Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jan - Mar 2019 / v.21 / n.1 / 001-008

http://dx.doi.org/10.1590/1806-9061-2018-0789

# *Performance and Serum Biochemical Profile of Broiler Chickens Supplemented with Piper Cubeba Ethanolic Extract*

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**Keywords**

Additives, antibiotic growth promoters, biochemical serum profile, pepper.



Submitted: 03/April/2018
Approved: 16/October/2018

## ABSTRACT

*Piper cubeba* is an Asian pepper used in popular medicine for its known antimicrobial, antiparasitic, and anti-inflammatory actions. The study evaluated the effects of dietary inclusion of *Piper cubeba* ethanolic extract (PE) as a replacement for anantibiotic growth promoter (AGP) on the performance and biochemical serum profile of 1- to 21-d-old broilers (Cobb®). Two hundred one-d-old broilers were housed in experimental battery cages and distributed in a completely randomized design. The following treatments were applied: negative control (NC) – basal diet; positive control (PC) – basal diet with addition of AGP; and the basal diet with inclusion of 0.17%, 0.34%, and 0.52% of PE (NCE1, NCE2, and NCE3, respectively). Growth performance, biochemical serum profile and internal organ weights were evaluated. Birds fed the AGP presented higher feed intake compared with the other treatments ($p<0.05$). The PC, NC, and NCE1 treatments presented higher weight gain compared with those fed NCE2 and NCE3 ($p<0.05$). The NC, NCE1, and NCE2 diets promoted better feed conversion ratio than the PC and NCE3 ($p<0.05$). Lower triglyceride serum levels were determined in broilers fed the NC and NCE1 diets. Amylase serum levels were lower in NCE1 and NCE2 treatments compared with the NC ($p<0.05$), whereas those obtained with the PC and NCE3 diets were not different ($p>0.05$) from the others. Organ relative weights were not influenced by the treatments. The inclusion of 0.17% of PE did not compromise the growth performance, biochemical serum profile or organ relative weights of 21-d-old broilers.

## INTRODUCTION

Antibiotic growth promoters (AGPs) have been used in broiler production since the 1950s to control pathogenic microorganisms present in the intestinal lumen of birds (Murugesan *et al.*, 2015). However, their indiscriminate use may generate microbial resistance against antibiotics used in human medicine, leading to the research of alternative products to replace them (Traesel *et al.*, 2011). These alternative products, in addition of promoting intestinal health, should not impair broiler performance. The addition of alternative performance enhancers to broiler diets, such as organic acids, enzymes, probiotics, prebiotics, symbiotics and phytogenic additives, should be able to promote similar performance as that obtained with antibiotic growth promoters, which reduce mortality rates and the incidence of subclinical infections (Jang *et al.*, 2007; Brenes & Roura, 2011).

Natural products are promising sources of new agents with therapeutic potential because they contain a large number of biologically-active metabolites and that may have synergic actions (Windisch *et al.*, 2008, Hong *et al.*, 2012). Plant extracts commonly have pleasant taste and odor, and improve animal performance by increasing feed palatability, stimulating the production of saliva and the secretion of digestive enzymes, and balancing the intestinal microflora, which aids in the control of subclinical infections (Toledo *et al.*, 2007).

*Piper cubeba* is an Asian pepper used as a spice or condiment or as medicinein traditional medicine for the treatment of abdominal pain, asthma, diarrhea, dysentery, enteritis, gonorrhea, and syphilis (Silva *et al.*, 2007; Maistro *et al.*, 2011). Studies have shown that *Piper cubeba* has several biological activities, which are mostly attributed to its lignans (Souza *et al.*, 2005; Medola *et al.*, 2007; Yam *et al.*, 2008). At least 24 lignans of *Piper cubeba* have been identified, out of which cubebin, hinokinin, and yatein are present in greater proportion (4-5%) (Elfahmi *et al.*, 2007). In spite of the many studies on the biological properties of *Piper cubeba*, there are no literature reports evaluating its inclusion in broiler diets. Therefore, the present study was carried out to evaluate the effects of different dietary inclusion levels of an ethanolic extract of *Piper cubeba* in replacement of an antibiotic growth promoter (AGP) on performance, biochemical serum profile and internal organ weights of 1- to 21-d-old broilers.

## MATERIAL AND METHODS

### Birds and management

The experiment was performed in the poultry sector of São Paulo State University (UNESP), Ilha Solteira campus, state of São Paulo, Brazil. All procedures involving the birds were approved by the Ethics Committee on the Use of Animals number 07/2013, of that University.

A total of 200 one-day-old Cobb® broilers (100 males and 100 females) were evaluated. Birds were housed in experimental battery cages for a period of 21 days. Each cage housed four males and four females and was equipped with a trough feeder and a trough drinker. During the entire experimental period, a continuous lighting program (24h/d) was applied. Water and feed were supplied *ad libitum*, as recommended by the Cobb® genetic line manual (Cobb, 2012). Birds were vaccinated by eye drop against infectious bursal disease at 7 days of age and against Newcastle disease in drinking water at 14 days of age.

### Experimental design

Birds were randomly assigned to five treatments, with five replicates of eight birds each (four males and four females).

The following treatments were evaluated: positive control (PC) - basal diet with the addition of an AGP at 0.01% (60% chloro-hydroxy-quinolone, Quixalud®, Novartis, Switzerland); negative control (NC) – basal diet containing no AGP; and basal diet with inclusion of 0.17%, 0.34%, and 0.52% of the ethanolic extract of *Piper cubeba* (NCE1, NCE2, and NCE3, respectively).

The ethanolic extract was obtained according to the methodology described by Laurentiz *et al*. (2015) from the seeds of *Piper cubeba* purchased from Floral Seed Company®, Dehradun, India.

An isonutritive basal diet was formulated (Table 1) to supply the nutritional requirements of 1- to 21-d-old broilers, according to Rostagno *et al*. (2011). The tested additives were included in the diets in replacement of washed sand as inert material.

### Growth performance

Feed intake (g/d), weight gain (g/d) and feed conversion ratio (FCR, g feed intake/g weight gain) were weekly evaluated between 1 and 21 days of age.

### Biochemical serum profile

Biochemical serum parameters were analyzed when birds were 21 days old. All birds were subjected to a 12-hour feed fasting period prior to blood sample collection. The blood of two males per experimental unit was collected by puncture of the ulnar vein into non-heparinized tubes, which were immediately submitted to analyses at the poultry sector of the same institution. Only males were used for the biochemical serum profile in order that sex influences did not interfere in the analysis.

Whole blood samples were centrifuged (2000x*g* for 10 minutes) and the resulting sera were stored at -20°C until further analyses. Blood parameters indicative of hepatic function (glucose, cholesterol, albumin, triglycerides, total protein levels and gammaglutamyl transferase-GGT and aspartate aminotransferase-AST activities), pancreatic function (amylase and lipase activities) and renal function (uric acid and urea levels) were determined. Analyses were performed in a semi-automatic biochemical analyzer (SX-3000M®, Sinnowa, Nanjing, China), using commercial kits (Labtest®, Minas Gerais, Brazil), according to the protocols described by the manufacturer.

### Internal organ weights

Internal organ weights were measured at the end of the experiment (21 days). After blood collection, one of the males of each experimental unit from which blood

**Table 1 –** Percentage composition of the experimental diets fed to broiler chickens from 1 to 21 days of age.

| Ingredients (%) | Treatments[1] | | | | |
|---|---|---|---|---|---|
| | PC | NC | NCE1 | NCE2 | NCE3 |
| Corn | 60.28 | 60.28 | 60.28 | 60.28 | 60.28 |
| Soybean meal | 34.00 | 34.00 | 34.00 | 34.00 | 34.00 |
| Dicalcium phosphate | 1.55 | 1.55 | 1.55 | 1.55 | 1.55 |
| Calcitic limestone | 0.88 | 0.88 | 0.88 | 0.88 | 0.88 |
| Vegetable oil | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 |
| Mineral and vitamin supplement[2] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| L-Lysine (78%) | 0.251 | 0.251 | 0.251 | 0.251 | 0.251 |
| DL-Methionine (99%) | 0.269 | 0.269 | 0.269 | 0.269 | 0.269 |
| Choline chloride (60%) | 0.080 | 0.080 | 0.080 | 0.080 | 0.080 |
| Coccidiostat[3] | 0.050 | 0.050 | 0.050 | 0.050 | 0.050 |
| AGP[4] | 0.010 | 0.000 | 0.000 | 0.000 | 0.000 |
| *Pepper cubeba* ethanolic extract | 0.000 | 0.000 | 0.170 | 0.340 | 0.520 |
| Inert material[5] | 1.000 | 1.010 | 0.840 | 0.670 | 0.490 |
| Total | 100 | 100 | 100 | 100 | 100 |
| | Calculated composition of diets | | | | |
| Metabolizable energy (kcal/kg) | 2,950 | | | | |
| Crude protein (%) | 21.00 | | | | |
| Calcium (%) | 0.85 | | | | |
| Available phosphorus (%) | 0.40 | | | | |
| Digestible lysine (%) | 1.17 | | | | |
| Digestible methionine +cystine (%) | 0.84 | | | | |
| Sodium (%) | 0.21 | | | | |

[1]PC: Positive Control; NC: Negative Control; NCE1, NCE2 and NCE3: Negative control diet with the inclusion of 0.17%, 0.34% or 0.52% of *Piper cubeba* ethanolic extract, respectively.
[2]Guaranteed levels per kg of product: Vit. A 5,500,000 IU, Vit.D3 1,000,000 IU, Vit. E 6,500 IU, Vit. K3 1250 mg, Vit. B1 500 mg, Vit. B2 2,500 mg, Vit B6 750 mg, Vit. B12 7,500 mcg, folicacid 251 mg, pantothenicacid 6,030 mg, biotin 25 mg, niacin 17,5 mg, calcium pantothenate 11.22 mg, copper 3000 mg, cobalt 50 mg, iodine 500 mg, iron 25 mg, manganese 32,5 g, selenium 100,5 mg, zinc 22,49 mg, antioxidant 12 mg, andvehicle (52,8%). [3]Coxistac® (12%) [4]AGP: antibiotic growth promoter - Quixalud® (60%) [5] Washed sand was included in the negative control diet to allow the inclusion of AGP and of the different levels *Piper cubeba* ethanolic extract.

was collected was euthanized, necropsied, and its proventriculus, gizzard, liver, pancreas, small intestine and large intestine were removed. Organs were weighed on an analytical scale (0.01-g precision), and their weights were calculated relative to live weight, according the equation: Relative organ weight (%): ([organ weight (g)/live weight (g)]x100). The length of both the small intestine and large intestine (SI + LI) was measured using a ruler.

### Statistical analysis

The results were submitted to analysis of variance using the SISVAR 5.1 software program (Ferreira, 2011). Treatment means were compared by the test of Tukey, and differences were considered statistically significant at $p<0.05$.

## RESULTS AND DISCUSSION

### Growth performance

The growth performance results obtained for the total experimental period (1 to 21 days of age) are shown in Table 2.

Broilers fed all *Piper cubeba* levels presented lower feed intake ($p<0.05$) compared with those fed the diet containing the AGP (PC), and similar to those fed the negative control diet (NC; $p>0.05$). However, the lowest *Piper cubeba* inclusion level (0.17%; NCE1) promoted similar weight gain ($p>0.05$) as the PC and the NC diets, the diets containing 0.34% and 0.52% *Piper cubeba* (NCE2 and NC3, respectively) resulted in lower weight gain ($p<0.05$) compared with the other treatments. Broilers fed the PC diet ($p<0.05$) presented worse FCR compared with those fed the NC, NCE1, and NCE2 diets, whereas the FCR of the NC3 birds was not statistically different ($p>0.05$) from the other treatment groups. The worse FCR results obtained with the PC diet relative to the NC and NCE1 diets are due to the 18% higher feed intake and similar weight gain of the birds fed the PC diet, and may be attributed to the high energy requirements for the metabolism of the AGP by the birds, as well as to its possible negative effects on the beneficial intestinal microflora, which aid nutrient absorption in the absence of health challenge (Settle *et al.*, 2014).

**Table 2 –** Growth performance of broilers fed diets with different inclusion levels of *Piper cubeba* ethanolic extract from 1 to 21 days of age.

| Parameters | Treatments[1] | | | | | CV (%) | *p value* |
|---|---|---|---|---|---|---|---|
| | PC | NC | NCE1 | NCE2 | NCE3 | | |
| Feed intake (g/d) | 1161[a] | 1043[b] | 1000[b] | 1011[b] | 989[b] | 3.83 | 0.0001 |
| Weight gain (g/d) | 901[ab] | 962[a] | 893[ab] | 882[b] | 828[b] | 4.05 | 0.0023 |
| Feed conversion ratio (g/g) | 1.288[a] | 1.084[b] | 1.119[b] | 1.146[b] | 1.195[ab] | 4.98 | 0.0014 |

Means followed by different letters in the same row differ by Tukey's test ($p<0.05$).

[1]PC: Positive Control; NC: Negative Control; NCE1, NCE2 and NCE3: Negative control diet with the inclusion of 0.17%, 0.34%, or 0.52% of *Piper cubeba* ethanolic extract, respectively. CV: coefficient of variation.

The broilers fed the diet with no inclusion of AGP (NC) presented the best performance. The absence of chemical compounds (antibiotics) or phytogenic feed additives in broiler diets saves the energy required for their metabolism, which can then be used for the utilization of dietary nutrients, consequently promoting better performance (Murugesan *et al*., 2015). Maiorka *et al*. (2002), evaluating the effects of mineral and vitamin supplementation infinisher broiler diets, observed that finisher broilers fed a diet not supplemented with minerals and vitamins presented better performance and lower liver specific weight than those fed supplemented diets. According to those authors, the low health challenge of the experimental conditions and the absence of compounds that required metabolism favored the utilization of dietary nutrients, resulting in better performance.

The performance results of the broilers fed lower *Piper cubeba* inclusion levels of (NCE1 and NCE2) was not statistically different from those achieved by those fed the NC diet possibly due to the stimulation of nutrient absorption by the phytogenic compounds present in the *Piper cubeba* ethanol extract (Amad *et al*., 2011), which may have compensated the energy spent for their metabolism.

The inconsistency between the performance results obtained in the present study and literature reports may be attributed to differences in the active principles of the phytogenic additives applied. Gonçalves *et al*. (2014) evaluated the effects of the inclusion of pink pepper in combination with different doses of two antibiotics in broiler diets and did not find any performance differences. Cardoso *et al*. (2012) did not observe any significant compromise in the performance of the broilers that received diets containing piperine extracted from *Piper nigrum*, however the broilers fed high piperine levels presented liver lesions, indicating a toxic effect. Amad *et al*. (2011), evaluating a commercial phytogenic additive composed of thyme and star anise essential oils, did not obtain any significant performance differences in starter broilers. Those results are different from those obtained in the present study possibly because purified and scientifically-tested extracts were applied in the mentioned studies. It should be noted that the dietary addition of the highest level of *Piper cubeba* ethanolic extract (0.52%) in the present experiment impaired broiler performance, suggesting that this level may have increased the utilization of energy for its metabolism or may have reduced feed palatability.

## Biochemical serum profile

The biochemical serum profile evaluated in 21-d-old broilers is shown in Table 3. The treatments did not affect glucose, cholesterol, albumin, and total protein serum values levels, or gamma-glutamyl transpeptidase (GGT) and aspartate aminotransferase (AST) activities ($p>0.05$). Although triglyceride and urea serum levels and amylase activity were influenced ($p<0.05$) by the treatments, however all the biochemical serum profile observed values are within the normal range established for broilers by Borsa *et al*. (2006) and Thrall *et al*. (2007).

When testing a therapeutic compound, it is extremely important to evaluate its systemic action and effects on the performance indexes, as this will determine the feasibility of its intended use for a particular species. According to Kuttappan *et al.* (2013), an increase in the AST and GGT serum levels indicates liver damage caused by the metabolism of dietary therapeutic compounds and additives. However, this was not observed in the present study, indicating that the *Piper cubeba* extract did not interfere with the liver function of broilers up to 21 days of age, although it significantly ($p<0.05$) affected serum triglycerides values. Broilers fed the diet supplemented with 0.34% *Piper cubeba* ethanolic extract (NCE2) presented higher serum triglyceride levels compared with those fed the NC and NCE1 diets, whereas those fed the PC and NCE3 diets presented intermediate levels. These changes in triglycerides serumalone do not confirm liver function impairment. Differently from the present

**Table 3 –** Serum biochemical profile of broilers fed diets with different inclusion levels of *Piper cubeba* ethanolic extract from 1 to 21 days of age.

| Variables | Treatments[1] | | | | | CV % | *p value* |
|---|---|---|---|---|---|---|---|
| | PC | NC | NCE1 | NCE2 | NCE3 | | |
| *Hepatic function* | | | | | | | |
| Glucose (mg/dL) | 219.5 | 212.6 | 186.5 | 193.9 | 193.8 | 13.30 | 0.3915 |
| Cholesterol (mg/dL) | 155.0 | 152.6 | 155.0 | 141.9 | 142.2 | 6.27 | 0.1361 |
| Albumin (g/dL) | 1.84 | 1.79 | 1.75 | 1.91 | 1.86 | 6.19 | 0.3284 |
| Triglycerides (mg/dL) | 36.9[ab] | 31.2[b] | 31.0[b] | 38.4[a] | 36.1[ab] | 9.64 | 0.0189 |
| Total proteins (g/dL) | 3.31 | 3.02 | 3.28 | 3.32 | 3.55 | 6.74 | 0.0638 |
| GGT (U/L)[2] | 25.5 | 20.2 | 19.5 | 23.7 | 22.0 | 15.62 | 0.1396 |
| AST (U/L)[2] | 203.8 | 202.1 | 166.4 | 212.5 | 195.5 | 12.56 | 0.1374 |
| *Pancreatic function* | | | | | | | |
| Amylase (U/L) | 893.5[ab] | 978.8[a] | 703.0[b] | 682.4[b] | 743.2[ab] | 16.26 | 0.0217 |
| Lipase (U/L) | 7.92 | 7.02 | 6.12 | 6.57 | 7.06 | 18.23 | 0.3825 |
| *Renal function* | | | | | | | |
| Uric acid (mg/dL) | 4.32 | 3.97 | 4.43 | 4.39 | 5.26 | 15.34 | 0.1601 |
| Urea (U/L) | 5.33[a] | 4.35[a] | 3.96[a] | 2.68[b] | 2.40[b] | 21.13 | 0.0005 |

Means followed by different letters in the same row differ by Tukey's test ($p<0.05$).

[1]PC: Positive Control; NC: Negative Control; NCE1, NCE2 and NCE3: Negative control diet with the inclusion of 0.17%, 0.34% or 0.52% of *Piper cubeba* ethanolic extract, respectively.

[2]GGT: Gamma-glutamyltranspeptidase; AST: aspartateaminotransferase.

study, Traesel *et al*. (2011) did not find any influence of different supplementation levels of a combination of essential oils and pepper (50, 100 e 150 mg/kg) on triglyceride serum levels in broilers, but the inclusion level of 150mg/kg increased the serum concentration of aspartate aminotransferase (AST), which may suggest hepatocellular disease (Thrall *et al*., 2007).

Among the serum parameters indicative of pancreatic function evaluated in the present study, only amylase activity was influenced by the treatments ($p<0.05$) (Table 3). Broilers fed the NC diet presented higher amylase activity than those fed the NCE1 and NCE2 diets (0.17 and 0.34% *Piper cubeba* extract, respectively), while the PC (AGP addition) and NCE3 (0.54% *Piper cubeba* extract) diets promoted intermediate values, which were not statistically different from the other treatments. Although Adil *et al.* (2010) and Amad *et al.* (2011) reported that essential oils or phytotherapeutic compounds stimulate the production and the activity of digestive enzymes, this effect was not observed in the present study, as amylase activity was reduced and lipase activity was not affected by the dietary inclusion of *Piper cubeba* extract. However, contrary to the findings of the present study, Traesel *et al* (2011) found that the inclusion of a combination of essential oils and pepper in the diet of broilers has no effect on the birds' serum amylase values, but significantly increased serum lipase values.

Urea and uric acid serum levels were used as renal function indicators (Table 3). The levels of uric acid serum were not affected ($p>0.05$) by the treatments. On the other hand, the birds fed the highest *Piper cubeba* extract levels (NCE2 and NCE3) presented lower urea serum levels ($p<0.05$) compared with those fed the PC, NC, and NCE1 diets. However, both the urea and uric acids serum levels determined in all treatment groups were within the normal range for broilers, of0-5 mg urea/dL and 0-15 mg uric acid/dL, according to Thrall *et al.* (2007) and Lumeij (1987). In contrast with the results of the present study, Hosseinzadeh *et al*. (2014) did not report any significant effect of increasing inclusion levels coriander seeds and extract in broiler diets on uric acid serum levels.

The urea serum levels determined in the bids fed the positive control diet (with AGP) were slightly higher than the normal reference values of broilers. However, this small increase is negligible, as this parameter is a meaningful diagnostic indicator of renal disease only when there is a simultaneous increase in uric acid levels (Capitelli & Crosta, 2013), which did not occur in the present study.

## Internal organ weights

Internal organ weights (Table 4) were not influenced by the treatments ($p>0.05$). These results are in partial agreement with those obtained by Adil *et al*. (2010), who fed broilers with organic acids and probiotics and observed no statistical differences in relative organ weights. Evaluating the addition of different levels of a commercial phytogenic additive containing thyme

**Table 4 –** Body weight (g), relative internal organ weights, and intestinal length of 21-d-old broilers supplemented with different levels of *Piper cubeba* ethanolic extract from 1 to 21 days of age.

| Variables | Treatments[1] | | | | | CV (%) | *p value* |
|---|---|---|---|---|---|---|---|
| | PC | NC | NCE1 | NCE2 | NCE3 | | |
| Body weight (g) | 952.0[ab] | 1038.7[a] | 908.5[ab] | 912.0[ab] | 831.7[b] | 7.52 | 0.012 |
| Proventriculus and gizzard (%)[2] | 3.63 | 3.32 | 3.71 | 3.67 | 3.73 | 9.43 | 0.4674 |
| Liver (%)[2] | 2.86 | 2.76 | 3.00 | 2.89 | 2.78 | 12.59 | 0.8805 |
| Pancreas (%)[2] | 0.29 | 0.25 | 0.32 | 0.28 | 0.26 | 12.42 | 0.0581 |
| Small intestine (%)[2] | 2.96 | 2.90 | 3.12 | 3.14 | 3.50 | 15.25 | 0.4550 |
| Large intestine (%)[2] | 0.70 | 0.74 | 0.83 | 0.97 | 0.92 | 17.64 | 0.0950 |
| SI+LI length (cm)[3] | 151.3 | 155.0 | 144.3 | 146.0 | 142.3 | 9.76 | 0.7154 |

Means followed by different letters in the same row differ by Tukey's test ($p<0.05$).

[1]PC: Positive Control; NC: Negative Control; NCE1, NCE2 and NCE3: Negative control diet with the inclusion of 0.17%, 0.34% or 0.52% of *Piper cubeba* ethanolic extract, respectively.

[2]Relative organ weight: organ weight as a percentage (%) of live weight.

[3]SI + LI: small intestine and large intestine length

and star-anise essential oils in broiler diets, Amad *et al*. (2011) found no statistical differences in liver, heart, kidney, and spleen weights.

Despite the lack of statistical significance, the broilers fed the diet with the lowest *Piper cubeba* extract inclusion level (NCE1) presented higher relative pancreas weight than those on other diets. According to Stringhini *et al*. (2006), pancreas weight increases as a result of the stimulation of the pancreatic functions, consequently enhancing the digestion process. The FCR values obtained in the birds fed the NCE1 diet suggest that 0.17% may be the best inclusion level of *Piper cubeba* extract in broiler diets.

The dietary inclusion of 0.17% *Piper cubeba* ethanolic extract did not compromise the growth performance, biochemical serum profile and internal organ weights of broiler chickens. This treatment inclusion can be explored to as a possible replacement for current performance enhancers, which has been a difficulty in the avian production in the last years.

## ACKNOWLEDGEMENTS

The authors wish to thank the Coordination of Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq) (Grant n. 482071/2013-3) for their financial support, and Sao Paulo Research Foundation (FAPESP) for the M.Sc. grant given to M.S.R. (FAPESP n. 2012/14107-9) and the scientific research grant given to E.S.M (FAPESPn. 2012/03562-7).

The authors express their gratitude to Dean's Office of Research, UNESP, IlhaSolteira campus, for the financial aid provided through the First Projects Program.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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