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A Comparative Study on Using Simple Pollen Substitutes to Improve Brood Rearing Activity and Characteristics of Honey Bee Workers

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ABSTRACT

Pollen substitutes are alternatives to natural pollens during dearth periods. In this study, three pollen substitutes prepared using simple materials were compared. The first one contained yeast as protein source (yeast), the second one contained corn flour (corn flour), and the third one contained corn flour plus turmeric (turmeric). These pollen substitutes were presented to bee colonies beside sugar candy without any protein source as a control group. Some parameters were subsequently measured under apiary and laboratory conditions. All feeding types were attractive to bee colonies but bees consumed significantly more yeast and sugar candy within 72h than corn and turmeric. All feeding types did not impair the ability of young larvae to develop into pupae. Bee colonies had high sealed brood area and number of bees in yeast group followed by sugar candy and corn flour. Each of corn flour and turmeric affected the survival ability of bee workers negatively unlike yeast and sugar candy. All the feeding types showed approximately the same effect on the ability of bees to tolerate low temperature. Some morphological characteristics (head width, forewing length and width, wax mirror longitudinal and wax mirror transversal) used as indicators to body size and the development of glands. These characteristics showed higher values in yeast group than the other groups. In light of this study, yeast feeding is promising and recommended as a simple pollen substitute followed by corn flour while turmeric is not preferred.

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INTRODUCTION

The development of bee colonies is highly impacted by feeding. The natural feeding for bees depends on nectar/pollen sources (Crailsheim *et al*. 1992; Huang, 2012; Abou-Shaara, 2017a). Thus, beekeepers establish their apiaries close to cultivated areas with suitable flowering plants for bees. Indeed, pollens represent the natural protein source for bees (Huang, 2012). Pollens are consumed in high amounts by bee colonies over the year (Crailsheim *et al*., 1992), and are important to help bees to fight pathogens (DeGrandi-Hoffman *et al*., 2016). Also, pollens provide bees with vitamins and amino acids which have role in bee immunity as shown from laboratory studies (Glavinic *et al*., 2017). Moreover, pollens are necessarily for brood rearing, causing high effect on colony development (Crailsheim *et al*., 1992; Babendreier *et al*., 2004). Bees showed no preference to consume more feeding (Pollens) with low protein contain than those with high protein content (Basualdo *et al*., 2013). Thus, the nutritional quality of bee pollens did not affect their consumption by nurse bees (Corby-Harris *et al*., 2018). Unfortunately, during dearth periods few flowering plants are available to bees causing negative effects on bees.

There are two types of feeding that can be used as alternatives to natural pollens: pollen supplements and pollen substitutes (Saffari *et al*., 2010a; Aly *et al*., 2014; Gamal-Eldin *et al*., 2018). Pollen supplements contain pollens while substitutes do not contain pollens but suitable alternatives to pollens including flour. Pollen substitutes can show better results than pollens for example acacia pod flour (De Jong *et al*., 2009). An example for the simple substitutes is the use of sugar syrup and whey. This feeding showed a possibility to improve colony parameters and productivity (Vrabie *et al*., 2019). However, there are a doubt about the effectiveness of animal protein sources in improving bee survival and characteristics. Also, in old studies whey showed less consumption rate by bees and did not improve brood rearing activity (Herbert and Shimanuki, 1978). Other trails were based on using alternatives to soybean in pollen substitutes including the use of the flour of black gram which showed ability to

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improve colony parameters (Abbas *et al*., 1995). In other studies, a mixture of components were used including different types of flour, dried yeast, turmeric, corn gluten, sugar, cinnamon, honey and water (Usha *et al*., 2014; Taha, 2015; Amro *et al*., 2016; Zaghloul *et al*., 2017; Islam *et al*., 2020). Such mixtures of components are expected to be costly and require efforts in preparation. Therefore, searching for simple substitutes based on protein from plant sources is necessarily.

The evaluation of pollen substitutes should include their attractiveness to bee workers, effects on bee characteristics, survival, and colony development (Nabors, 2000; Rogala and Szymas, 2004; El-Wahab and Gomaa, 2005; Peng *et al*., 2012; Taha, 2015; Lamontagne-Drolet *et al*., 2019). Therefore, this study aimed to compare the ability of three simple pollen substitutes to improve bee colonies and worker characteristics. These substitutes contained one protein source (yeast, corn flour or turmeric) beside powdered sugar and water. Such feeding types can be easily prepared by beekeepers and were compared with sugar candy as a control group. Based on this study the best feeding type was specified and recommended for usage.

MATERIALS AND METHODS

Pollen substitutes

Three simple pollen substitutes were compared in this study at an apiary in Damanhur city, Egypt. The component per each 100g was 30g inactive Brewer's yeast and 70g powdered sugar for the first feeding type (abbreviated as yeast), 30g corn flour and 70g powdered sugar for the second feeding type (abbreviated as corn flour), 20g corn flour, 10g turmeric and 70g powdered sugar for the third feeding type (abbreviated as turmeric) while the control feeding was sugar candy only without any protein source. Sugar syrup (2 sugar: 1 water w/l) was used to mix the components of each feeding type. The experiment was conducted during autumn period and up to 12 weeks.

Bee colonies

The experiments started with 16 small hybrids of Carniolan bee colonies containing only three combs (two food combs and one sealed brood comb), and new combs were added to each colony according to its need over the study period (10 weeks during autumn). Each feeding type was assessed using four colonies (replicates).

Apiary experiments

Attractiveness and consumption rates

The attractiveness and preference of bees to a specific feeding type was assessed utilizing a choice experiment. In this experiment, four colonies with equal strength were used. All feeding choices were placed above frames (100 g per each feeding type). The number of bees attracted to each feeding type was counted after 5 min from adding the feeding types to evaluate the attractiveness degree. Also, the consumption rates were assessed after 72h to find out the preference of bees to the used feeding types.

Development of immature stages

Areas containing 100 one day old larvae were marked in each colony and inspected after 7 days to count the number of sealed cells. During this period it was expected that bee larvae were fed by nurse workers using the tested feeding types. Therefore, any deleterious effects of feeding types on larval development can cause death of larvae and hence reducing the number of sealed cells than 100. This experiment was done to test the safety of each feeding type to bee larvae.

Colony development

The areas of sealed brood and bees were measured at the end of the experiment using frame divided into square inches (Jeffree, 1958). Then, the number of bees was calculated by multiplying the area of bees by 10 (Abou-Shaara *et al*., 2013a).

Laboratory experiments

Survival rates

The survival ability of bees fed on different feeding types was assessed using a survival experiment. Four jars with perforated covers (Abou-Shaara, 2017b) were used in this experiment per each treatment (a total of 16 jars). In each jar 30 bees were placed with a total of 120 bees per feeding type. The feeding (about 8 g) was presented to caged bees and was renewed daily. Subsequently, the number of dead bees was counted daily and up to seven days. This experiment was performed under room conditions at temperature about 30°C.

Tolerance to low temperature

In this experiment, caged bees of 35 bees per perforated plastic jar and 4 jars per feeding type were used. These bees were left without food for 12h then food was presented (10 g) for 12h and then the bees were left again without food for 1h. Subsequently, the bees were placed in low temperature (about 8°C) until all bees showed not movements. The times at which no movements occurred were recorded and compared among treatments. This experiment was done according to Abou-Shaara (2017a).

Body size and glands development

There is a relationship between body weight and bee morphology (Abou-Shaara *et al*., 2013b). Thus, some

morphological characteristics were used as indicators for the development of bee workers. From each colony 30 nurse bees were collected with a total of 120 per each feeding group. Then, forewing length and width were measured to evaluate the size of bees, and to find out any effects of the used feeding types on body size. Also, head width was measured as an indication to the development of hypopharyngeal glands. Moreover, wax mirror longitudinal and transversal were measured as an indication to the development of wax glands. Measurements of head width and wax mirrors were taken to 15 bees per colony (a total of 60 bees per treatment). The measurements were taken according to Ruttner *et al*. (1978). Scan photo method (El-Aw *et al*., 2012) based on a scanner and a measuring program was utilized to take the measurements.

Statistical analysis

Parametric and non-parametric tests were performed on the data based on their normality, which was tested using Shapiro-Wilk test. Data with normal distribution were subjected to ANOVA followed by Tukey test (parametric tests) while those without normal distribution were subjected to Kruskal-Wallis (non-parametric tests). The survival data were analyzed using Kaplan-Meier analysis (Breslow test), which is perfect for survival analysis (Abou-Shaara, 2018). SPSS v. 16 (Chicago, USA, 2007) was used in the analysis considering $p \leq 0.05$ as significant.

RESULTS

Attractiveness of bee and their consumption rates

The bees attracted and consumed feeding types at various degrees. The number of bees attracted to each feeding type after 5 min showed the absence of significant differences (ANOVA: df=3, F= 1.40, P=0.28>0.05). The mean numbers of attracted bees to each feeding type ranged from 50.75 to 66.25 bees (Fig. 1). Bees according to their attraction to feeding types can be arranged in descending order as yeast, control, corn flour and finally turmeric.

The bees consumed more yeast feeding and sugar candy (control) than corn and turmeric feeding (Fig. 2). The variations between yeast and control in side and corn and turmeric in the other side were significant (ANOA: df=3, F=28.97, p=0.000<0.05). The bees were able to consume more yeast feeding than the other feeding types by 8.67, 40.9, and 42.77g for control, corn flour, and turmeric, respectively.

Development of immature stages

All bee larvae showed ability to complete their development into pupae as the number of sealed cells was 100 in all colonies. Thus, all feeding types had not any negative effects on the development of bee larvae. This indicates the safety of all pollen substitutes used in this experiment to bee larvae.

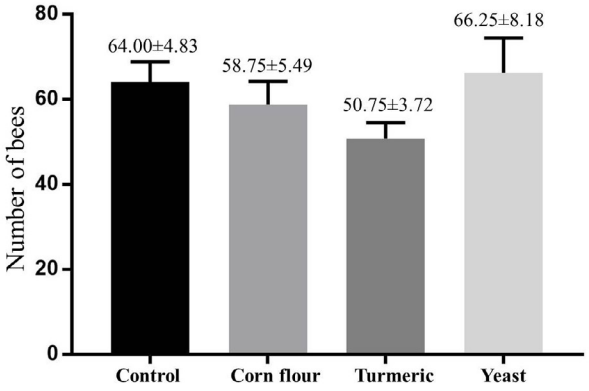

Fig. 1. Number of bees attracted to each feeding type after 5 min (mean±SE).

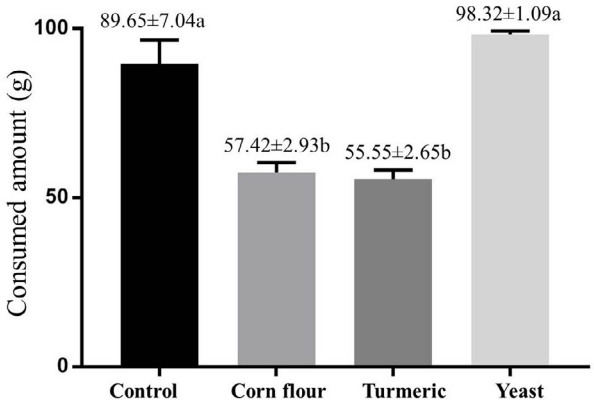

Fig. 2. The amount of each feeding type consumed after 72h (mean±SE). Letters denote the significant differences between feeding types according to Tukey test.

Colony development

Significant differences were detected between all groups in brood area (df=3, F= 21.455, p=0.00<0.05) and number of adult bees (df=3, F= 20.505, p=0.00<0.05). Yeast recorded significantly the highest means in number of bees and brood area (Fig. 3). The control group recorded the second rank after yeast without significant variations than corn flour. The turmeric recorded the last rank with the lowest mean number of bees and brood area than other groups significantly.

Survival rates under laboratory condition

The bees fed with test feeding types showed dissimilar survival rates (Fig. 4). Sugar feeding only (control) and

yeast showed similar survival abilities without significant differences between them (Breslow: Chi-Square=0.367, significance=0.545>0.05). The survival of bees in the control group was better than corn and turmeric groups with significant differences (Breslow for corn: Chi-Square=3.926, significance=0.048<0.05; Breslow for turmeric: Chi-Square=12.112, significance=0.001<0.05). Also, the yeast group was better than corn flour and turmeric groups with significant difference than turmeric group (Breslow: Chi-Square=8.720, significance=0.003<0.05). The results of corn flour feeding was intermediate between turmeric and yeast without significant difference than turmeric (Breslow: Chi-Square=2.589, significance=0.108>0.05) and yeast (Breslow: Chi-Square=1.984, significance=0.159>0.05).

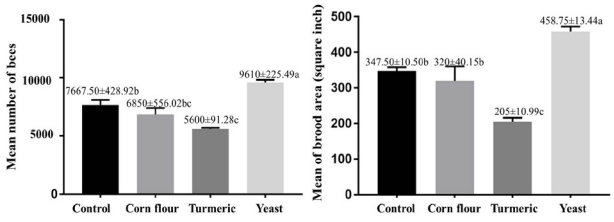

Fig. 3. Variations in mean number of bees and brood area between the four feeding groups. Means±SE are presented, and letters present significant variations between groups according to Tukey test.

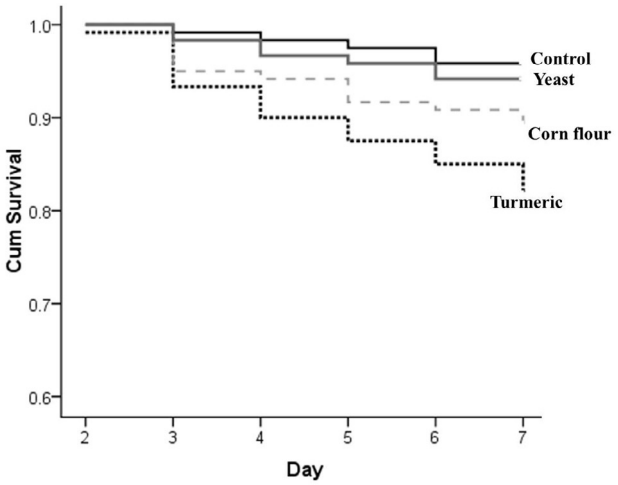

Fig. 4. Cumulative survival of bees fed with different feeding types over one week.

Tolerance to low temperature

The bees after feeding on all feeding types showed similar ability to tolerate low temperature (Fig. 5). The tolerance time until no bees were able to move ranged from 81.50 min (control) to 84.75min (yeast) with difference of only 3.25 min. The treatments can be arranged in descending order as yeast, corn flour, turmeric, and control, respectively. The variations between all treatments were not significant (ANOVA: df=3, F=2.198, p=0.141>0.05).

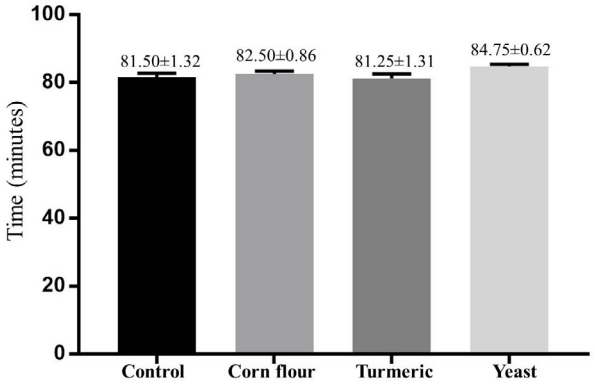

Fig. 5. The time (mean±SE) at which bees stopped moving after exposure to low temperature.

Body size and glands development

Morphological characteristics as indicators to body size showed the presence of significant differences between feeding groups (ANOVA for forewing length: df=3, F=16.094, p=0.00<0.05; ANOVA for forewing width: df=3, F=34.574, p=0.00<0.05). Also, those used as indicators to glands development showed significant variations (ANOVA for head width: df=3, F=5.315, p=0.001<0.05; ANOVA for Wax mirror longitudinal: df=3, F=7.136, p=0.00<0.05; ANOVA for Wax mirror transversal: df=3, F=14.393, p=0.00<0.05). Bees in yeast group had the highest means followed by corn flour, then control group and turmeric (Table I). Yeast differed significantly than control group and turmeric in all measured characteristics, while than corn flour group in forewing width only.

DISCUSSION

Attractiveness of bee and their consumption rates

The bees attracted to all feeding types without significant differences. This can be explained by the presence of sucrose sugar in all feeding types with percentage from 70 to 100%. It is well known that sucrose is highly attractive to bees due to its sweetness (Hough and Phadnis, 1976; Barker, 1977). The bees are able to utilize sucrose from different types of feeding either liquid or solid as shown from a previous experiment (Abou-Shaara, 2017a). Also, the attraction of bees to all feeding types confirmed that protein materials: yeast, corn and turmeric are palatable to bees. The consumption rates showed clear significant

Table I. Measured morphological characteristics (Means±S.E.) of bee workers from treatment groups.

Characteristic	Control	Corn flour	Turmeric	Yeast
Forewing length	8.71±0.01b	8.75±0.01a	8.71±0.008b	8.79±0.009a
Forewing width	3.02±0.01b	3.04±0.01b	2.97±0.009c	3.12±0.01a
Head width	3.53±0.01b	3.56±0.009ab	3.55±0.01b	3.59±0.01a
Wax mirror longitudinal	1.32±0.007b	1.33±0.008ab	1.31±0.007b	1.36±0.007a
Wax mirror transversal	1.89±0.01b	1.95±0.01a	1.90±0.01b	1.99±0.01a

* Means followed by the same letters are not significantly different according to Tukey test.

variations between feeding types. It was clear that bees were able to consume yeast feeding rapidly within 72h followed by control, corn flour and turmeric. Thus, the bees were able to utilize yeast in high rates than corn and turmeric. Perhaps the yeast particles are more preferable to bees than corn flour and turmeric which required more time than 72h to be completely consumed by bees.

Development of immature stages

Larvae are fed by bee workers on secretions from head glands, protein material (pollen or its alternatives), and nectar according to larval age (Crailsheim *et al*., 1992; Babendreier *et al*., 2004). Thus, yeast, corn flour and turmeric reached to bee larvae during feeding by nurse bees. This experiment showed that all bee larvae were able to complete their development into pupae (sealed brood). Therefore, this experiment confirmed the safety of the used protein materials on bee larvae. In fact, the safety of the feeding type to honey bees is very important especially that some carbohydrates in pollen substitutes showed toxicity to caged bees (Barker, 1977). In a similar way, the tested pollen substitute was consumed by bees without negative effects on the development of larvae into pupae (Van der Steen, 2007).

Colony development

Pollen substitute showed good results in improving colony performance and was recommended to be used with bee packages and small colonies to improve their strength (Nabors, 2000). In the present study, the strength of bee colonies based on brood area and number of bees was significantly better in case of yeast group than other treatments. This can be explained by the ability of bees to consume and utilize yeast in brood rearing more than corn flour and turmeric. Adding Brewer's yeast to bee diet at percentage of 25% showed enhancement in brood rearing (El-Wahab and Gomaa, 2005). Also, bees fed with diet containing 40% brewer's yeast beside other components showed enhancement to colony growth (Taha, 2015). This previous study confirmed the ability of yeast diet used in this study to improve colony strength.

Also, control group and corn group showed similar results, suggesting the low effects of corn flour on colony development. In line with the obtained results, Saffari *et al*. (2010b) found higher sealed brood and bee population in colonies fed pollen or pollen substitutes over control group without any pollen alternatives. A study showed the lowest results of corn flour and chickpea feeding in sealed brood area than skimmed soybean feeding, all these main protein sources were mixed with sugar, dry yeast, cinnamon, and honey (Zaghloul *et al*., 2017). Pollen substitutes using corn (Maize) flour mixed with honey and water occupied the second rank after soybean flour mixed with honey and water in measured parameters (Usha *et al*., 2014).

Also, a study compared a mixture of components including corn flour, turmeric and yeast in one feeding type showed less results in sealed brood area and colony strength than feeding contained soybean flour, yeast, turmeric beside other components (Islam *et al*., 2020). Thus, corn flour in this feeding mixture showed low results than using soybean flour. Additionally, corn gluten showed less brood rearing activity than other used protein diets (Amro *et al*., 2016). Turmeric showed the least ability to improve colony development. Indeed, the availability of diet is important for brood rearing activity (Herbert and Shimanuki, 1982), and brood rearing is impacted by the amount of diet consumed (DeGrandi-Hoffman *et al*., 2008). However, in this study all diets were presented weekly and at the same weight and were consumed within week. Thus, the variations are basically due to diet efficiency in improving brood rearing. Moreover, the protein percentages in dried yeast are about 40% (feedipedia.org), about 10% in corn flour (Kerr, 2017), and about 9.4% in turmeric (Ikpeama *et al*., 2014). Therefore, yeast diet has the highest protein content followed by the mixture of corn flour and turmeric, then corn flour diet, and finally the control group. However, all feeding types were better than turmeric feeding, confirming the less ability of this feeding type to improve colony strength.

Survival rates of bees under lab condiions

This experiment showed that adult bees were able

to survive with similar rates in control group and yeast group, suggesting the absence of any harmful effects of yeast on adult bees. Also, corn showed somewhat similar rates to yeast. Thus, corn flour showed no harmful effects on bees. Indeed, turmeric showed the lowest survival rates than the other test groups. This reflects the low ability of adult bees to utilize turmeric during feeding. Altaye *et al*. (2010) explained the variations between protein diets in the survival of caged bees due to the differences in diet components. The results of this experiment are consistent to colony strength found from the apiary experiments. These feeding types showed no harmful effects on bee larvae but showed dissimilar effects on adult bees. This can be explained by the amount consumed by larvae and adult bees. In fact, tiny amounts of protein feeding can reach to bee larvae during their development while in the survival experiment bees were directly provided with each feeding type. A short longevity in bee workers from colonies fed with two protein supplements suggested their limited suitability in bee feeding (Lamontagne-Drolet *et al*., 2019). This supports that turmeric is not perfect as bee feeding due to its significant negative effect on survival of bees.

Tolerance to low temperature

The bees showed similar ability to tolerate low temperature after feeding on all feeding types. It is known that survival to low temperature depends on the availability of carbohydrate sources for bees (Abou-Shaara, 2017a). Thus, this experiment indicated that the bees were able to utilize the carbohydrate sources for all feeding types without any problems. In other words, the additions of protein materials to sugar did not affect the ability of bees to absorb nutrients.

Body size and glands development

It is known that the morphological characteristics can be impacted by various factors including feeding (Abou-Shaara *et al*., 2013b). It was clear that yeast had noticeable effects on bee characteristics, and had the highest means of all measured characteristics. In fact, the development of bee glands and body size is impacted by protein feeding (Peng *et al*., 2012; Omar *et al*., 2017; Hendriksma *et al*., 2019). Thus, yeast was the best protein source presented to bees. The corn flour feeding occupied the second rank followed by turmeric and sugar candy without a protein source. This indicates that corn flour as source of protein is better than a mixture of corn flour and turmeric. Also, the turmeric feeding type showed no better results than sugar candy, suggesting their similar effects on bee development. In a laboratory study gland and muscle development in caged bees were higher in case of feeding bees on essential amino acids than control group (Hendriksma *et al*., 2019). This supports the role of protein feeding in improving bee characteristics through their contents of amino acids. In light of the above mentioned experiments, bees were able to utilize sugar from all feeding types. Thus, bees offered sugar candy alone or turmeric candy were able to utilize sugar without noticeable benefit from protein source. Perhaps, the addition of turmeric to corn flour affected the ability of bees to utilize the protein source (corn flour) efficiently. To what extent, the larger bees in yeast group can be expected to be more productive than other test groups. Especially, a correlation between body size of bee workers including forewing length/ width and colony productivity was confirmed in previous studies (Waddington, 1989; Edriss *et al*., 2002; Abou-Shaara *et al*., 2013b).

CONCLUSION

The present study showed a comparison between three simple pollen substitutes (yeast, corn flour, and turmeric). All these feeding types were attractive to bees but yeast was consumed within short period by bee colonies. Also, all of them showed the same effect on larval development and the ability of bees to tolerate low temperature. Yeast showed better results in regard to colony development, bee survival, and body characteristics than the other two feeding types and the control group (sugar feeding only). Therefore, beekeepers are advised to use yeast feeding to boost the development of their colonies while corn flour is considered as the second rank. In light of this study, turmeric is not recommended in bee feeding as the main source of protein.

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Statement of conflict of interest

The author has declared no conflict of interests.

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