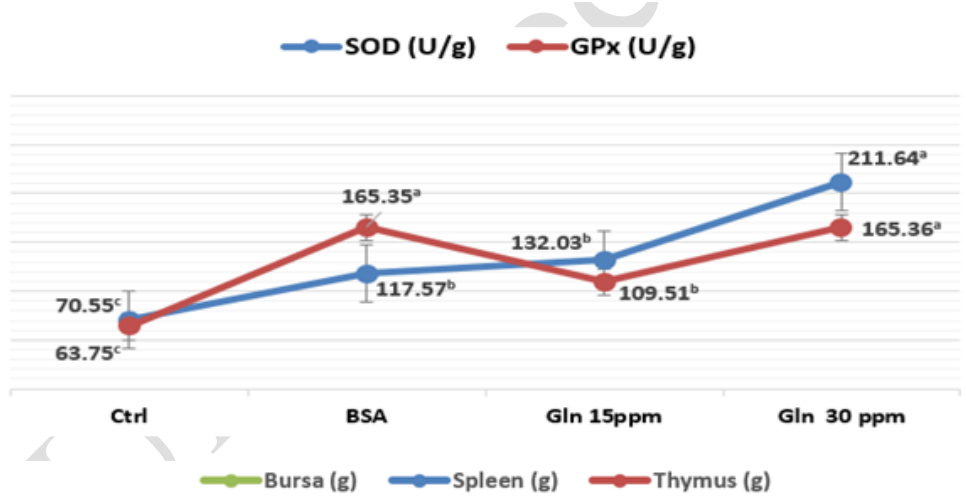

Fig. 3: Influence of in-ovo feeding bovine serum albumin (BSA) and glutamine (Gln) to Japanese quail on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in breast muscles.

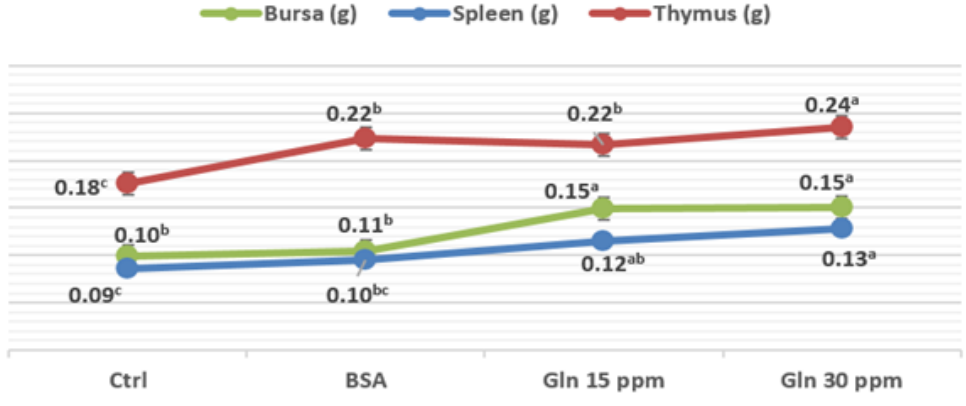

Fig. 4: Influence of in-ovo feeding bovine serum albumin (BSA) and glutamine (Gln) to Japanese quail on relative weight of lymphoid organs.

status and saves muscle protein from mobilization, resulting in improved hatchability and growth. This is most likely related to the in-ovo injection reaction, which is influenced by heredity, hatching egg quality, and incubation conditions (Uni and Ferket 2004). In broiler chicks, Rufino et al. (2019) found that Gln in-ovo injection increased hatchability by 0.5 percent than the control (non-injected group). In-ovo feeding of Gln has been demonstrated to enhance body weight, weight gain, and feed consumption (Shafey et al. 2014; Salmanzadeh et al. 2016 and 2020). In addition, Gln supplementation improved the growth performance of broilers (Salmanzadeh and Shahryar 2013a,b). According to Al-Daraji et al. (2012), Japanese quails produced from eggs injected with various doses of arginine exhibited significant increase in body weight and weight gain.

Carcass Characteristics

The carcass characteristics recorded in this study are in agreement with those of Chen et al. (2009) and Salmanzadeh and Shahryar (2013a,b), who found that in-ovo Gln supplementation significantly increased quail carcass weight compared to the control. Likewise, Glutamine supplementation (5 and 10g/kg diet) improved the carcass weight of broilers (Dai et al. 2009).

Blood Biochemical Parameters

Our findings regarding the effects of IOF on blood biochemical parameters in hatched chicks are consistent with those of Rufino et al. (2019), who found that 0.5 percent L-glutamine might be used as a serum biochemical metabolism regulator in in-ovo chick embryos.

Antioxidant Activities

Superoxide dismutase and GSH-Px are the principal antioxidants in the body cells (Zhang et al. 2015). Similar to findings of this study, Zhu et al. (2011) concluded that IOF of Gln can boost tissue antioxidant capacity and minimize oxidative stress in chicks. Furthermore, Gln supplementation boosted the activities of GSH-Px and SOD, as well as GSH and TAC (Bai et al. 2019).

Relative Weight of Lymphoid Organs

It is well known that the immune tissue development is the basis of immune functionally. In addition, relative weight of lymphoid organs reflects the immunity status of an individual. When 1% Gln was supplied in the feed of broilers, the relative weights of the thymus and spleen were significantly higher compared to the control group (Bartell and Batal 2007). Salmanzadeh et al. (2020) found that treatment of broiler breeder eggs in-ovo with 10, 20, 30, 40, and 50 mg of Gln resulted in increased immunological response in birds hatched from these eggs.

Conclusion

Results of the present study show that in-ovo feeding with BSA, Gln 15 or 30ppm significantly ($P<0.05$) increased body weight, weight gain and improved feed conversion ratio without any adverse effect on the bone, kidney, liver functions of Japanese quails. In addition, there was significant ($P<0.05$) improvement in plasma total antioxidants capacity, and superoxide dismutase and glutathione peroxidase activities in both RBCs lysates and breast muscles. Furthermore, the best treatment is IOF with Gln 30ppm in Japanese quails in terms of productive performance, antioxidants status and relative weights of spleen, bursa and thymus.

Authors' Contribution

All the authors equally contributed to the study design, methods, interpretation of results, and preparation of the manuscript.

Declarations

There is no conflict of interest to declare in this study.

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