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Technical Cashew Nutshell Liquid in Diets of Growing Meat-Type Quails

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ABSTRACT

The aim of this study was to evaluate the inclusion of technical cashew nutshell liquid (TCNSL) in European quails (*Coturnix coturnix coturnix*) diets from 1 to 35 days of age on the performance, carcass traits, and development of the digestive organs. 575 quails were distributed in a completely randomized design with five treatments (0%, 0.25%, 0.50%, 0.75%, and 1.00% TCNSL), five replicates, and 23 birds per experimental unit. Live performance was evaluated by weight gain, average feed intake, and feed to conversion ratio. The relative weight of the digestive organs (proventriculus, gizzard, pancreas, liver, small intestine, and large intestine), length of the small and large intestine, and carcass and parts yield (breast, wing, and leg) were determined at the end of the experimental period (35 days). The supplementation of quail diets with TCNSL did not influence live performance or carcass and parts yields. The relative weights of the proventriculus, gizzard, pancreas, small intestine, and large intestine, and the length of the small intestine and large intestine were not affected by the dietary inclusion of TCNSL. However, TCNSL supplementation had a quadratic effect ($p<0.05$) on liver relative weight, with the lowest relative weight predicted at 0.33% TCNSL. Quails fed 1% TCNSL showed higher liver relative weight compared with those fed the TCNSL-free diets. The dietary inclusion of up to 1% TCNSL did not influence the performance, carcass traits, or development of the digestive organs of European quails.

INTRODUCTION

The concern of consumers about the possibility of antibiotic residues in animal food products as well as the ban on antibiotics as a performance enhancer in many European countries have recently boosted research on natural additives. Among the several additives studied, functional oils have aroused interest because of their secondary plant metabolites, compounds that act to protect the plant against pathogens. When added to feed, these substances can improve both health and animal performance, in addition to their nutritional value (Murakami *et al.*, 2014).

Cashew nutshell liquid (CNSL), obtained from cashew fruit peel, contains cardanol, anacardic, cardol, and 2-methylcardol acids in its composition (Trevisan *et al.*, 2006), which confer many biological activities, such as antimicrobial (López *et al.*, 2012) and antioxidant properties (Abreu *et al.*, 2017). In general, these substances help to balance the intestinal microbiota by inhibiting the growth of pathogenic bacteria by inducing cellular apoptosis (Muzaffar & Chattoo, 2017) and favoring the growth of beneficial bacteria that aid digestive and absorptive processes. Moreover, these compounds can act as free radical scavengers, acting not only in the initial stage of the oxidative process but also in their propagation, with antioxidant activity at the cellular level in the animal organism (Toyomizu *et al*., 2000) or in animal food products, extending their shelf life (Abreu *et al*., 2015).

In fact, some studies have demonstrated positive performance results of broiler chickens when co-products from the processing of cashew nuts or even cashew nut meal were incorporated in diets (Ojewola *et al*., 2004; Tanod *et al*., 2015), and reduced *Escherichia coli* counts in the intestinal contents (López *et al*., 2012). Bess *et al*. (2012), studying a commercial mixture of functional oils containing CNSL, observed better performance parameters in broilers, even when the birds were fed a diet with a reduction of 100 kcal metabolizable energy/kg, demonstrating that the dietary inclusion of the product improved nutrient digestibility. In addition, some authors (Toyomizu *et al*., 2003; Murakami *et al*., 2014) evaluated CNSL as a natural anticoccidial and found a reduction of the severity of cecal lesions of chickens infected with coccidia, suggesting they act as ionophores. However, despite these positive effects, Farias *et al*. (2017) did not observe any changes in the performance traits and gastrointestinal development of laying quails fed fresh or 180-d stored cashew nut meal.

This heterogeneity of results may be related to the concentration of these compounds, which vary with the extraction method used. When obtaining CNSL by using solvents, there is a high concentration of anacardic acid, whereas heat processing favors the decarboxylation of this acid, resulting in a high content of cardanol (Mazzetto *et al*., 2009), and it is named technical CNSL (TCNSL). In this context, anacardic acid has higher antioxidant and enzyme-inhibiting capacities relative to the other compounds in CNLS (Trevisan *et al*., 2006) due the presence of three double bonds in the alkyl side-chain (Stasiuk & Kozubek, 2010). Nevertheless, *in-vitro* assays demonstrated that TCNSL may have a protective effect against oxidative stress (Andrade *et al*., 2011), but there are still few studies on the effects of feeding TCNSL and its effects on the animals' organism.

Considering the above, the aim of this study was to evaluate the effects of feeding different TCNSL levels to European quails on their performance, carcass yield, and relative weight of the digestive organs.

MATERIAL AND METHODS

The experiment was performed at the Poultry Research Center of the Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil. The experimental procedures complied with the ethical principles for animal experimentation established by the Brazilian Association for Laboratory Animal Science (SBCAL) and the National Council for the Control of Animal Experimentation (CONCEA).

The poultry house was divided in floor pens, measuring 2.12 m^2 each, covered with new wood-shavings litter, and equipped with tube feeders and bell drinkers. House temperature was controlled by a negative pressure system and infrared heating lamps. House temperature and relative humidity were daily measured using digital thermo-hygrometers (Instrutemp, ITHT 2250, São Paulo, Brazil) placed at birds' height. Average maximum and minimum temperatures were 31.0 and 26.2°C, respectively. Average maximum and minimum relative humidity were 75.5 and 57.6%, respectively.

In all, 575 one-d-old male and female European quails (*Coturnix coturnix coturnix*) were distributed according to a completely randomized design into five dietary treatments (0%, 0.25%, 0.50%, 0.75%, or 1.00% of TCNSL) with five replicates of 23 birds each. Birds were reared until 35 days of age.

The TCNSL was purchased from the company Usibras (Aquiraz, Ceará, Brazil) and stored under refrigeration until the preparation of the experimental diets. According to a report of the manufacturer, the concentrations of anacardic, cardanol, cardol, and 2-methylcardol acids were 10.03 mg/g, 540.77 mg/g, 102.34 mg/g, and 19.17 mg/g of product, respectively.

The isoenergetic and isoprotein experimental diets were formulated to meet the nutritional requirements proposed by Rostagno *et al*. (2011) for the phases of 1 to 14 and 15 to 35 days of age (Table 1). Throughout the experimental period, water and feed were supplied *ad libitum*. A continuous lighting program was adopted (24 h of natural + artificial light).

Performance parameters (weight gain, average feed intake, and feed conversion ratio) were determined per experimental unit for the periods of 1 to 14 days (phase 1) and from 15 to 35 days of age (phase 2). Weight gain (g) was calculated by subtracting initial body weight (BW) from final BW, and feed intake (g) was determined as the difference between feed offer and feed residues in the feeders at the end of each phase. Feed conversion ratio was calculated by dividing average feed intake by average weight gain. Average feed intake and feed conversion were daily adjusted for mortality.

Table 1 – Ingredients and calculated nutritional composition of the experimental diets.

	Period (days)	
Ingredients (%)	1 to 14	15 to 35
Corn	47.186	54.477
Soybean meal, 45%	46.220	38.700
Soybean oil	2.700	3.620
Limestone	1.040	0.930
Dicalcium phosphate	1.350	0.980
NaCl	0.381	0.331
Inert material*	0.100	0.100
DL- Met, 98%	0.385	0.352
L-Lys sulfate, 50,7%	0.182	0.260
L-Thr, 98%	0.206	0.000
Supplement minerals and vitamins[1]	0.250	0.250
Total	100	100
Calculated nutritional composition		
Crude protein (%)	25	22
Metabolizable energy (kcal/kg)	2900	3050
Calcium (%)	0.850	0.700
Available phosphorus (%)	0.380	0.300
Digestible Met + Cys (%)	1.040	0.940
Digestible Lys (%)	1.370	1.230
Digestible Thr (%)	1.040	0.760
Digestible Trp (%)	0.290	0.250

[1] Mineral and vitamin supplement (content per kg of product): folic acid 80 mg; pantothenic acid 2200 mg; copper 3600 mg; choline 40 mg; iron 18 mg; butylated hydroxy--toluene 150 mg; iodine 300 mg; manganese 30 g; niacin 6000 mg; selenium 120 mg; vitamin A 2800000 IU; vitamin B_1 340 mg; vitamin B_{12} 2800 cg; vitamin B_2 1000 mg; vitamin B_6 560 mg; vitamin D_3 600000 IU; vitamin E 2000 IU; vitamin K_3 480 mg; zinc 28g.

* The technical cashew nutshell liquid was added to diet in replacement of inert material (kaolin).

On d 35, two males per replicate (average BW ± 5%) were fasted for six hours and then slaughtered by decapitation between the occipital and atlas bones. The relative weight (as a % of BW) of the gastrointestinal organs (proventriculus, gizzard, small intestine, large intestine, pancreas, and liver) and the length (cm) of the small and large intestine (colon, rectum, and cecum) were determined. Carcass yield was calculated as the ratio of the hot eviscerated carcass (without head, feet, neck, and abdominal fat) to BW at slaughter. Parts yield (breast, wing, and leg) was calculated as their weight relative to eviscerated carcass weight.

The results were submitted to analysis of variance, and Dunnett's test was applied to compare the differences between the control treatment (0% TCNSL) and each TCNSL inclusion level. Data were also submitted to regression analysis by polynomial decomposition of the degrees of freedom. All the statistical analyses were performed using the SAEG – System for Statistical and Genetic Analysis (2007), and a probability of <0.05 was considered significant.

RESULTS AND DISCUSSION

Although CNSL is typically used as a raw material for manufacturing waterproofing agents, paints, varnishes, plastics, and other products (Akinhanmi *et al*., 2008), its phenolic composition (cardanol, anacardic, cardol, and 2-methylcardol acids) has attracted the interest of nutritionists because it is correlated to several biological activities, such as antimicrobial (López *et al*., 2012) and antioxidant properties (Abreu *et al*., 2017).

According to the literature, the concentration of compounds in the final product depends on the processing conditions. Extraction by maceration of the cashew nutshell with solvents at room temperature produces a liquid with high anacardic acid level, whereas roasting at high temperatures favors the decarboxylation of anacardic acid, increasing the final content of cardanol, which is classified as technical CNSL (Paramashivappa *et al*., 2001; Kumar *et al*., 2002). According to a report from the manufacturer, the concentrations of anacardic, cardanol, cardol, and 2-methylcardol acids found in the CNSL used in the experiment were 10.03 mg/g, 540.77 mg/g, 102.34 mg/g, and 19.17 mg/g, respectively, characterizing it as TCNSL.

Among the compounds isolated from CNSL, anacardic acid is the most frequently lipid reported in the literature, since it is biologically more efficient than the other compounds identified (Himejima & Kubo, 1991; Trevisan *et al*., 2006). Nevertheless, other CNSL components, such as cardanol, including one of its main compounds, cardanol monoene, are also capable of benefiting the animal's body due to their antioxidant, bactericidal, and anti-cancer activities (Andrade *et al*., 2011; Su *et al*., 2017).

Several modes of action of the bioactive compounds identified in CNSL have been described. The amphipathic character of the acids allows them to pass through the lipid bilayer of bacteria, inhibiting the activity of enzymes involved in the synthesis of energy and lipids (Murata *et al*., 1997; Parasa *et al*., 2011). Their effectiveness is greater against gram-positive than gram-negative bacteria due to the lower structural complexity of the membrane of the former (Himejima & Kubo, 1991). Therefore, they are able to modulate the intestinal microbiota, causing the death of specific microorganism groups considered pathogenic to the animal. In addition, these compounds may inhibit the pro-oxidative enzymes involved in the production of free radicals and have a binding specificity to some metals, such as Fe^{2+} and Cu^{2+}, reducing their availability for both bacteria and for the conversion of reactive-oxygen species (Hemshekhar *et al*., 2011; Parasa *et al*., 2011).

Despite the previously reported beneficial effects of biological substances, the dietary TCNSL supplementation did not influence (p>0.05) quail performance or carcass and parts yields (Tables 2 and 3) at any of the evaluated levels.

The relative weight of the proventriculus, gizzard, pancreas, small intestine, and large intestine, and the length of the small intestine and large intestine of 35-d-old quails were not affected (p>0.05) by the dietary inclusion of TCNSL at any levels (Table 4). However, TCNSL supplementation had a quadratic effect (p<0.05) on liver relative weight, with the lowest relative weight predicted at 0.33% TCNSL (Figure 1). When comparing each inclusion level against the control, it was observed that quails fed 1% TCNSL had higher liver relative weight compared with those fed TCNSL-free diets (Table 4).

Table 2 – Body weight gain, feed intake, and feed:gain of growing meat-type quails fed diets with different levels of technical cashew nutshell liquid (TCNSL).

	1 to 14 days of age			15 to 35 days of age		
TCNSL level (%)	Body weight (g)	Feed intake (g)	Feed:gain	Body weight (g)	Feed intake (g)	Feed:gain
0.00	67.595	129.961	1.929	132.848	453.545	3.409
0.25	69.811	134.736	1.927	135.530	430.026	3.172
0.50	64.874	135.985	2.091	138.016	421.250	3.055
0.75	67.863	136.538	2.019	134.184	426.396	3.183
1.00	69.327	131.498	1.893	132.470	430.967	3.256
CV (%)	8.00	11.63	8.72	4.95	7.19	5.90
P Dunnett's test	0.720	0.958	0.453	0.701	0.616	0.126
P Regression analysis						
Linear	0.803	0.836	1.000	0.770	0.343	0.394
Quadratic	0.529	0.465	0.133	0.207	0.217	0.180

CV = coefficient of variation; Not significant by Dunnett's test (p>0.05)

Table 3 – Carcass and parts yields (%) of growing meat-type quails at 35 days of age fed diets with different levels of technical cashew nutshell liquid (TCNSL).

TCNSL level (%)	Carcass yield (CY) (% BW)	Breast yield (% CY)	Leg yield (% CY)	Wing yield (% CY)
0.00	70.62	40.93	24.38	10.87
0.25	72.07	39.69	25.58	11.28
0.50	72.35	41.58	23.71	10.74
0.75	71.43	41.05	23.94	11.04
1.00	70.45	40.56	24.69	9.84
CV (%)	2.68	5.03	6.33	15.26
P Dunnett's test	0.443	0.675	0.378	0.696
P Regression analysis				
Linear	0.721	0.832	0.650	0.334
Quadratic	0.073	0.788	0.649	0.400

CV = coefficient of variation; Not significant by Dunnett's test (p>0.05).

The lack of influence of the dietary inclusion of TCNSL on quail performance and carcass yield parameters observed in the present study may be due to a several intrinsic and extrinsic factors, including experimental methods (Windisch *et al*., 2008). In the current experiment, the optimal rearing environmental conditions, reduced health challenge, and adequate management conditions may account for these results. In addition, the supply of highly-digestible diets may have reduced the availability of substrates for bacterial proliferation (Lee *et al*., 2003), and therefore, the expected benefits were not expressed. In fact, Murakami *et al*. (2014), evaluating the effect of the inclusion of functional oils (CNSL and castor oil) in the diets of broilers challenged with *Eimeria* spp., observed that those fed diets containing oils showed greater economic feasibility and reduced lesions caused by the protozoan, evidencing the benefits of substances derived from functional oils under challenging environments.

Furthermore, there is limited information on the interaction between the biological activity of natural

Table 4 – Relative weight of gastrointestinal organs (%), and length of the small and large intestine (cm) of growing meat-type quails fed diets with different levels of technical cashew nutshell liquid (TCNSL).

	Relative weight (% BW)						Length (cm)	
TCNSL level (%)	Proventriculus	Gizzard	Liver	Pancreas	Small intestine	Large intestine	Small intestine	Large intestine
0.00	0.387	1.876	1.899	0.218	1.922	0.603	50.300	17.950
0.25	0.455	2.119	1.885	0.247	2.338	0.637	57.300	19.950
0.50	0.430	2.046	1.895	0.234	2.329	0.563	55.350	19.700
0.75	0.404	2.068	1.874	0.265	2.195	0.628	51.350	19.450
1.00	0.412	1.985	2.350*	0.253	2.449	0.668	55.200	20.250
CV (%)	15.67	10.26	12.42	19.15	14.74	15.64	7.38	14.12
P Dunnett's test	0.544	0.422	0.024	0.572	0.155	0.530	0.057	0.715
P Regression analysis								
Linear	0.990	0.574	0.018	0.205	0.066	0.387	0.502	0.304
Quadratic	0.285	0.122	0.032[1]	0.625	0.426	0.365	0.224	0.607

CV = coefficient of variation; Significant by Dunnett's test ($p<0.05$); [1]$Y = 1.93788228 – 0.72798168 x + 1.08486560 x^2$ ($R^2 = 0.84$) (Minimum point: 0.33% of TCNLS).

additive compounds and nutritional factors, including nutrient levels and diet type (Zeng *et al*., 2015). Jamroz *et al*. (2006) observed higher jejunal villi in chickens fed on maize diet supplemented with plant extract than in those fed diets based on wheat and barley.

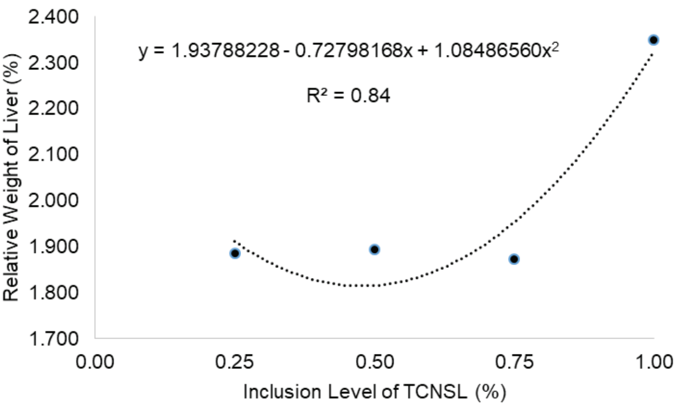

Figure 1 – Effect of different levels of technical cashew nutshell liquid (TCNSL) on liver relative weight (%) of growing meat-type quails.

Technical cashew nutshell liquid in diets of growing meat-type quails; Lorena Mari Sanches, Cinthia Eyng, Rodrigo Garófallo Garcia, Gislaine Paganucci Alves, Gabriela Glaeser Sangalli, Ricardo Vianna Nunes

The analysis of the relative weight of the digestive organs can be used as an indirect indicator of their health and functionality. The liver is the organ responsible for the metabolism of substances harmful to the organism, and therefore, any possible interference in the hepatic metabolism may change its size (Simsek *et al*., 2007). In fact, anacardic acid and, to a lesser extent cardanol (Andrade *et al*., 2011) may modulate liver antioxidant activity by acting on the uncoupling oxidative phosphorylation in mitochondria and causing a small inhibition in respiration rate (Toyomizu *et al*., 2000; Hamad & Mubofu, 2015). In this context, the observed effect on the liver relative weight may be related with beneficial changes in liver metabolism, including low lipid peroxidation and to the absence of hepatic dysfunction, since no toxic effects were observed with the use of isolated cardanol, the compound with the highest concentration in the TCNSL used (Leite *et al*., 2015). Braz *et al*. (2017) did not observe any changes in the concentration of non protein sulfhydryl groups in the liver of laying hens fed diets containing CNSL, indicating no toxic effects of its compounds. Contrary to the observations in this study, López *et al*. (2012) and Farias *et al*. (2017) did not observe any changes in liver relative weight when feeding TCNSL to broilers and cashew nut meal to quails, respectively.

CONCLUSION

The dietary inclusion of up to 1% TCNSL did not influence the performance, carcass traits, or development of the digestive organs of European quails.

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