

ANIMAL PRODUCTION

SYMBIOTIC AND NATURAL EXTRACTS IN DIETS FOR
JAPANESE QUAILS AT LAYING PERIOD

JANAINA DELLA TORRE DA SILVA^{1*}, ALEXANDRA DA SILVA MATOS¹, FABRICIO HIROTA HADA¹,
RODRIGO ANTONIO GRAVENA¹, RAFAEL HENRIQUE MARQUES¹, VERA MARIA BARBOSA MORAES¹

¹Departament of Animal Science – FCAV/UNESP Jaboticabal – SP – Brazil
e-mail: janainadts@yahoo.com.br

ABSTRACT

The objective of this study was to assess the performance, tonic immobility time (TIT), intensity of injuries (II) and heterophil to lymphocyte ratio (H:L) by the addition of the symbiotic and plant extract (*Aloe vera* and *Symphytum officinale*) to the diet of Japanese quails. Ninety quails were used, distributed in randomized blocks with 3 treatments (0 – control; 250 and 750 mg symbiotic and plant extract/kg of diet), five repetitions and six birds per cage. Feed intake, feed conversion, production and weight

of eggs, viability, TIT, II and H:L were evaluated. Results showed that the addition of the product to the diet did not affect the performance, however, it decreased the TIT, II and the H:L of quails which received the highest level of the product in the diet. Thus, the use of symbiotic and plant extract in the diet has been promising regarding the behavioral and physiological parameters, decreasing the stress of the animals, mainly for the level of 750 mg/kg diet.

KEYWORDS: *Aloe vera*; *Coturnix coturnix japonica*; prebiotic; probiotic, *Symphytum officinale* L.

SIMBIÓTICO E EXTRATOS NATURAIS NA DIETA DE CODORNAS JAPONESAS NA
FASE DE POSTURA

RESUMO

Objetivou-se avaliar o desempenho, tempo de permanência em imobilidade tônica (TIT), intensidade de ferimentos (IF) e relação heterófilo:linfócito (H:L) de codornas japonesas alimentadas com ração contendo simbiótico e extrato vegetal (*Aloe vera* e confrei). Noventa codornas em postura foram distribuídas em delineamento em blocos ao acaso e submetidas a três tratamentos (0 – controle; 250 e 750 mg de simbiótico e extrato vegetal/kg de ração), com cinco repetições e seis aves por parcela.

Avaliou-se o consumo de ração, conversão alimentar (consumo/dúzia e kg de ovos), produção e peso dos ovos, viabilidade, TIT, IF e H:L. Os resultados obtidos mostraram que a adição do produto na dieta não influenciou o desempenho, porém, diminuiu o TIT, a IF e a H:L das aves que receberam a maior dosagem do produto na dieta. Dessa forma, pode-se concluir que o uso de simbiótico e extrato vegetal na dieta foi promissor quanto aos parâmetros comportamentais e fisiológicos,

tornando os animais mais calmos, principalmente para a dosagem de 750 mg do produto/kg de ração.

PALAVRAS-CHAVE: *Aloe vera*; *Coturnix coturnix japônica*; prebiótico; probiótico; *Symphytum officinale* L.

INTRODUCTION

Quail rearing for egg production has been growing each year in Brazil. This growth is due to the low initial investment and the fast financial return of this kind exploration because this bird presents rapid growth, good feed conversion and reaches sexual maturity early (MURAKAMI & ARIKI, 1998). However, it is easily stressed, and this factor is responsible for the emergence of undesirable behaviors such as feathers pecking, social deviance and depression, affecting the birds' welfare and productivity (MILLS & FAURE, 1990; JONES, 1996).

Likewise other birds, quails show peculiar behavior when threatened, known as a state of tonic immobility, which is manifested when the birds are exposed to adverse situations, especially those that cause fear or stress (JONES et al., 1988). From this behavior and the intensity of head and the body injuries, it is possible to evaluate the stress in this species through induction of tonic immobility, the permanence time of the birds in this state and the observation and measurement of injuries.

Another way to assess the stress in birds is through the heterophils and lymphocytes ratio, which indicates chronic stress (ELROM, 2000), being the most reliable means to estimate the level of stress in broilers (GROSS & SIEGEL, 1983).

Studies with anxiolytic and sedative phytotherapy of passionflower, valerian and chamomile have been carried out in order to reduce stress in quail and, thus, improve these birds' performance and welfare (GRAVENA et al., 2010; MARQUES et al., 2010; SILVA et al., 2010a).

Another way to control stress in birds would be through tryptophan supplementation in the diet. Tryptophan is an amino acid precursor of the neurotransmitter serotonin, a sedative and tranquilizer substance, able to reduce stress and promote well-being. GUANDOLINI et al. (2005) and RIZZO et al. (2008) supplemented quails' diets with different levels of tryptophan; however, they did not find any effect on performance, time spent in tonic immobility and severity of the injury.

Currently, researches of new additives and

ingredients that promote integrity, development and proper functioning of the intestinal mucosa have been carried out, since this mucosa is responsible for nutrients digestion and absorption, has an important role in protecting the immune system (SILVA et al. 2010b) and also synthesizes about 80-90% of the serotonin required for the proper functioning of the organism. Prebiotics and probiotics fit in such features, as they help in the maintenance, balance and integrity of the intestinal mucosa, promoting the growth of beneficial bacterial flora and protecting the body from pathogenic microorganisms.

The symbiotic and plant extract consisting of aloe vera (*Aloe vera*) and comfrey (*Symphytum officinale*) could be alternatives to improve quails' performance and alleviate the stress that affects these birds because they improve the intestinal flora and thereby increase serotonin synthesis in the intestine, providing a feeling of calmness and well-being to the quail.

Both aloe vera and comfrey have anti-inflammatory, healing, anti-microbial and bactericidal properties (LORENZI & MATOS, 2006). In this context, the addition of the symbiotic and plant extract could improve performance and reduce nervous behavior / agitated as well as the incidence of injury in laying quails.

Therefore, this work aimed to evaluate the effect of different inclusion levels of symbiotic and plant extracts in the diet of quails at the laying phase on performance, tonic immobility ("righting time"), intensity of injury and the heterophil:lymphocyte ratio.

MATERIAL AND METHODS

The experiment was carried out at the Poultry Sector of the Animal Science Department, Faculty of Agriculture and Veterinary Sciences - Jaboatocabal Campus - UNESP.

Ninety quails at laying phase (42 days old) were weighed and housed in cages measuring 32 x 36 x 16 cm, where they remained for one week for adaptation. Laying cages were arranged in steps at 70 cm from the floor of the shed. Nipple drinkers were used and feed was supplied in continuous galvanized sheet feeder.

The quails were distributed in randomized blocks for weight control at the beginning of the experiment and submitted to three treatments (0 - control, 250 and 750 mg of symbiotic and plant extracts / kg of diet), with five replicates of six quails in each plot. We adopted this density per cage because the birds were not beak trimmed. In order to keep a constant population density, another 12 quails were equally divided into two cages per treatment, and received the same experimental diets for replacement of birds in the event of death.

The symbiotic consisted of prebiotic (mannan oligosaccharides - MOS) and probiotic (polyprobiotic: *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *L. sp*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecium*) and the plant extract was constituted of aloe vera, comfrey, antioxidant, flavor and vehicle.

The diets supplied were isonitrogenous (18% CP) and isocaloric (2800 kcal ME/kg). For balancing rations, ingredient composition tables proposed by ROSTAGNO et al. (2005) were used, with the nutritional requirements according to the ones proposed by MURAKAMI et al. 1993. The light program used was 17 hours light per day.

Egg production was recorded daily, beginning after a period of 12 days in which the birds were adapted to the experimental diets. Thereafter, every 14 days a new production cycle was established until the completion of six cycles.

At the end of each cycle, feed leftover and eggs of each replicate were weighed, and the eggs were weighed on the last three days of each cycle. Thus, it was possible to evaluate feed intake, feed conversion (feed intake / dozen eggs and feed intake / egg mass), egg production and viability.

The tonic immobility (TI) and severity of injuries to the head and body were evaluated at the end of each production cycle, in a total of six evaluations.

For the evaluation of TI, all birds in the same cage were placed in a box and one quail at a time was abruptly turned and placed in dorsal recumbency on a table. Before taking the hand off of the bird, a slight pressure was made on the animal for approximately three seconds. After this procedure, the time the animal remained motionless was counted with the aid of a digital chronometer watch. The bird should remain still for at least 10 seconds so that it would be

considered in tonic immobility state (JONES & FAURE, 1981).

The severity of injury was assessed on the same days of tonic immobility evaluations, following the methodology described by SAVORY et al. (1999). For this evaluation, injuries to the head, back, tail and wings were observed, these last three being considered as the body. Evaluations were made by scores as follows:

- Score 0: no lesion;
- Score 1: mild lesion (affected area presenting some feathers and without injury);
- Score 2: moderate lesion I (area completely without feathers and without injury);
- Score 3: moderate lesion II (area with few injuries);
- Score 4: severe injury (area with many injuries);
- Score 5: very severe injury (area with bleeding).

At 70, 105 and 140 days of age, blood samples were collected from two birds per plot, totaling 30 samples in each collection. The blood was used for blades manufacture in order to count heterophils and lymphocytes, as described by CAMPO & DÁVILA (2002).

Statistical analyzes of results were performed by the GLM procedure of SAS[®] (STATISTICAL ANALYSIS SYSTEM, 1995), except for the results of intensity of injury, which were obtained by Chi-square test. In order to verify the significance between treatment means, Tukey test at 5% probability was used.

RESULTS AND DISCUSSION

There were no significant differences ($P > 0.05$) in performance results among the different treatments (Table 1). These results are consistent with those found by RIZZO et al. (2008), who evaluated the performance of quail submitted to different additions of tryptophan to the diet.

Studies with different levels of probiotics in the diet of laying hens showed similar results to those found in this study; however, no improvements in feed intake, feed conversion and egg production were verified (PEDROSO et al., 2001; GIAMPAULI et al., 2005).

Likewise, the use of probiotics in broiler diets did not influence the performance of the batch (CAVALVANTI et al., 2003; SOUZA et al. 2010)

Table 1. Mean values for feed intake (FI), feed conversion (feed intake / dozen and feed intake / kg eggs) - FC / dozen and FC / kg egg, egg weight, egg production and viability of quails submitted to different levels of symbiotic and plant extract in the diet during the laying phase

Symbiotic and Plant Extract (mg / kg)	FI (g)	FC/ Dozen	FC / kg egg	Egg weight (g)	Production (%)	Viability (%)
0	26.50	0.34	2.53	11.30	92.57	99.43
250	26.62	0.36	2.64	11.40	90.47	98.87
750	28.04	0.37	2.61	11.74	90.60	97.83
P value	0.5281	0.2608	0.5765	0.0691	0.8286	0.6039
CV (%)	8.47	6.17	5.97	0.96	6.65	2.50

Similar results were observed by HERNÁNDEZ et al. (2004), who used oregano and Labiatae to replace antibiotics in feed for broilers from 21 to 42 days of age and did not find any differences between treatments for performance results.

By evaluating the use of herbal remedies, such as garlic and oregano, to replace antibiotics in broilers' diet, FREITAS et al. (2001) and FUKAYAMA et al. (2005) found no effect of treatments on feed intake, weight gain and feed conversion.

Some authors associate the inefficiency in the use of probiotics and prebiotics with the good sanitary conditions to which the lot was submitted during the experiment. Thus, the lack of challenge provided by an adequate following and excellent management conditions may have precluded obtaining results favorable to the use of agents (JUNQUEIRA, 1997; PEDROSO et al., 2001).

There was a significant effect ($P < 0.05$) among treatments regarding the duration of tonic immobility - TI (Table 2). The birds fed the highest dose of symbiotic and plant extract in the diet had the lowest values of TI when compared to birds fed the control diet, showing that the level of stress decreased in these animals. This result might be a reflection of increased production of serotonin by the intestine, as the use of probiotics and prebiotics improve intestinal integrity.

In a study of laying quails receiving passionflower in the diet, SILVA et al. (2010a) found that birds fed the highest doses of passionflower (500 and 750 mg passionflower / kg) presented lower TI in relation to birds that did not receive it in the diet. This shows that passionflower was able to calm and relieve the stress of the quails, which have agitated and nervous behavior.

Table 2. Average values for tonic immobility (TI) of quails submitted to different levels of symbiotic and plant extract in the diet, during the laying phase

Symbiotic and Plant Extract (mg / kg of ration)	TI (seconds)
0	26.29 b
250	22.32 ab
750	16.01 a
P value	0.0340
CV(%)	23.36

Means followed by the same letter in the same column do not differ by Tukey test ($P > 0.05$).

In contrast, research with the inclusion of valerian and chamomile in the diet of Japanese quail showed that these herbal medicines in the dosages of 0, 250, 500 and 750 mg / kg diet did not influence the remaining time of the birds in tonic immobility (GRAVENA et al., 2010; MARQUES et al., 2010).

For the intensity of injuries, it was found in this study that the birds receiving the highest dosage of the product consisted of phytotherapeutic, prebiotic and probiotic showed less injuries (Table 3).

These results are similar to those found by MARQUES et al. (2010) and SILVA et al. (2010a), who evaluated the effect of different doses of chamomile and passionflower on scores of injuries presented by quail. However, GUANDOLINI et al. (2005) found no effect of addition of tryptophan on the intensity of the head and the body injuries of quail in the laying phase, as well as GRAVENA et al. (2010) who found no effect of the inclusion of valerian on the intensity of injury in quails.

Throughout the literature we could not find any report about the activities of symbiotic and plant extract on quail behavior, as well as on the time remained in tonic immobility and severity of injury.

Table 3. Scores of lesions on the head and body of quails submitted to different levels of symbiotic and plant extract in the diet during the laying phase

Symbiotic and Plant extract (mg/kg of ration)	Without lesion		With lesion	
			Head	
0	24.86 a		75.14 b	
250	27.38 a		72.62 b	
750	36.81 b		63.19 a	
Chi-Square Value			34.70	
Probability			0.0001	
			Body	
0	22.03 a		77.97 b	
250	26.89 b		73.11 b	
750	38.21 c		61.79 a	
Chi-Square Value			55.19	
Probability			0.0001	

Means followed by the same letter in the column do not differ by Kruskal-Wallis test ($P \leq 0.05$).

There were differences among treatments for heterophil:lymphocyte ratio, because the birds that received the highest levels of symbiotic and plant extract in the diet showed the lowest heterophil:lymphocyte ratio when compared with birds fed the control diet (Table 4), indicating less chronic stress in these birds (ELROM, 2000).

SILVA et al. (2010a) evaluated the effect of passionflower on the stress of Japanese quails, and did not observe any differences between treatments for the heterophil: lymphocyte ratio. Likewise, GRAVENA et al. (2010) and MARQUES et al. (2010) found no differences in this parameter when submitted quails in the laying phase to different doses of valerian and chamomile, respectively.

According to GROSS & SIEGEL (1983), the heterophil:lymphocyte ratio is the most reliable measure to indicate the level of stress in birds, since there is an increase in the number of heterophils and a decrease in the number of lymphocytes in response to stress.

CAMPO & DÁVILA (2002) also found no significant differences for this parameter in chickens reared under different lighting programs. MCFARLANE & CURTIS (1989) tested different situations of stress in chickens and found no significant effect on heterophil:lymphocyte ratio when birds were submitted to beak trimming, coccidiosis and noise.

Table 4. Mean values for the heterophil: lymphocyte ratio (H:L) of quails submitted to different levels of symbiotic and plant extract in the diet, during the laying phase

Symbiotic and Plant extract (mg/kg of ration)	H:L
0	0.528 b
250	0.499 ab
750	0.487 a
P value	0.0230
CV(%)	11.43

Means followed by the same letter in the same column do not differ by Tukey test ($P > 0.05$).

CONCLUSION

By the results obtained in this study, it was concluded that the symbiotic plant extract were effective in reducing stress in quails, the best results being observed when the highest doses of product were applied per kg of feed. Furthermore, the use of symbiotic and plant extract does not affect the birds production.

REFERENCES

- CAMPO, J.L.; DÁVILA, S.G. Influence of mating ratio and group size on indicators of fearfulness and stress hens and cocks. **Poultry Science**, v.81, n.8, p.1099-1103, 2002.
- CAVALCANTI, J.S.; SOARES, A.T. Probióticos e farinhas de carne e osso com diferentes contaminações bacterianas para frangos de corte. **Revista Ciência e**

- Agrotecnologia**, Lavras, v.27, n.04, p.927-933, 2003.
- DUNCAN, I.J.H. Animal behaviour and welfare. In: **Environmental aspects of housing for animal production**. J.A. Clark (ed.), London., p.445-470. 1981. (Resumo).
- ELROM, K. Review: Handling and transportation of broilers welfare, stress, fear and meat quality. **Journal of Veterinary Medicine**, v.56, n.1, p.39-45, 2000.
- FREITAS, R.; FONSECA, J.B.; SOARES, R.T.R.N.; ROSTAGNO, H.S., SOARES, P.R. Utilização do alho (*Allium sativum* L.) como promotor de crescimento de frangos de corte. **Revista Brasileira de Zootecnia**. Viçosa, v.30, n.3, p.761-765, mai/jun, 2001.
- FUKAYAMA, E.H.; BERTECHINI, A.G.; GERALDO, A.; KATO, R.K.; MURGAS, L.D.S. Extrato de orégano como aditivo em rações de frango de corte. **Revista Brasileira de Zootecnia**, v.34, n.6, p.2316-2326, 2005.
- GIAMPAULI, J.; PEDROSO, A.A.; MORAES, V.M.B. Desempenho e qualidade de ovos de poedeiras após a muda forçada suplementadas com probiótico em diferentes fases de criação. **Ciência Animal Brasileira**, v.6, n.3, p.179-186, jul/set, 2005.
- GRAVENA, R.A.; MARQUES, R.H.; SILVA, J.D.T.; HADA, F.H., SILVA, V.K.; MALHEIROS, R.D.; MORAES, V.M.B. Efeitos fisiológicos e comportamentais do uso do extrato de valeriana em dietas de codornas em crescimento. **Veterinária e Zootecnia**, v.17, p.407-414, 2010.
- GROSS, W.B.; SIEGEL, H.S. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. **Avian Diseases**, v.27, n.4, p.972-979, 1983.
- GUANDOLINI, G.C.; RIZZO, P.V.; COSTA, M.J.R.P.; MORAES, V.M.B. Avaliação de imobilidade tônica e agressividade em codornas nas fases de recria e postura: efeitos da inclusão de triptofano na alimentação. **Revista Brasileira de Ciência Avícola**, v. 7, p.96, 2005.
- HERNÁNDEZ, F.; MADRID, J.; GARCIA, V.; ORENGO, J.; MEGIAS, M.D. Influence of two plant extracts on broilers performance, digestibility, and Digestive organ size. **Poultry Science**, v.83, n.2, p.169-174, 2004.
- JONES, R.B. Fear and adaptability in poultry: insights, implications and imperatives. **World's Poultry Science Journal**, v.52, p.131-174, 1996.
- JONES, R.B.; BEUVING, J.; BLOKHUIS, H.J. Tonic immobility and heterophil/lymphocyte responses of the domestic fowl to corticosterone infusion. **Physiological Behaviour**, v.42, n.3, p.249-253, 1988.
- JONES, R.B.; FAURE, J.M. Sex and strain comparisons of tonic immobility ("righting time") in the domestic fowl and the effects of various methods of induction. **Behaviour Processing**, v.6, n.1, p.47-55, 1981.
- JUNQUEIRA, O. M. . Enzimas sintéticas e probióticos na alimentação de aves. In: IMENES, S.L.; TUCCI, E.C.; POTENZA, M.R. (Org.). Anais do II Ciclo de Palestras sobre agricultura orgânica. Campinas: , 1997, p.78-87.
- LORENZI, H.; MATOS, F.J.A. **Plantas medicinais no Brasil: nativas e exóticas**. Nova Odessa-SP Instituto Plantarum, 2006. 868p.
- MARQUES, R.H.; GRAVENA, R.A.; SILVA, J.D.T.; HADA, F.H.; SILVA, V.K.;MUNARI, D.P.; MORAES, V.M.B. Camomila como aditivo fitoterápico para codornas na fase de postura. **Revista Brasileira de Saúde e Produção Animal**, v.11, p.990-998, 2010.
- McFARLANE, J.M.; CURTIS, S.E. Multiple concurrent stressors in chicks. 3. Effects on plasma corticosterone and the heterophil:lymphocyte ratio. **Poultry Science**, v.68, n.4, p.522-527, 1989.
- MILLS, A.D.; FAURE, J.M. Panic and hysteria in domestic fowl: a review. In: ZAYAN, R., DANTZER, R. (Ed.), **Social Stress in Domestic Animals**, Dordrecht: kluwer Academic Publisher, 1990. p.248-272.
- MURAKAMI, A.E.; ARIKI, J. **Produção de Codornas Japonesas**. FUNESP, Jaboticabal, 1998. v.1. 79 p
- MURAKAMI, A.E.; MORAES, V.M.B.; ARIKI, J. Níveis de proteína e energia em dietas de codornas japonesas (*Coturnix coturnix japonica*) em postura. **Revista Brasileira de Zootecnia**, v.22, n.4, p.541-551, 1993.
- PEDROSO, A.A.; MORAES, V.M.B.; ARIKI, J. Desempenho e qualidade de ovos de poedeiras de 50 a 66 semanas de idade suplementadas com probiótico. **Ciência Rural**, v. 31, n. 4, p. 683-686, 2001.
- RIZZO, P.V.; GUANDOLINI, G.C.; AMOROSO, L.; MALHEIROS, R.D.; MORAES, V.B.M. Triptofano na alimentação de codornas japonesas nas fases de recria e postura. **Revista Brasileira de Zootecnia**, v.37, n.6, p.1017-1022, 2008.
- ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L.; GOMES, P.C.; OLIVEIRA, R.F.; LOPES, D.C.; FERREIRA, A.S.; BARRETO, S.L.T. **Tabelas brasileiras para aves e suínos: Composição de alimentos e exigências nutricionais de aves e suínos**. 2ª edição. Universidade Federal de Viçosa, Viçosa, MG, 2005, 186p.
- SAVORY, C.J.; MANN, J.S.; MACLEOD, M.G. Incidence of pecking damage in growing bantams in relation to food form, group size, stocking density, dietary tryptophan concentration and dietary protein source. **British Poultry Science**, v.40, n.5, p.579-584, 1999.
- SILVA, J.D.T.; GRAVENA, R.A.; MARQUES, R.H.; SILVA, V.K.; HADA, F.H.; MORAES, V.M.B.; MALHEIROS, R.D. Effect of passionflower in diets of Japanese quails rearing and laying periods. **Revista Brasileira de Zootecnia**, v.39, n.7, p.1530-1537, 2010a.
- SILVA, V.K.; SILVA, J.D.T.; GRAVENA, R.A.; MARQUES, R.H.; HADA, F.H.; MORAES, V.M.B. Yeast extract and prebiotic in pre-initial phase diet for broiler chickens raised under different temperatures.

Revista Brasileira de Zootecnia, v.39, n.1, p.165-174, 2010b. como promotores de crescimento para frangos de corte. **Colloquium Agrariae**, v.6, n.2, p.33-39, 2010.

SOUZA, L.F.A.; ARAÚJO, D.N.; ASTOLPHI, J.L.L.; DIAS, L.B.M.; AMBIEL, A.C.; SANTOS, L.S.; CARMO, A.J.; SILVA, P.C.G. Probiótico e antibiótico STATISTICAL ANALYSIS SYSTEMS INSTITUTE. *User's guide*, Version 6, 4^{ed}. Cary: SAS[®]/STAT, SAS Institute Incorporation, 1995. 365p.

Submitted on February, 12, 2009. Accepted on January, 30, 2012.