



## Laying performance, egg quality and hatchability traits of Japanese quail (*Coturnix coturnix japonica*) fed a chromium yeast feed additive Layplus<sup>(R)</sup>

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### Abstract

A 12-week study was conducted at the Teaching and Research Farm of the Obafemi Awolowo University, Ile-Ife to evaluate the effect of dietary inclusion of LAYPLUS® (LP) on fertility and egg parameters of female Japanese quails, *Coturnix coturnix japonica*. Two hundred and ten birds of average weight of 167.75±3.94g were used for this study, and they were allocated randomly into seven treatment groups and replicated thrice. Group 1 served as control without inclusion of LAYPLUS®, while groups 2, 3, 4 and 5 had 250 mg/kg, 500 mg/kg, 750mg/kg and 1000mg/kg inclusion of LP and group 6 had 240mg/kg of vitamin E, respectively, and group 7 clomiphene citrate. Eggs were collected daily and egg production was calculated on a bird-day basis. The data obtained were analyzed using one-way ANOVA. There was significant increase ( $p<0.05$ ) in the egg production parameters namely egg weight, egg laid per hen and percentage laying rate of LP500mg/kg compared with other groups. The highest percentage egg production was by the LP500mg/kg at 57% while the lowest was by the LP250mg/kg at 41%. There was no significant difference in the egg weight and Haugh unit among the supplemented birds and the control group. There was significant difference among the treatments in the percentage hatchability of the eggs with LP500mg/kg eggs recording the highest value of 95.68%. It can be concluded from this experiment that supplementation of the feed with LP at 500mg/kg had beneficial effect on quail production through improved effect on egg production and hatchability.

**Key words:** quail, fertility, egg parameters, layplus®,

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### Introduction

The Japanese quail (*Coturnix coturnix japonica*) is the smallest avian species being farmed and has been considered as a source of animal protein and kept as a dual purpose breed for meat and egg production. Japanese quails are good model for research due to their small size, early sexual maturity (6-8 weeks), ability to produce three or four generations a year, and the relative ease of maintaining a colony (Wilson *et al.*, 1961; Vilchez *et al.*, 1990). Gebreil (2002) emphasized that for the optimum fertility ratio of Japanese quails; required male-female ratio is between 1:1 and 1:3. The fertility could highly vary even within the same breed mainly due to poor management, improper proportion of males or poor ability of males in the flock to produce viable sperms (Malago and Baitilwake, 2009). Several studies have shown that vitamin E is used in the poultry diet because of its antioxidant properties (Sahin and Kucuk 2001, Sahin *et al.*, 2002, Ramnath *et al.*, 2008). Franchini *et al.* (1991) reported that dietary vitamin E increases the level of sex hormones.

The research with different avian species has shown that increased level of dietary vitamin E increased hatchability and fertility (Lin *et al.*, 2004, Fitri *et al.*, 2012). LAYPLUS® is a powdery plant product containing chromium yeast and *Leptadinea reticulata* (3mg and 100mg/kg respectively) formulated in the poultry industry to improve egg production and hatchability in layer. This product is easily available in the Nigerian market and the recommended dose in layers is 500mg/tonne of feed. Chromium is essential for activating certain enzymes such as digestive enzyme trypsin and for stabilization of proteins and nucleic acids (Anderson, 1994). Sahin *et al.* (2001) observed that chromium picolinate supplementation of Japanese quail diets increased albumen index and albumen weight.

The practice of using synthetic hormones as stimulators of reproductive performance in farm animals have been questioned in many quarters because of its negative cumulative effect in animal products meant for consumption and human health (Nikolova *et al.*, 2010 ; Surdjiska *et al.*, 2005). Alternative measures now

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recommended for facilitating reproductive efficiency of animals is the application of organic extract of plants like leaves, seeds, stem and roots (Nikolova *et al.*, 2010, Kitanov *et al.*, 2003, Petkova *et al.*, 2008). Extracts of plants have been used as natural feed additives and have generally been proven to be effective and non-toxic when consumed by humans and animals. *Leptadenia reticulata* is very valuable medicinal plant belonging to family Asclepiadaceae popularly known as Jivanti. Extract of roots and leaves of the plant act as antibacterial and anti-fungal agent (Patel and Dantwala, 1958). The plant possesses the potent lactogenic, anabolic and galactagogue effect (Anjaria *et al.*, 1974; Anjaria *et al.*, 1975; Ravishankar and Shukla, 2007). It is specially known for its stimulant and restorative properties; it possesses appetizer, aphrodisiac and antibacterial properties (Bawra *et al.*, 2010). This product has not been evaluated in quail birds to establish its potential to boost layers efficiency; therefore this study was designed to assess the effects of varying level of the product to enhance the performance and egg quality characteristics of Japanese quails.

### Materials and Methods

#### Location of the Experiment

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife, Osun state, situated in the South-western part of Nigeria. Ethical clearance was received from the Department of Animal Sciences Ethical board of Obafemi Awolowo University, Ile-Ife, Nigeria

#### Housing and Management

A total of two hundred and ten 3weeks old Japanese quails (147 females and 63 males) were obtained from a reputable farm and were placed in a well-designed cage and adapted for two weeks. Feed and water were provided *ad-libitum* for consumption. Multi-vitamin drugs were given to the birds on arrival on the farm to serve as an anti-stress and during the period of their acclimatization to stabilize their condition.

#### Experimental design

After two weeks of adaptation the birds were weighed and randomly allotted into seven experimental treatments replicated three times. LAYPLUS® contains chromium yeast and *Leptadinea reticulata* (3mg and 100mg/kg respectively) formulated in the poultry industry to improve egg production and hatchability in layer. Each treatment contains 7 female birds

with 3 male birds in each group. The experimental design was Randomized Complete Design. Treatment 1 served as the control, Treatment 2 comprised 250mg/kg LAYPLUS®, Treatment 3 comprised 500mg/kg LAYPLUS®, Treatment 4 comprised 750mg/kg LAYPLUS®, T5 comprised 1000mg/kg LAYPLUS®, T6 comprised 250mg/kg of vitamin E and T7 comprised 1.5mg/kg of clomiphene citrate.

#### Performance measurements

Eggs were collected daily and egg production was calculated on a bird-day basis. Eggs were weighted individually daily. Feed not consumed was weighed daily and average bird consumption was then calculated by dividing total feed consumed during 7 day by number of birds per cage. Feed conversion ratio was calculated as kg feed per kg egg.

#### Determination of physical characteristics of eggs

A total of 54 intact, newly laid eggs (9 per treatment group) were collected from the experimental birds once in a week from 9weeks to 12weeks of their age for determination of physical characteristics. Egg quality traits were measured following standard procedures as described by Monira *et al.* (2003) and Fayeye *et al.* (2005). Egg was weighed individually using electric kitchen scale, then broken into a flat surface and vernier calliper was used to determine the height of the thick albumen that immediately that surround the yolk. Albumen weight was determined based on the difference between the egg weight, yolk and shell weight. The length and maximum width of each egg was measured using vernier calliper to the nearest 0.1cm. The yolk was collected into a calibrated graduated beaker and weigh on a sensitive scale.

#### Yolk index

Yolk index was calculated as the ratio of yolk height to yolk width to determine egg freshness (Sharp and Powell, 1930)

$$\text{Yolk index (\%)} = \frac{\text{Yolk height (cm)}}{\text{Yolk width (cm)}} \times 100$$

#### Haugh unit

The height correlated with the weight determines the haugh unit. The higher the number, the better the quality of egg. Haugh unit =  $100 \times \log (h - 1.7w^{0.37} + 7.6)$

Where H = albumen height in millimetres ; W= weight of egg in grams.

**Eggshell weight and thickness**

The egg shell was cleaned and weighed on sensitive scale while the shell thickness was determined using micrometer gauge with a ball anvil to the nearest 0.01mm. The broken surface of the shell was wiped cleaned with a tissue moistened in distilled water, and then the measurement of egg shell thickness was taken from the mid region.

**Yolk colour**

Yolk colour was determined using a Roche color fan, which is a series of 15 color plastic tabs arranged as a fan corresponding to the range of yolk colours found in eggs.

**Reproduction parameters**

The birds were housed in cages at a ratio of three males to seven females. A total of 180 eggs at 15 week of age were collected and used to investigate reproduction parameters. Eggs collected daily over a 3 days period were numbered and weighed individually before taken to hatchery unit. Eggs were incubated at a temperature of 37.8°C with 55% RH for 14 days. They were then transferred at random to Hatcher trays (which were located in the bottom of the same incubator) and were maintained at 37.2°C and 75% RH until hatching.

**Traits measured**

After hatching, chicks were weighed, the un-hatched eggs were broken to investigate fertile and infertile eggs. Fertility was determined as

the proportion of fertile eggs to the number of eggs set. Hatchability of fertile eggs was estimated at the proportion of chicks hatched from fertile eggs. Hatchability of set eggs was calculated as the proportion of the number of chicks hatched out of the total number of eggs set.

**Results**

There was marked improvement in laying rate (average percentage of egg produced throughout the period of the study) and total number of eggs produced by birds fed supplemented diets of *Leptadenia reticulata* and Chromium yeast (LAYPLUS®) combination. Group supplemented with 500mg/kg LP had significantly higher laying rate and egg number when compared with other treated and control group as shown in Table 1.

There was significant ( $p < 0.05$ ) decrease in egg weights of birds fed diet T3 (250mg/kg LAYPLUS®) compared to those recorded in other groups during the entire period of experiment. In addition, the results showed a significant ( $p < 0.05$ ) difference among treatments on egg mass. The highest egg mass was recorded in T4 (500mg/kg LP group) (238.33±26.0) while group with 1.5mg/kg diet clomiphene citrate (T7) had the lowest egg mass (116.33±12.42). Average egg weight of supplemented quails at 750mg/kg LAYPLUS® (9.904g) was higher numerically but not statistically significantly different to the control group (9.847g).

**Table 1: Effect of LAYPLUS® on egg production traits**

TREATMENTS	Egg Production traits			
	Laying rate (%)	Egg Number/hen	Egg weight (g)	Egg mass (g)
T1 (CTR)	48.29±7.02 <sup>b</sup>	23.66±3.44 <sup>bc</sup>	9.66±0.17 <sup>a</sup>	189.33±27.53 <sup>bc</sup>
T2 (VIT E)	40.73±3.44 <sup>c</sup>	19.95±1.68 <sup>cd</sup>	9.61±0.13 <sup>ab</sup>	159.66±13.5 <sup>cd</sup>
T3 (LP250)	35.62±5.61 <sup>d</sup>	17.45±2.75 <sup>d</sup>	8.87±0.15 <sup>d</sup>	139.66±22.0 <sup>de</sup>
T4 (LP500)	60.79±6.63 <sup>a</sup>	29.79±3.25 <sup>a</sup>	9.37±0.18 <sup>bc</sup>	238.33±26.0 <sup>a</sup>
T5 (LP750)	53.48±6.03 <sup>ab</sup>	26.20±2.97 <sup>ab</sup>	9.41±0.14 <sup>abc</sup>	209.66±23.7 <sup>ab</sup>
T6 (LP1000)	41.75±5.80 <sup>c</sup>	20.45±2.84 <sup>c</sup>	9.28±0.10 <sup>c</sup>	163.66±22.74 <sup>cd</sup>
T7 (CC)	29.67±3.16 <sup>c</sup>	14.54±1.55 <sup>e</sup>	9.21±0.10 <sup>c</sup>	116.33±12.42 <sup>c</sup>

<sup>a,b,c</sup> Means within each column with different superscripts are significantly different at ( $p < 0.05$ ); CTR- Control (No feed supplement) VIT E- Vitamin E 240mg/kg LP- LAYPLUS®, CC- Clomiphene citrate 1.5mg/kg

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**Table 2: Effect of LAYPLUS® supplementation on the egg quality parameters of Japanese quails**

PARAMETERS	T1	T2	T3	T4	T5	T6	T7
Average egg weight (g)	9.847±0.21 <sup>a</sup>	9.628±0.09 <sup>ab</sup>	9.099±0.15 <sup>c</sup>	9.780±0.19 <sup>a</sup>	9.904±0.15 <sup>a</sup>	9.297±0.08 <sup>bc</sup>	9.389±0.09 <sup>bc</sup>
Albumen height (mm)	6.028±0.14 <sup>ab</sup>	5.778±0.21 <sup>b</sup>	6.083±0.19 <sup>ab</sup>	6.458±0.11 <sup>a</sup>	6.203±0.21 <sup>ab</sup>	6.333±0.13 <sup>a</sup>	5.958±0.13 <sup>ab</sup>
Albumen weight (g)	4.714±0.09 <sup>b</sup>	4.783±0.06 <sup>ab</sup>	4.368±0.06 <sup>c</sup>	4.796±0.09 <sup>ab</sup>	5.000±0.08 <sup>a</sup>	4.564±0.06 <sup>c</sup>	4.434±0.07 <sup>c</sup>
Yolk height (mm)	8.736±0.25 <sup>b</sup>	9.097±0.25 <sup>ab</sup>	8.922±0.21 <sup>ab</sup>	9.458±0.15 <sup>a</sup>	9.375±0.16 <sup>a</sup>	9.042±0.13 <sup>ab</sup>	9.097±0.12 <sup>ab</sup>
Yolk diameter (mm)	26.94±0.22 <sup>a</sup>	26.58±0.19 <sup>ab</sup>	26.69±0.33 <sup>ab</sup>	27.25±0.14 <sup>a</sup>	26.81±0.27 <sup>ab</sup>	26.04±0.21 <sup>b</sup>	26.86±0.09 <sup>ab</sup>
Yolk weight (g)	3.235±0.08 <sup>a</sup>	3.127±0.06 <sup>a</sup>	3.063±0.06 <sup>a</sup>	3.175±0.12 <sup>a</sup>	3.081±0.08 <sup>a</sup>	3.009±0.05 <sup>a</sup>	3.196±0.04 <sup>a</sup>
Shell weight (g)	0.912±0.01 <sup>bc</sup>	0.889±0.01 <sup>bc</sup>	0.822±0.01 <sup>d</sup>	0.925±0.02 <sup>a</sup>	0.958±0.02 <sup>a</sup>	0.882±0.01 <sup>bc</sup>	0.871±0.01 <sup>c</sup>
Shell thickness (mm)	0.956±0.02 <sup>c</sup>	0.975±0.02 <sup>c</sup>	0.974±0.03 <sup>c</sup>	1.354±0.102 <sup>a</sup>	1.132±0.03 <sup>b</sup>	0.988±0.05 <sup>c</sup>	0.967±0.03 <sup>c</sup>
Yolk index (%)	26.61±1.03	28.18±0.90	27.58±1.05	28.06±0.49	28.21±0.47	28.08±0.33	27.38±0.48
Haugh unit (%)	98.20±0.61 <sup>ab</sup>	97.16±0.95 <sup>b</sup>	98.89±0.84 <sup>ab</sup>	100.04±0.41 <sup>a</sup>	98.55±1.09 <sup>ab</sup>	99.88±0.50 <sup>a</sup>	98.08±0.55 <sup>ab</sup>

Notes= Means within each row with different superscripts are significantly different ( $P < 0.05$ ). T1= Control (No feed supplement) ; T2 = Vitamin E 240mg/kg, T3= LAYPLUS® at 250mg/kg; T4= LAYPLUS® at 500mg/kg; T5= LAYPLUS® at 750mg/kg; T6= LAYPLUS® at 1000mg/kg; T7= Clomiphene citrate

The result obtained in T2, T4, and T5 were similar to the control group whereas T3 had lowest average egg weight (Table 2). The result obtained in groups fed supplemented diets of LP (T3-T7) was similar to that of control group except T2 that had the lowest albumen height. There were significant increase in the albumen weight at 240mg/kg vitamin E, LP 500mg/kg and 750mg/kg supplemented diets. Yolk height was significant ( $p < 0.05$ ) higher with supplemented diets of LP 500mg/kg and 750mg/kg (T4, T5) respectively. There were no significant difference ( $p < 0.05$ ) in the yolk diameter of the birds supplemented with diets of LP 500mg/kg, 750mg/kg, 250mg/kg, 240mg/kg vitamin E and 1.5mg/kg Clomiphene citrate compared to the control group (T1). There was no significant difference ( $p > 0.05$ ) in the yolk weight across all the treatment groups. There was significant increase ( $p < 0.05$ ) in the shell weight of birds supplemented with 500mg/kg (T4) diet compared to the rest of the treatments. There was no significant difference ( $p < 0.05$ ) in the yolk index across all treatment groups.

The haugh unit results obtained was similar across the treatment groups except in vitamin E 240mg/kg supplement which had the lowest value recorded. Hatchability percentage of quail eggs fed supplemented diets was significantly ( $p < 0.05$ ) higher in birds with vitamin E supplement diet (T2) ( $96.0 \pm 0.23$ ) followed by T4 (LP500mg/kg) ( $95.68 \pm 0.02$ ) compared to the control (T1). Hatch rate (%) was significantly higher in eggs of quails on T2 (240mg/kg vitamin E), followed by T4 (*Leptadenia reticulata* and Chromium yeast combination at 500mg/kg) where as the lowest value was obtained in eggs of birds supplemented with 1.5mg/kg clomiphene citrate (T7). Fertility percentage was significantly ( $p < 0.05$ ) higher in birds supplemented with LP1000mg/kg (T6) and 240mg/kg Vitamin E (T2) compared to other groups. Birds in T4 and T5 recorded similar ( $p < 0.05$ ) values. Weight of the hatched chicks of quails fed LP500mg/kg supplemented diet was similar ( $p < 0.05$ ) to the control; hatching weight of the other groups were lower than that of the control.

**Table 3:** Effect of LAYPLUS® on the reproduction parameters of Japanese quails

TRAITS	T1 (CTR)	T2 (VIT E)	T3 (LP250)	T4 (LP500)	T5 (LP750)	T6 (LP1000)	T7 (CC)
Hatchability (%)	66.73±0.55 <sup>d</sup>	96.07±0.23 <sup>a</sup>	68.89±0.35 <sup>c</sup>	95.68±0.02 <sup>a</sup>	93.30±1.00 <sup>b</sup>	69.14±0.34 <sup>c</sup>	62.59±0.48 <sup>e</sup>
Hatch rate (%)	64.08±0.22 <sup>e</sup>	95.89±0.11 <sup>a</sup>	64.12±0.29 <sup>e</sup>	85.56±0.58 <sup>b</sup>	80.12±0.24 <sup>c</sup>	69.02±0.22 <sup>d</sup>	46.49±0.58 <sup>f</sup>
Fertility (%)	71.18±0.28 <sup>e</sup>	99.84±0.16 <sup>a</sup>	92.85±0.05 <sup>b</sup>	86.95±0.22 <sup>c</sup>	87.56±0.62 <sup>c</sup>	99.83±0.17 <sup>a</sup>	73.12±0.85 <sup>d</sup>
Chick weight (g)	5.805±0.01 <sup>a</sup>	5.590±0.01 <sup>b</sup>	5.236±0.06 <sup>d</sup>	5.725±0.02 <sup>a</sup>	5.166±0.03 <sup>d</sup>	5.533±0.03 <sup>b</sup>	5.367±0.03 <sup>c</sup>

Means within each row with different superscripts are significantly different ( $P < 0.05$ ). CTR- Control (No feed supplement) ; VIT E- Vitamin E 240mg/kg ; LP- LAYPLUS® ; CC- Clomiphene citrate 1.5mg/kg

### Discussion

Extracts of plants have been used as natural feed additives and have generally been proven to be effective and non-toxic when consumed by humans and animals. Rapid increase in egg production of Japanese quails on treatment T3-T6 diets could be as a result of antioxidants, vitamins and minerals present in *Leptadenia reticulata* which are known to increase growth and stimulate reproduction in humans and animals (Machebe *et al.*, 2011, Nwangwa *et al.*, 2007). The data obtained in this study agrees with the results of other studies on laying Japanese quail (Sahin *et al.*, 2001; 2002) and laying hens (Lien *et al.*, 1999; Uyank *et al.*, 2002; Yildiz *et al.*, 2004) fed diet supplemented with vitamin E and vitamin C combination. This observation may be due to the fact that *Leptadenia reticulata* has tocopherol (Bawra *et al.*, 2010) and it has been confirmed to possess immunomodulatory and antioxidant activities (Pravansha *et al.*, 2012). The egg weight result agreed with Kim *et al.* (1997) who reported that supplementing 800 ppb Cr from Cr picolinate to laying hen diets resulted in higher hen-day production and egg weight, it also agreed with Bayram *et al.* (2007) who reported that egg weight was reduced when quail were fed anise seeds an antioxidant at inclusion levels of 3% and 5%. The significant increase in egg mass is probably the antioxidant activity of the active ingredients in the feed additive. The significant increase in egg number/hen fed supplemented diets could be as a result of antioxidant and minerals present in the feed additive. Bozkurt *et al.* (2009) reported that broiler breeder hens given the lower level of herbal essential oil mixture (24 and 48 mg/kg diet from day one till

46 week of age) produced a higher number of eggs. The improvement in laying rate of supplemented group could be as a result of antioxidant present in LP, similar result was reported by Cabuk *et al.* (2014) who found that herbal essential oil mixture had beneficial effects on the laying rate of Japanese quails.

The reduction in the egg weight obtained in this study was in contrast to the result of some researchers who found positive effect of dietary vitamin C supplementation on egg weight (Altan *et al.*, 1999; Konca and Yazgan, 1999). Single or combined dietary supplementation with vitamin C and vitamin E in laying hens diet had significantly improved egg weight (Ajakaiye *et al.*, 2011). There was higher value of yolk height, yolk diameter, shell weight, shell thickness and haugh unit in group supplemented diet of 500mg/kg *Leptadenia reticulata* and Chromium yeast combination but this was in contrast with the results of Saldanha (2008) who found no differences in egg quality of laying hens fed diets supplemented with different levels of Zn. The similarity of the result obtained for the yolk weight and yolk index across treatment groups however agreed with the report of Abdel-Azeem *et al.* (2005) who found that laying hens fed diets with Yea-Sacc or Lacto-Sacc supplementation had no effect on yolk weight and yolk index. Zinc supplementation of quail diets did not also influence egg weight, haugh unit, yolk index or albumen index (Cruz and Fernandez, 2011).

The significant increase in the hatchability percentage of the birds supplemented diet (LP 500mg/kg) agreed with the report that broiler breeder hens given the lower level of oil mixture of medicinal herbs (including lavender, *Lavandula* spp, eucalyptus *Eucalyptus oblique* and peppermint) at 24 and 48mg/kg diet from

day one till 46 week of age produced a higher number of eggs, fertility and hatchability percentages. King'ori (2011) reported that weight, shell thickness and shape index are the most egg parameters that affect hatchability percentage; hatchability for small eggs is lower than medium and large eggs, which is in accordance with the present results of LAYPLUS supplemented group that hatchability percentage depends on size, shell thickness of eggs. Abiola *et al.* (2008) and Malago and Baitilwake (2009) showed that there was a close correlation between egg size and chick hatching weight. Breeder factors that affect hatchability include health, nutrition, egg size, weight and quality (King'ori, 2011).

The significant increase in the fertility percentage was in agreement with the result of Contreras *et al.* (2000) who found that supplementation of Japanese quail diets with chromium methionine improved egg fertility. Fertility percentage was superior with LP 1000mg/kg (T6) supplemented diets compared to the control. The improvement observed in the fertility rate could be as a result of the stimulant and tonic properties of *Leptadenia reticulata* (Dadarkar *et al.*, 2005). Senapati *et al.* (1996) reported positive correlation between egg weight and hatchability. This agrees with the results obtained in LP500. King'ori (2011) reported that among the most influential egg parameters of hatchability are weight, shell thickness and shape index. Hatchability for small eggs is lower than medium and large eggs, which is in accordance with the results obtained for quails on LP250, LP750, LP1000 and CC which recorded depressed egg hatchability along with the lowest chick hatching weight. Abiola *et al.* (2008) and Malago and Baitilwake (2009) showed that there was close correlation between egg size and chick hatching weights.

### Conclusion

At the end of the experiment, it can be concluded that the group fed diet supplemented with LAYPLUS® at 500mg/kg bw had the best hatchability, fertility and egg production percentage. It is recommended that *Leptadenia reticulata* and Chromium yeast combination (LAYPLUS®) at 500mg/kg feed is optimum for egg production, egg quality, reproduction parameters.

### Conflict of interest

Authors declare that conflicts of interest do not exist in this work.

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