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INFLUENCE OF FENNEL IN JAPANESE QUAIL DIET OVER EGG QUALITY AND BEHAVIOR ASPECTS

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Abstract

The objective of this research was to evaluate the effect of *Pimpinella anisum* (fennel) on the performance, stress, and quality of Japanese Quail eggs in the second laying cycle (73 to 77 weeks). One hundred sixty-eight female quails from Japanese sub-species (*Coturnix coturnix japonica*) at 73 weeks of age, divided and submitted to four treatments (0; 250; 500 and 750mg of fennel/kg of feed) were used in a randomized block design. (RBD), with seven replications and six birds per experimental plot, totaling 28 plots. Performance, behavioral, and egg quality parameters were evaluated. Bird performance was not influenced (p>0.05) by the treatments tested. The highest egg weight and albumen weight were found in birds fed 750 mg of fennel, while a higher albumen height was observed for the estimated dose of 669 mg (p<0,05). The estimated doses of 554.09 mg, 634.10 mg, and 613.10 mg of fennel were efficient in reducing agitated behaviors by riding, pecking, and stirring, respectively. However, non-aggressive variables were not influenced by the addition of fennel to the diet (p>0.05). The tonic immobility test had a decreasing linear effect (p<0.05), which indicates a lower time in seconds in tonic immobility to birds receiving 750 mg of fennel. Fennel added to the diet did not interfere with performance, but it influenced essential parameters related to egg quality and was able to alter characteristics related to bird behavior.

Key-words

Coturnix coturnix japonica, behavior, stress, Pimpinella anisum, egg quality

INFLUÊNCIA DA ERVA-DOCE NA DIETA DE CODORNAS JAPONESAS SOBRE A QUALIDADE DOS OVOS E ASPECTOS COMPORTAMENTAIS

Resumo

O objetivo desta pesquisa foi avaliar o efeito da *Pimpinella anisum* (erva-doce) sobre o desempenho, estresse e qualidade dos ovos de codornas japonesas no segundo ciclo de postura (73 a 77 semanas). Foram utilizadas 168 codornas fêmeas da subespécie japonesa (*Coturnix coturnix japonica*) com 73 semanas de idade, divididas e submetidas a quatro tratamentos (0; 250; 500 e 750mg de erva-doce/kg de ração), em delineamento em blocos casualizados (DBC), com sete repetições e seis aves por parcela experimental, totalizando 28 parcelas. Foram avaliados os parâmetros de desempenho, comportamentais e qualidade dos ovos. O desempenho das aves não foi influenciado (p>0,05) pelos tratamentos testados. O maior peso dos ovos e peso do albúmen foram encontrados nas aves alimentadas com 750 mg de erva-doce, enquanto a maior altura do albúmen foi observada pela dose estimada de 669 mg (p<0.05). As variáveis comportamentais agressivas foram consideradas diferentes entre os níveis analisados (p<0,05). As doses estimadas de 554,09 mg, 634,10 mg e 613,10 mg de erva-doce foram eficientes na redução dos comportamentos agitados montando, bicando e agitada, respectivamente. No entanto as variáveis não agressivas não foram influenciadas pela adição de erva-doce na dieta (p>0,05). O Teste de imobilidade tônica teve efeito linear (p<0,05) decrescente, que indica um menor tempo em segundos em imobilidade tônica para as aves que receberam 750 mg de erva-doce. A erva-doce adicionada à dieta não interfere no desempenho, porém melhorou à qualidade dos ovos e foi capaz de alterar características relacionadas ao comportamento das aves.

Palavras-chave

Coturnix coturnix japonica, comportamento, estresse, *Pimpinella anisum*, qualidade dos ovos

INTRODUCTION

In the Brazilian poultry industry, the co-culture has been gaining. This condition reflects positively on this Brazilian economic sector, in the view of the accelerated development (ARAÚJO et al., 2015). In the past, japanese quail farming was basically for subsistence (SAKAMOTO et al., 2016). Nowadays, production has been more technical, guaranteeing better incomes for animal farmers (GERON et al., 2014).

This use has brought advantages to the production of japanese quails due to the low initial investment cost (VIEIRA FILHO et al., 2016). They are enabling a quick return on invested capital, beyond to show an early sexual maturity (KAR et al., 2017). Other characteristics were high productivity (TEIXEIRA et al., 2013), good feed conversion, easy handling, and resistance to diseases (MARQUES et al., 2010). In addition to offering meat and eggs rich in nutrients, especially proteins (NEPOMUCENO et al., 2014; RÉHAULT-GODBERT et al., 2019; TUNSARINGKARN et al., 2013).

Several factors can affect the performance and quality of life of these birds. Due to the intensification of production, laying quails can suffer a zootechnical performance drop. This occurs because the japanese quails are easily affected by stress, which triggers a series of undesirable behaviors (MILLS and FAURE, 1990; NAZAR and MARIN, 2012).

The concern with animal welfare has been a relevant topic that has been increasingly studied and discussed (YOU et al., 2014). Measures to control animal stress have become fundamental to the success of breeding, reflecting positively on the production and welfare of japanese quails.

The use of phytotherapy in animal nutrition proved to be efficient in controlling stress. These ingredients have a positive effect on improving life quality and lower aggression between the japanese quails (SILVA et al., 2010).

Pimpinella anisum, popularly known as fennel, is a specie from Apiaceae (Umbelliferae) botanical family (DANTAS, 2008). The seeds of *P. anisum* have approximately 1.5 to 6.0% of their mass with volatile oils, consisting mainly of trans-anetol. This compound is the most representative essential oil in the species, ranging from 57.4 to 93.9% according to the different parts of the plant (SHOJAII and FARD 2012).

Studies with the inclusion of fennel for poultry diets have been reported in broilers (SAKI et al., 2014; RAGAB et al., 2013; TEIXEIRA et al., 2014), turkeys (BHAISARE and THYAGARAJAN, 2012) and japanese quails (BUĞDAYCI et al., 2018; MAHMUD, 2014). However, there are few studies using fennel as an anti-stressor agent in the japanese quail diet.

The objective of this study was to evaluate the effect of the inflorescence of the fennel plant (*Pimpinella anisum*), dried and ground in the feeding of Japanese quails in the second cycle of posture as a stress modulator, evaluating performance, behavioral parameters, and egg quality.

MATERIAL AND METHODS

This experiment was conducted in the Experimental Farm at the Federal University of Mato Grosso (UFMT). The research was approved for the Ethics Committee on the Use of Animals (CEUA) of the UFMT under protocol number 23108.187860/2016-11.

It was used 168 female quails of the japanese quail subspecies (*Coturnix coturnix japonica*), with 73 weeks age, housed in a conventional shed. Previously, quails were submitted to forced molting by the fasting method when they reached 65 weeks of age.

The female japanese quails were housed in the peak posture of the second cycle (6 to 10 weeks), totaling 28 experimental days. The 168 quails were distributed in galvanized wire cages with dimensions of 50 x 38 x 21 cm (316.6 cm^2/bird). Four treatments were evaluated (0; 250; 500 and 750 mg of fennel/kg of feed), in a randomized block design (RBD) to control of the initial weight, with seven repetitions and six birds per experimental unit, totaling 28 experimental units.

Fennel seeds were added to the diets after harvest. The seeds were submitted to the shade drying method and the gamma radiation sterilization process. After that, it was finely ground, forming a hygroscopic powder of brown color with pleasant aromatic fragrance. This powder contains anethole as the predominant active ingredient. The diets were formulated by the recommendations of Rostagno et al. (2017), according to Table 1.

The feed was supplied *ad libitum*, twice a day, in metal feeders type trough. The feeder was divided according to each treatment and repetition. The water was supplied *ad libitum* in a trough-type drinking fountain with water exchange twice a day.

The daily management consisted of collecting and counting the eggs, providing the feed, cleaning the egg trimmers, and performing the temperatures (maximum and minimum) and relative humidity (RH). It was provided 16 hours of light daily throughout the experimental period.

The performance variables studied were feed intake, weight gain, feed conversion, feed conversion per dozen eggs, feed conversion by mass of eggs produced, and percentage of laying. Three random eggs were collected every seven days from each experimental plot to assess the quality of the eggs. The specific gravity was analyzed by the method of immersing the eggs in saline solution, ranging from 1.050 to 1.100. For the egg weight and yolk weight, an

Table 1. Composition percentage of the rations provided to the birds in the laying phase according to each treatment

	Fennel (mg/kg feed)			
Ingredients%	0	250	500	750
Corn	54.17	54.14	54.12	54.09
Soybean meal	34.70	34.70	34.70	34.70
Limestone	7.01	7.01	7.01	7.01
Dicalcium phosphate	1.15	1.15	1.15	1.15
Sodium chloride	0.36	0.36	0.36	0.36
Posture core [1]	1.50	1.50	1.50	1.50
Soybean oil	1.11	1.11	1.11	1.11
Fennel	0	0.025	0.050	0.075
Total	100	100	100	100
Calculated Composition				
Metabolizable energy (kcal/kg)	2800.00	2800.00	2800.00	2800.00
Crude protein (%)	19.46	19.46	19.46	19.46
Digestible lysine (%)	1.080	1.080	1.080	1.080
Digestible methionine+Cystine (%)	0.94	0.94	0.94	0.94
Digestible Tryptophan (%)	0.23	0.23	0.23	0.23
Digestible threoin (%)	0.68	0.68	0.68	0.68
Calcium (%)	3.07	3.07	3.07	3.07
Available match (%)	0.30	0.30	0.30	0.30
Sodium (%)	0.16	0.16	0.16	0.16
Crude fiber (%)	2.74	2.74	2.74	2.74

[1]Laying core - Composition/kg of the product: Calcium (min) 80g/kg (8%), Calcium (max) 100g/kg (10%), Phosphorus (min) 37g/kg (3.7%), Sodium (min) 20g/kg, Methionine (min) 21.5g/kg, Lysine (min) 18g/kg, Vitamin A (min) 125000IU/kg, Vitamin D3 (min) 25000UU/kg, Vitamin E (min) 312UI/kg, Vitamin K3 (min) 20mg/kg, Vitamin B1 (min) 20mg/kg, Vitamin B2 (min) 62.5mg/kg, Vitamin B6 (min) 37.5mg/kg, Vitamin B12 (min) 200mcg/kg, Folic Acid (min) 6.25mg/kg, Pantothenic Acid (min) 125mg/kg, Biotin (min) 1.25mg/kg, Choline (min) 1700mg/kg, Niacin (min) 312mg/kg, Copper (min) 125mg/kg, Iron (min) 68 0mg/kg, Iodo (min) 8.75mg/kg, Manganese (min) 937mg/kg, Selenium (min) 3.75mg/kg, Zinc (min) 500mg/kg, Fluoride (max) 370mg/kg.

analytical balance with an accuracy of 0.0001g was used. Yolk thickness and albumen height were measured using a digital caliper (150mm MTX®). For yolk color, a fan calorimeter (DSM Yolk Color Fan, DSM-YCF®) was used with scores ranging from 1 to 15. The yolks were separated from the eggs and placed on a flat surface for evaluation. The evaluation was performed by the same person, in the same place and the same light (VALENTIM et al. 2019).

The evaluation of the tonic immobility time (TIT) and the behavioral evaluation were performed in the last week of the experiment (21 to 28 days of the experiment). The TIT assessment was carried out according to the methodology proposed by Heiblum et al. (1998).

Behavioral assessments followed the ethogram: aggressive behaviors (walking, pecking, and shaking) and non-aggressive behaviors (feeding, drinking, idleness, defecating, and scratching). The focal observations lasted 5 minutes and were carried out in all experimental plots always in the morning, by the same person and at the same time (SAVORY et al. 1999).

The parametric variables of the performance, as the behavior and the TIT were submitted to the statistic F test and subsequently analyzed by the polynomial regression procedure and normality by the Shapiro-Wilk multivariate test ($p<0.05$). The nonparametric data of the yolk color were calculated by the chi-square test ($p<0.05$). All data were analyzed using the R Core Team program (2015).

RESULTS AND DISCUSSION

The maximum and minimum temperatures inside the shed during the experimental period was 39.2 ºC and 23.8 ºC, respectively. Relative humidity ranged from 65.8% to 80.5%. The performance variables were not affected significantly ($p>0.05$) with the addition of fennel in the japanese quails diet in the second laying cycle (Table 2). No mortality was observed in the entire experimental period.

Table 2. Total feed intake (TFI), daily feed intake (DFI), feed conversion per dozen eggs (FCDE), feed conversion by egg mass (FCEM) laying percentage (LP) of Japanese Quails submitted to different levels of fennel in the diet.

Variables	Fennel (mg/kg feed)				CV[1] (%)	P-valor
	0	250	500	750		
TFI (g)	1404.2	1440.1	1491.4	1273.1	11.04	0.0898
DFI (kg)	0.679	0.691	0.650	0.686	5.94	0.2741
FCDE (kg/dz)	0.125	0.134	0.146	0.139	14.54	0.2577
FCEM (kg/kg)	1.22	1.19	1.178	1.14	14.85	0.8671
LP (%)	80.95	76.19	70.23	72.02	14.56	0.2925

[1]CV = Coefficient of variation

Corroborating of those results, Marques et al. (2010) worked with different chamomile inclusions (*Matricaria camomila*), and Silva et al. (2010) studied different levels of kava-kava (*Piper methysticum*) not found effects of phytotherapics on performance variables in japanese quails. Buğdaycı et al. (2018) evaluating 0.3, 0.6, and 0.9% of fennel in the japanese quail diet, also found no differences between treatments for feed intake. However, Silva et al. (2010) found the best result to feed conversion for dozens of eggs in japanese quail fed with 250 mg of passionflower/kg of feed, followed by treatments 500 and 750 mg of passionflower.

The use of phytotherapy is broad; however, the results are contradictory, asserting the need for research about the use of these products. Other researchers are necessary with the goal of improving the performance and well-being of animals, especially the japanese quails.

New researches are also necessary on the second production cycle since there are few studies with the addition of anxiolytic phytotherapeutics in this production stage.

The egg quality parameters, as the egg weight, the albumen weight, and the albumen height were influenced by the addition of fennel on the diet (P= 0.00044, P=0.00078, and 0.04202, respectively). However, the fennel addition no influenced the egg density, the yolk thickness, the yolk weight, and the yolk color (Figure 1). All quality data were considered normal by the Shapiro-Wilk test ($p<0.05$).

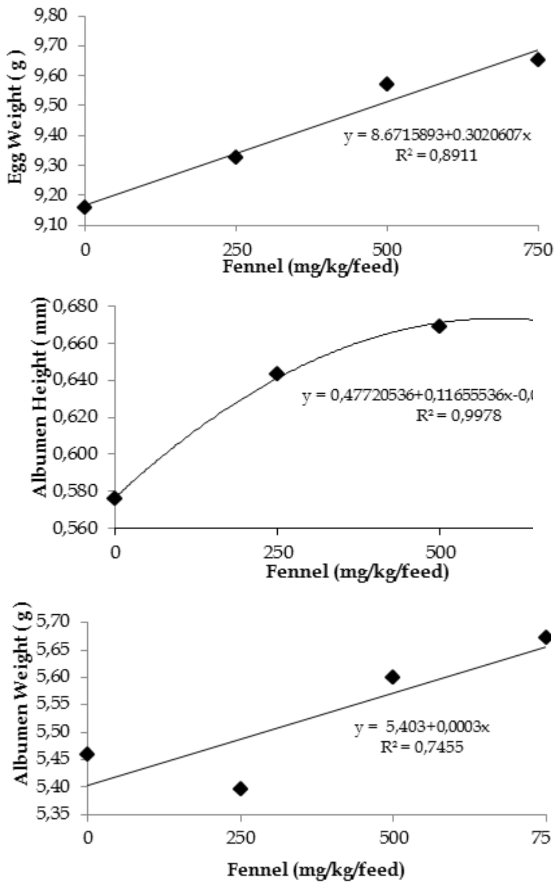

Figure 1. Egg weight (g), Albumen height (mm), and Albumen weight (g) of Japanese quails fed fennel in the second production cycle.

The egg weight was linearly affected, represented by equation $\hat{Y} = 8.6715893 + 0.3020607x$; $R^2 = 0.8911$. The height of the albumen obtained a quadratic effect, represented by equation $\hat{Y} = 0.47720536+0.11655536x - 0.01729464x^2$; $R^2 = 0.997$, from which it is estimated the inclusion of 669 mg of fennel/kg on the diet to greater albumen height. For the weight of the albumen was a linear effect, represented by equation $\hat{Y} = 5.403 + 0.7455$; $R^2 = 0.7455$.

Similar results of egg weight were found by Majidzadeh Heravi and Vakili (2016), who studied the addition of plant extracts and flaxseed for laying hens. Unlike the results obtained on this study, Gharaghani et al. (2015), using three levels of fennel (0, 10 and 20 g/kg of diet) for laying hens, not obtained significant results for egg weight and albumen height, even in concentrations greater than the current study. Özeku et al. (2011) reported a significant improvement in albumen height and Haugh unit values in the laying of chicken eggs fed with a mixture of essential oils of oregano, bay leaf, sage, fennel, and citrus.

The yolk color and fennel concentrations were independent by the Chi-square test (P=0.2902), which was being similar to the study of Kazemi-Fard et al. (2013). For Rodriguez-Amaya (1997), the fennel has a low number of carotenoids, which can be attributed to lower yolk color; however, the level of inclusion of this ingredient was low. Disagreeing with the results of this research, Majidzadeh Heravi and Vakili (2016) obtained an egg color index of chickens, receiving thyme extract and flaxseed treatment, which was significantly greater than fennel seed treatment. According to the Danish food composition database (2008), the concentration of carotenoids present in thyme and flaxseed is greater than fennel, which justifies such a result.

The variables of aggressive behavior (walking, pecking, and agitated) were considered different between treatments (P = 0.002318, P = 0.00003, and P = 0.00003, respectively). Non-aggressive variables (feeding, drinking, idleness, defecation, and scratching) were not influenced by the addition of fennel to the diet ($p > 0.05$), as shown in Figure 2. This result shows that fennel provides an anxiolytic effect on japanese quails. According to Nazareno et al. (2011), these acts that the express birds can be used as direct measures of welfare.

The inclusion of fennel showed a quadratic effect on the perching behavior ($\hat{Y} = 9.27500000 - 0.03261429x + 0.00002943x^2$; $R^2 = 0.9024$), estimating the inclusion of 554.09 mg of fennel/kg of feed, which the birds spend less time perching. The pecking behavior had a quadratic effect for the addition of fennel to the diet ($\hat{Y} = 3.955714286 - 0.01231429x + 0.00000971x^2$; $R^2 = 0.9314$), estimating the inclusion of 634.10 mg of fennel/kg of feed proportioned a lower pecking behavior. It was possible to observe a quadratic effect in relation to the agitated behavior ($\hat{Y} = 9.43214285 - 0.02698571x + 0.00002200x^2$; $R^2 = 0.9792$).

Similar to these results, Marques et al. (2010) found that japanese quails that received the highest dosage of chamomile (750 mg/kg feed) showed less perching behavior, especially when compared to birds that did not receive chamomile. Likewise, Silva et al. (2010) concluded that the kava-kava used in feeding laying japanese quails causes a reduction in the stress. Corroborating the results, Tenório et al. (2017) stated that the inclusion of 1.8 g of chamomile/kg of feed to japanese quails provides a condition of bird welfare. Different results were found by Gravena et al. (2009), using valerian extract in the feeding of japanese quails in the laying phase, concluded that valerian was not able to reduce aggressive behaviors.

These discrepancies in results are still a factor that must be taken into account. The

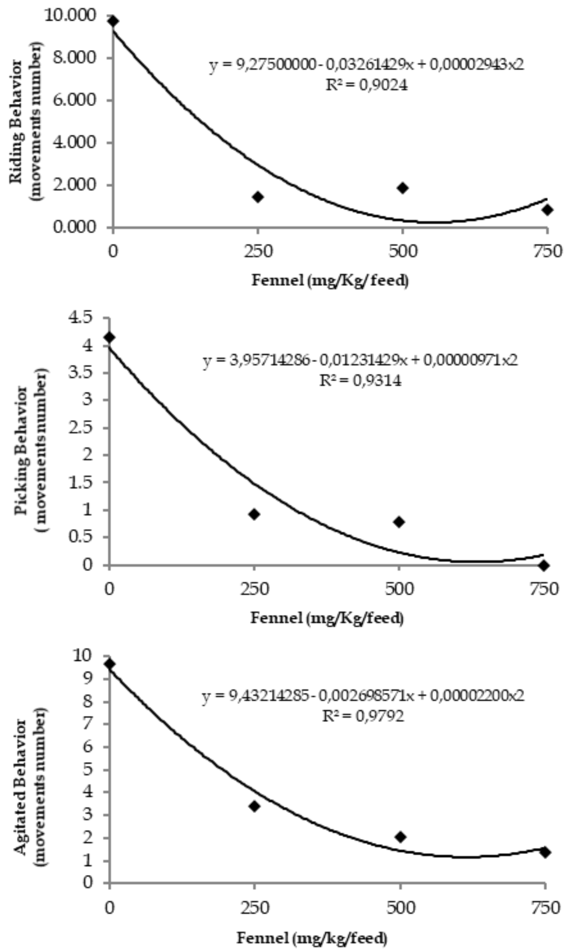

Figure 2. Aggressive behaviors riding, pecking, and agitated of Japanese quails submitted to different levels of fennel in the diet.

Herbal Medicines show agents with a greater action potential, but the number of studies is still small, mainly with fennel as an anxiolytic agent.

Non-aggressive behaviors did not differ between treatments ($p<0.05$). Most studies highlight the importance of using phytogenic additives, and little are studies about the effect of phytotherapics concerning the calming properties under domestic animals. This is the reason, which can justify the lack of literature and data since there are divergences between the same medicine herbal and the same species studied.

The TIT belongs to the defense behavior category, is preceded primarily by coping behavior and responses evoked by a stressful situation (MARQUES et al., 2010). This behavior is the last anti-predatory defense response of some species (PITTET et al., 2019). It is characterized by pretending to be dead to achieve an escape opportunity by inducing relaxation of the predator's attention (HUMPHREYS and RUXTON, 2018). The tonic immobility test was significant ($P = 0.01635$), evidenced by linear effect ($\hat{Y} = 31.314286 - 4.224429x$; $R^2 = 0.8624$). This indicates a lower time in seconds in tonic immobility of birds that received greater levels of fennel, suggesting a decreasing line as a function of treatment and time (Figure 3).

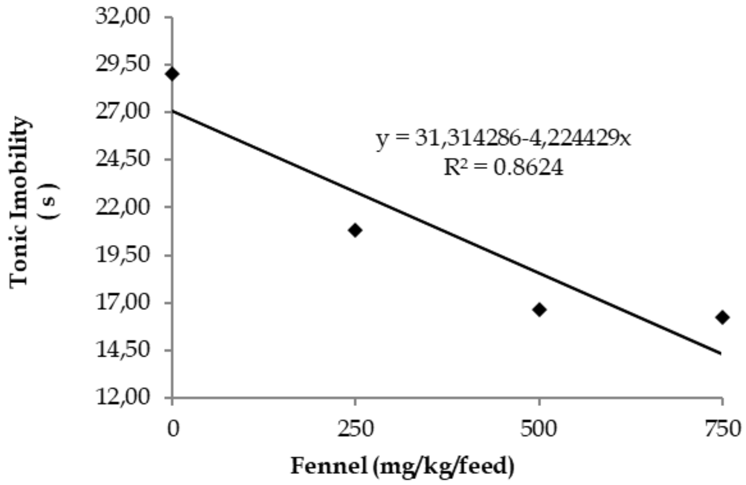

Figure 3. Tonic immobility time of Japanese quails submitted to different levels of fennel in the diet.

The results corroborate with Silva et al. (2010) that used a dry extract of passiflora leaves (*Passiflora alata*) and using kava-kava (*Piper methysticum*) for laying japanese quails, both reporting a decrease in tonic immobility time in japanese quails. Gravena et al. (2009) not observed significant effects for parameters of tonic immobility with the use of valerian (*Valerian officinalis*), using the same amount used in this experiment.

The TIT is considered a natural behavior related to stress (HEIBLUM et al., 1998). In this context, the results obtained by this study established that the japanese quails fed with greater amounts of fennel were less stressed concerning the Japanese quails that did not receive phytotherapy. This evidence the positive effect of the fennel in japanese quails in the second cycle of posture.

CONCLUSION

The use of fennel in the Japanese Quails diet in the second production cycle no influenced the performance parameters. The inclusion from 500 up to 750 mg of fennel improve eggs quality, and decrease the frequency of aggressive behaviors and the tonic immobility time, suggesting the use of this herbal medicine as a stress modulating agent.

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