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Use of liquid vinasse as a feed additive for Japanese quails¤

Uso de vinaza líquida como aditivo alimenticio para la codorniz japonesa

Vinhaça líquida como aditivo alimentar para codornas japonesas

Poliana C Martins[1*], MSc; María C de Oliveira[1,2], PhD; Diones M da Silva[2], MSc; Sabina A Mesquita[2], BVM; Higor C Oliveira[2], PhD; Wilson A Marchesin[3], PhD.

[1]*Instituto Federal Goiano, Rio Verde, Brasil.*

[2]*Universidade de Rio Verde, Rio Verde, Brasil.*

[3]*Cooperativa Agroindustrial dos Produtores Rurais do Sudoeste Goiano (COMIGO).*

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Abstract

Background: Liquid vinasse (LV) has probiotic properties and low pH. It is fed to animals as a solution to the disposal problem of this by-product. **Objective:** To evaluate the effects of increasing dietary levels of LV on growing performance, egg quality and economic viability for Japanese quails. **Methods:** One hundred sixty Japanese quails were included in a randomized study with five dietary treatments and four replicates per treatment. The treatments consisted of adding 0, 2.5, 5, 7.5, or 10% LV to a commercial quail feed for 84 d. **Results:** The LV inclusion resulted in a linear decrease in daily intake of dry matter (DM), crude protein (CP), gross energy, calcium, phosphorus, and in the feed conversion ratio. However, it resulted in a linear increase in egg-specific weight, eggshell weight, CP content in the DM, and a quadratic change in the ether extract content of the egg. The price per dozen eggs decreased linearly with the inclusion of LV. **Conclusion:** The best results were obtained by adding 10% LV, which made egg production more profitable.

Keywords: *chemical composition, egg quality, layers, organic acids, sugarcane by-products.*

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* Corresponding author: Poliana C Martins. IF Goiano, Rio Verde, GO. Rod. Sul Goiano, Km 1, Zona Rural, Rio Verde, GO. 75901-000. Tel.: 55 (64) 3620-5600. E-mail: polianamartinsvet@hotmail.com

Resumen

Antecedentes: La vinaza líquida (LV) tiene propiedades probióticas y bajo pH, por lo que se ha utilizado como alimento para animales como una solución para el problema de la eliminación de este sub-producto. **Objetivo:** Evaluar los efectos del aumento de los niveles de la LV en el desempeño productivo de la codorniz japonesa, la calidad interna y externa del huevo y la viabilidad económica de la utilización de este sub-producto. **Métodos:** Ciento sesenta codornices japonesas fueron incluidas en un estudio al azar con cinco tratamientos y cuatro repeticiones cada uno. Los tratamientos consistieron en la adición de 0, 2,5, 5, 7,5 o 10% de LV a un alimento comercial para codornices durante 84 d. **Resultados:** La inclusión de LV resultó en una disminución lineal en la ingestión diaria de materia seca (DM), proteína bruta (CP), energía bruta, calcio, fósforo, y en la tasa de conversión del alimento. Sin embargo, dio lugar a un aumento lineal en el peso específico del huevo, el peso de la cáscara, el contenido de CP en la DM y cuadráticamente alteró el contenido de extracto etéreo de los huevos. El precio de una docena de huevos disminuyó linealmente con la inclusión de LV. **Conclusión:** Los mejores resultados se obtuvieron con un nivel de LV de 10%, que resultó en la producción de huevos más rentable.

Palabras clave: *ácidos orgánicos, calidad del huevo, composición química, ponedoras, sub-producto de caña de azúcar.*

Resumo

Antecedentes: A vinhaça líquida (LV) tem propriedades probióticas e pH baixo, e tem sido utilizada como alimentos para animais; uma solução para o problema da eliminação deste co-produto. **Objetivo:** Avaliar os efeitos de níveis crescentes de LV sobre o desempenho produtivo de codornas japonesas, qualidade interna e externa do ovo, e a viabilidade econômica da utilização deste coproduto. **Métodos:** cento e sessenta codornas japonesas foram utilizadas em delineamneto inteiramente casualizado, com cinco tratamentos e quatro repetições cada um. Os tratamentos consistiram de adição 0, 2,5, 5, 7,5 ou 10% LV à ração comercial para codornas, durante 84 d. **Resultados:** A inclusão de LV resultou em uma redução linear no consumo diário de matéria seca (DM), proteína bruta (CP), energia bruta, cálcio, fósforo, e da taxa de conversão alimentar. No entanto, resultou em um aumento linear no peso específico do ovo, peso da casca e teor de CP na DM e alterou de forma quadrática o teor de extrato etéreo dos ovos. O preço da dúzia de ovos dimuniu linearmente com a inclusão de LV. **Conclusão:** Economicamente, os melhores resultados foram obtidos com o nível de 10% de LV, o que tornou a produção de ovos mais rentável.

Palavras chave: *ácidos orgânicos, composição química, co-produto da cana-de-açúcar, poedeiras, qualidade do ovo.*

Introduction

Feed comprises 70-75% of the total costs in quail production, deserving the attention of nutritionists in search of cost reduction. However, using alternative products should consider their abundance and geographical location (Braga *et al*., 2005). Price and quality are also essential when choosing a byproduct. Tests to determine the best inclusion level in the diet are also necessary, along with measuring performance and egg quality (Sakamoto *et al*., 2006).

Liquid vinasse (LV) is the final residue of ethyl alcohol production. It is a distillery effluent with high polluting capacity and high fertilizer value. Between 12 and 20 L of vinasse are produced per liter of ethanol (Cazetta and Celligoi, 2006).

Inclusion of LV in animal feed may increase productive performance because it contains organic acids and B-complex vitamins that improve the use of nutrients and keep the intestinal flora in balance. LV inclusion increased viability, body weight, and development of the reproductive system (oviducts and follicles) in replacement pullets. The literature has described better uniformity of the flock with the use of LV at a rate of 14 mL/bird/d for layers in the rearing period, and an increase in egg production in laying hens supplemented with 15 mL LV/d (Hidalgo *et al*., 2009; Hidalgo *et al*., 2011).

The present study evaluated productive performance and egg quality of laying Japanese quails fed commercial feed with increasing levels of LV as well as to evaluate the economic viability of using this byproduct in egg production.

Materials and methods

Ethical considerations

This study was approved by the ethics committee for Animal Experiments of Universidade de Rio Verde (Brazil) protocol 0001-11, following guidelines established by Brazilian law 11.794 (October 08, 2008) and the Brazilian National Council for Control of Animal Experimentation.

Location, animals, and treatments

The experiment was performed at Universidade de Rio Verde (Goiás, Brazil). One-hundred-sixty 150 d-old female laying Japanese quail, weighing 188.96 ± 4.47 g, with 93.37 ± 1.46% initial egg production rate were used. The experimental period consisted of 84 d divided into three cycles of 28 d each and 4 additional days to collect the eggs and perform bromatological analysis. Animals were randomly allotted to five treatments with four replicates and eight birds per experimental group. The treatments consisted of increasing inclusion levels of LV in commercial sorghum-based feed (SBF) for laying quails (COMIGO, Rio Verde, GO, Brazil). Feed composition is presented in Table 1.

Vinasse [pH 4.0; 1,029 Kcal/Kg of gross energy (GE); 0.58% calcium (Ca); 0.01% total phosphorus (P); 2.73% crude protein (CP) on a dry matter (DM) basis of 3.17%] was donated by an alcohol-producing plant. The levels of LV in the feed were 0, 2.5, 5, 7.5, and 10%. The nutritional composition of the feed supplemented with LV was determined in the laboratory (Table 2).

Because of its potential use as a source of organic acids, LV was included as a feed additive. Since LV composition is mostly water, high levels were tested. To prepare the diets, the feed was weighed, the percentage of LV was calculated for each treatment, and LV was incorporated into the feed using a mixer. The diets were weekly prepared and stored in plastic buckets to promote its conservation and avoid deterioration.

The quails were housed in 25 ´ 15 ´ 33 cm (length ´ height ´ width) metal cages with a tray for egg collection and feeding and drinking troughs. Each drinking trough supplied four cages. Water and feed were available *ad libitum*, with feed offered twice daily, at 8 am and 5 pm, times when the eggs were counted and collected. The birds were submitted to a lighting program since the 40th d of age.

Table 1. Composition of the commercial feed (g/Kg) before LV inclusion.

Component	Quantity (g/Kg)
Sorghum	521.250
Soybean meal	380.000
Common salt	3.000
Limestone	74.000
Dicalcium phosphate	13.000
Vitamin and mineral mixture	5.000
Vitamin E	0.020
L-lysine	0.270
DL-methionine	1.430
Zinc bacitracin	0.500
Antifungal	1.000
Mycotoxin adsorbent	0.500
Antioxidant	0.030
Calculated nutrient composition (according to the manufacturer)	
Crude protein (%)	22.00
Fat (%)	3.830
Crude fiber (%)	3.291
Ash (%)	11.558
Calcium (%)	3.378
Available phosphorus (%)	0.512
Metabolizable energy (Kcal/Kg)	2823
Available lysine (%)	1.355
Available methionine + cysteine (%)	0.907
Choline chloride (g/Kg)	1.823
Linoleic acid (%)	1.709
Sodium (%)	0.153
Vitamin A (IU/g)	12.500
Vitamin D3 (IU/g)	3.750
Vitamin E (mg/g)	25.000
Total biotin (µg/Kg)	205.051

Table 2. Analyzed composition of the commercial feed supplemented with liquid vinasse (based on fresh matter).

Parameters	Liquid vinasse levels (%)				
	0.0	2.5	5.0	7.5	10.0
Dry matter (%)	91.10	88.80	85.60	80.90	78.20
Crude protein (%)	20.17	20.03	18.97	19.14	18.60
Gross energy (cal/g)	3,188	3,042	2,957	2,784	2,762
Ether extract (%)	2.57	2.79	1.88	1.31	1.48
Mineral matter (%)	14.20	13.45	13.37	13.58	10.15
Calcium (%)	4.02	3.99	3.54	3.01	3.15
Phosphorus (%)	0.61	0.58	0.57	0.54	0.48

At first, 14 h of light were provided daily. The light duration increased weekly in increments of 30 min until quails were exposed to 17 h of light/d, and this level was maintained until the end of the experiment.

Evaluated parameters

Productive performance included the evaluation of laying rate, egg mass, daily feed intake [as DM and fresh matter (FM)], feed conversion ratio/Kg and per dozen eggs as FM and DM, and the daily intake of CP, GE, Ca, and P, as DM.

Of the eggs produced in the last 3 d of the experimental period, two were used to determine egg weight, morphometry (height and diameter), weight of yolk and albumen, and weight and thickness of the eggshell. The height and diameter of the yolk and dense albumen were measured with a manual caliper and the other eggs were used to determine specific weight. Based on the resulting values, Haugh units, yolk, albumen, and eggshell percentages were determined. Haugh units were obtained using the formula:

$$HU = 100 \times \log (H - 1.7 \times P^{0.37} + 7.6)$$

Where:

H: Is albumen height (mm).
P: Is the whole egg weight (g).

The birds received treatment during four more days so that their eggs could be collected for bromatological analysis, which was performed for 25 eggs from each replicate using the method of Silva and Queiroz (2002) to determine DM, CP, ether extract (EE), and MM content.

Feed costs were calculated by multiplying feed conversion rate (Kg DM/Kg and Kg DM/dozen) by the price of 1 Kg feed (2.79 USD).

Statistical analysis

The results were subjected to analysis of variance and polynomial regression analysis, and the F-test was used to determine significance of the effects. The analyses were performed using SISVAR software version 5.3 (DEX/UFLA, Lavras, MG, BR), and the significance level was set at 5%.

Results

The laying rate, egg mass, daily feed intake, and feed conversion ratio in Kg/Kg and in Kg FM/dozen were not influenced by LV levels ($p>0.05$). However, daily intake of DM $p<0.003$), CP ($p<0.03$), GE ($p<0.03$), Ca ($p<0.001$), and P ($p<0.01$), and the feed conversion ratio (Kg DM/dozen) decreased linearly as LV increased (Table 3).

The LV levels had no influence ($p>0.05$) on egg weight, Haugh units, weight and percentage of yolk and albumen, or eggshell percentage and thickness. However, specific weight ($p<0.003$) and eggshell weight ($p<0.4$) increased linearly with the level of LV in the diet (Table 4).

There was no effect ($p>0.05$) of treatment on CP content (as FM) or on MM content (as DM and FM) of the eggs. However, DM content decreased ($p<0.004$) and CP content (as DM) increased ($p<0.014$) linearly with LV. The EE content of the eggs, both as DM ($p<0.014$), and as FM ($p<0.001$), showed a quadratic effect, with the lowest EE content associated with the 10% level of LV (Table 5).

There was no difference ($p>0.05$) in the price of a Kg of eggs, but the price of a dozen eggs decreased ($p<0.003$) linearly with the inclusion of LV (Table 6).

Table 3. Productive performance of Japanese quail consuming commercial feed supplemented with increasing levels of liquid vinasse.

Parameter	Liquid vinasse level (%)					CV (%)
	0.0	2.5	5.0	7.5	10.0	
Laying rate (%)	94.24	93.91	93.28	92.45	88.42	4.01
Egg mass (g/bird/d)	11.69	11.99	12.11	11.27	11.17	4.65
Daily feed intake (g FM/d)	34.35	31.36	31.77	32.52	30.34	8.38
Daily DM intake (g DM/d)[1]	29.52	26.44	26.00	26.05	23.53	8.31
Daily CP intake (g/d)[2]	5.91	5.75	5.22	5.52	4.82	8.23
Daily GE intake (Kcal/d)[3]	103.30	90.57	89.81	89.65	83.13	8.36
Daily Ca intake (g/d)[4]	1.31	1.19	1.08	0.97	0.95	8.20
Daily P intake (g/d)[5]	0.197	0.175	0.175	0.175	0.147	8.46
Feed conversion ratio (Kg FM/Kg)	2.93	2.61	2.62	2.89	2.71	8.65
Feed conversion ratio (Kg FM/dozen)	0.437	0.401	0.408	0.422	0.411	7.22
Feed conversion ratio (Kg DM/Kg)	2.53	2.21	2.15	2.32	2.10	8.58
Feed conversion ratio (Kg DM/dozen)[6]	0.354	0.317	0.312	0.312	0.282	8.30

CV: Coefficient of variation. DM: Dry matter. GE: Gross energy. FM: Fresh matter. [1]Linear effect ($\hat{Y} = 28.78 - 0.494x$; $r^2 = 0.84$). [2]Linear effect ($\hat{Y} = 5.93 - 0.09x$; $r^2 = 0.77$). [3]Linear effect ($\hat{Y} = 99.54 - 1.65x$; $r^2 = 0.78$). [4]Linear effect ($\hat{Y} = 1.28 - 0.04x$; $r^2 = 0.96$). [5]Linear effect ($\hat{Y} = 0.19 - 0.004x$; $r^2 = 0.79$). [6]Linear effect ($\hat{Y} = 0.345 - 0.0059x$; $r^2 = 084$).

Table 4. Egg quality of Japanese quail consuming commercial feed supplemented with increasing levels of liquid vinasse.

Parameters	Liquid vinasse levels (%)					CV (%)
	0.0	2.5	5.0	7.5	10.0	
Egg weight (g)	12.42	12.77	13.00	12.18	12.65	4.60
Specific weight (g/cm^3)[1]	1.073	1.073	1.076	1.078	1.078	0.26
Haugh unit	93.63	94.74	96.40	94.41	94.81	2.51
Yolk						
Weight (g)	4.01	4.00	4.41	4.17	3.96	7.73
Percentage (%)	32.33	31.37	33.91	34.28	31.31	6.16
Albumen						
Weight (g)	6.60	6.22	6.62	6.06	6.62	10.22
Percentage (%)	47.38	48.78	50.95	49.56	51.61	16.15
Eggshell						
Weight (g)[2]	1.82	2.03	1.96	1.96	2.12	7.25
Percentage (%)	14.80	15.89	15.13	16.14	16.53	8.56
Thickness (mm)	0.197	0.222	0.212	0.215	0.217	5.94

CV: Coefficient of variation. [1]Linear effect ($\hat{Y} = 1.073 + 0.583x$; $r^2 = 0.84$). [2]Linear effect ($\hat{Y} = 1.88 + 0.020x$; $r^2 = 0.57$).

Table 5. Nutritional value of eggs from Japanese quail consuming diets supplemented with increasing levels of liquid vinasse.

Parameter	Liquid vinasse levels (%)					CV (%)
	0.0	2.5	5.0	7.5	10	
DM (%)[1]	26.4	26.2	26.1	26.1	24.3	2.62
EE (% DM)[2]	33.0	36.7	34.7	34.1	31.9	5.56
EE (% FM)[3]	8.7	9.6	9.1	8.9	7.7	6.21
CP (% DM)[4]	42.1	42.1	42.5	42.4	44.2	2.31
CP (% FM)	11.1	11.0	11.1	11.1	10.8	3.51
MM (% DM)	8.0	8.1	8.8	8.0	7.7	13.18
MM (% FM)	2.1	2.1	2.3	2.1	1.9	13.38

DM: Dry matter; EE: Ether extract; FM: Fresh matter; CP: Crude protein; MM: Mineral matter; CV: Coefficient of variation. [1]Linear effect ($\hat{Y} = 26.65 - 0.167x$; $r^2 = 0.59$). [2]Quadratic effect ($\hat{Y} = 33.59 + 0.945x - 0.115x^2$; $R^2 = 0.74$). [3]Quadratic effect ($\hat{Y} = 8.79 + 0.320x - 0.042x^2$; $R^2 = 0.90$). [4]Linear effect ($\hat{Y} = 41.78 + 0.1757x$; $r^2 = 0.63$).

Table 6. Price per kilogram of eggs and a dozen eggs produced by Japanese quail supplemented with liquid vinasse.

Price (USD)	Liquid vinasse levels (%)					CV (%)
	0.0	2.5	5.0	7.5	10	
Kilogram of eggs	7.05	6.6	5.9	6.7	5.85	8.63
Dozen eggs[1]	0.8	0.8	0.7	0.7	0.4	8.31

CV: Coefficient of variation. [1]Linear effect ($Y = 0.31 - 0.0053x$; $r^2 = 0.84$).

Discussion

Inclusion of LV was expected to improve the productive performance because it is an acidifying product with probiotic properties. Although the intake of DM, CP, GE, Ca, and P decreased as LV increased, the feed conversion ratio (Kg DM/dozen) was the only affected variable. It suggests dietary nutrients were used more efficiently as a result of supplementation with LV.

Similarly, Swiatkiewicz *et al.* (2010) found that laying hens supplemented with prebiotics and organic acids showed no change in egg mass compared with hens fed non-supplemented diets. However, Bahnas (2009) reported an increase in the laying rate of Japanese quail supplemented with 0.05% malic acid.

Dahiya *et al.* (2016) concluded that laying hens supplemented with salts of organic acids had higher hen-d egg production. Hidalgo (2009) reported an increase of up to 16% in egg production when birds ingested LV with the feed. Dietary supplementation with organic acids for laying hens also increased egg mass (Soltan, 2008).

The DM content of LV is very low (3 to 4%). Thus, the higher the level of LV added, the lower the DM of the diet, accounting for a linear decrease in daily DM intake while daily feed intake remained unaltered. Gallo *et al.* (1986) also observed a decrease in feed intake of birds receiving LV in the drinking water.

Ribeiro *et al.* (2010) added organic acids associated with mannan oligosaccharides to the diet of laying hens and observed a decrease in feed intake. According to the authors, this result was caused by inhibition of microbial development and the influence of organic acids on nutrient availability. Bahnas (2009) provided 0.05% malic acid to Japanese quail and observed a decrease in feed intake, together with an increase in egg mass, indicating the economic efficiency of this practice.

The reduction in nutrient and energy intake did not affect feed conversion values, indicating dietary nutrient usage was optimized, possibly because of the presence of organic acids in the LV. Evaluating the effect of organic acids on productive performance of laying hens, Lala *et al.* (2016) observed a decline in feed conversion (g/g) when humic acid was added to the diet of laying hens, compared to the control group.

Acidifying substances also act as antibiotics, and supplementing birds with these substances is a strategy for improving animal performance. However, because of the differences in the modes of action, environmental conditions, used doses, and the conditions evaluated for this supplementation, conflicting results have been reported regarding response to the use of these substances (Viola and Vieira, 2007). These differences could explain the small effect of LV on quail performance in the present experiment. The animals did not face health challenges, considering the clean and comfortable environment in which they were housed. According to Ricke (2003), the antibacterial mechanism of acidifiers is not completely understood and variations may occur depending on the organism and the environment.

Acidifying substances may also positively influence egg weight (Soltan, 2008). On the other hand, Kaya *et al.* (2014) found no difference in shape index, shell thickness and weight, yolk and albumen index and Haugh unit in eggs laid by hens receiving a mixture of organic acids.

The yolk contains the greatest concentration of nutrients in the egg, so that increasing its size is desirable. Similarly to the present results, Soltan (2008) observed that supplementing the diets of laying hens with organic acids produced eggs with similar yolk weights. The characteristics of the albumen were unaltered, but adding organic acids to the diet of laying hens increased the albumen percentage and simultaneously decreased the percentage of yolk in the egg (Rahman *et al*., 2008).

Specific weight is an estimate of eggshell density. It is related to the resistance of the eggshell, and according to Güçlü *et al*. (2008), an increase in eggshell density indicates an improvement in eggshell quality. Integrity and resistance of the eggshell are essential for maintaining the nutritional and microbiological properties of the egg (Murata *et al*., 2009). Given that Ca is the main component of eggshell, the quality of this structure may be compromised when the diet provides low levels of Ca (Paz *et al*, 2009).

Considering a mean feed intake of 32 g/bird/d, Ca intake should be 0.89 (NRC, 1994), 0.95 (Silva e Costa, 2009), or 0.77 g/bird/d (Rostagno *et al*., 2011) for good eggshell quality. In this study, even the lowest level of Ca intake (0.95 g/bird/d) met the requirement of the bird. Thus, the best eggshell quality obtained by including 10% LV could be the result of the acidification of the diet and the greater Ca absorption in the intestines that results from acidification, as Suiryanrayna *et al*. (2012) reported. A similar effect was also observed by Soltan (2008), who supplemented the diets of laying hens with organic acids and observed increased eggshell thickness and weight, although the change in the latter was not significant.

Reduction in the DM content of eggs is not desirable in the egg industry. The DM content of the eggs remained relatively stable (approximately 26%) up to 7.5% LV inclusion. This reduction could be associated with lower daily DM intake, given that bird nutrition can influence egg quality. The values we obtained are similar to those reported by Genchev (2012), who evaluated the chemical composition of eggs from two lines of Japanese quail and found DM values of 26.72 and 26.06%.

The highest EE values were obtained at levels of 4.16 and 3.76% LV, and the lowest at 10% LV in the diet. Although jejunum is the main site for fat absorption (Krogdahl, 1985), the duodenal region may have been acidified with 10% LV supplementation. Moreover, the optimum pH for pancreatic lipase activity is close to 8, and these enzymes are inactivated at pH lower than 6 because low pH precipitates biliary acids, interfering with fat digestion and absorption (Ros, 2000) and consequently with its deposition in eggs.

The linear increase observed in CP content associated with LV is desirable because biological value of egg protein is high. As previously mentioned, duodenum acidification may improve the activity of intestinal proteolytic enzymes, whose optimum pH is between 3.5 and 4 (Goldberg *et al*., 1969). In addition, duodenal acidification can stimulate secretion of pancreatic juice, trypsin, and chymotrypsin (Thaela *et al*., 1998), improving protein digestion and absorption so that protein can be deposited in the egg. The linear increase in CP in this experiment could be explained by amino acid supplementation (Shafer *et al*., 1998) owing to their increased availability and digestibility offered by organic acids (Partanem and Mroz, 1999).

Although the effects of LV on egg production costs have not been thoroughly described in the literature, economic benefits of using LV in other animal species have been reported. Oliveira *et al*. (2013) found that the gross margin was greater when LV is added to the diet of rabbits.

Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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