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Effects of cashew nut shell liquid (CNSL) on the performance of broiler chickens

[*Efeitos do líquido da casca da castanha de caju (LCC) sobre o desempenho de frangos de corte*]

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ABSTRACT

The effects of cashew nut shell liquid (CNSL) on growth performance, carcass yield, relative weight of internal organs and microbiology of digestive tract of broiler chickens were investigated. Five hundred and forty male broiler chicks at one day of age were arranged in a completely randomized design with six treatments and six repetitions with 15 broiler chicks each. The treatments were: control (T1 – without growth promoter virginiamycin and CNSL); inclusion levels of 0.10mL (T2), 0.20mL (T3), 0.30mL (T4) and 0.40mL (T5) of CNSL/kg of feed; and commercial promoter virginiamycin (T6). At 21 and 40 days of age, body weight, feed intake, feed conversion and viability of birds were similar in all treatments. Carcass yield was higher in the treatment with the growth promoter when compared to the control treatment. There was a linear increase in carcass yield when the level of CNSL was increased in the diet. The relative weight of the intestine was lower in the treatment containing virginiamycin when compared to the treatment without the inclusion of additives. The relative weight of the intestines decreased when the levels of inclusion of CNSL were increased. There was a gradual reduction of *Escherichia coli* concentration reaching the lowest number on the CNSL level of 0.30mL/kg. It was concluded that CNSL showed similar performance and slaughter yield as the growth promoter and reduced the concentration of *Escherichia coli* in the intestinal contents.

Keywords: feeding, feed conversion, nutrition, vegetable extract

RESUMO

Um estudo foi conduzido para investigar os efeitos do líquido da casca da castanha de caju (LCC) sobre o desempenho, o rendimento de carcaça, o peso relativo dos órgãos internos e a microbiologia do trato digestivo de frangos de corte. Foram utilizados 540 pintos machos de um dia de idade, distribuídos num delineamento inteiramente casualizado, com seis tratamentos e seis repetições de 15 aves cada. Os tratamentos consistiram em: controle (T1 – sem promotor comercial e sem LCC), níveis de inclusão de 0,10mL (T2), 0,20mL (T3), 0,30mL (T4) e 0,40mL (T5) de LCC/kg de ração e T6 (promotor comercial – virginiamicina). Aos 21 e 40 dias de idade, o peso corporal, o consumo de ração, a conversão alimentar e a viabilidade das aves foram semelhantes em todos os tratamentos. O rendimento de carcaça foi superior no tratamento com promotor de crescimento em relação ao tratamento-controle. Ocorreu uma resposta linear de incremento no rendimento de carcaça com o aumento do nível de LCC na dieta. O peso relativo dos intestinos foi menor no tratamento com virginiamicina em relação ao tratamento-controle. O peso relativo dos intestinos diminuiu com o aumento do nível de inclusão do LCC. Houve uma redução gradual da concentração de Escherichia coli*, cuja menor concentração atingiu o nível de 0,30mL/kg. Conclui-se que o LCC mostrou desempenho e rendimento de abate semelhantes ao promotor de crescimento e reduziu a concentração de* Escherichia Coli *no conteúdo intestinal.*

Palavras-chave: alimentação, conversão alimentar, nutrição, extrato vegetal

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INTRODUCTION

The most widely used additives in poultry feed are the antibiotic growth promoters (AGP), which act in the intestine of birds reducing the population of certain types of commensal bacteria, promoting better growth performance. However, the use of AGP is being banned in animal feed due to the possibility of the emergence of resistant microorganisms. Therefore, it is necessary to study *in vivo* various natural additives that have similar characteristics to the growth promoters, in order for them to be used in poultry feeding in the future (Langhout, 2000).

The cashew (*Anacardium occidentale* L.) is a tropical plant, native of Brazil, scattered in almost all its territory. The fruit of the tree consists of an outer shell (epicarp), a tight-fitting inner shell (endocarp) and a strongly vesicant cashew nut shell liquid (CNSL). The CNSL is contained between the inner and outer shell (pericarp) in a honeycomb matrix. CNSL is a unique natural source of meta-alkyl phenols with a variable degree of unsaturation attached to the benzene ring. Based on the mode of extraction from the cashew nut shell, CNSL is classified into two types: solvent-extracted immature CNSL (iCNSL) and technical CNSL. A typical solvent-extracted material contains anacardic acid (60-65%), cardol (15-20%), cardanol (10%) and traces of 2-methyl cardol. Depending on the conditions of the roasting process, the composition of the technical CNSL can change and have higher cardanol content (83-84%), less cardol (8-11%) and maintain polymeric material at 10% and 2-methyl cardol content at 2% (Kumar *et al*., 2002).

Laboratory pharmacological tests showed the antiseptic properties of CNSL acting against the microorganisms responsible for tooth decay (*Streptococcus mutans*) and acne (*Propionibacterium acnes*). In addition, the CNSL is moderately effective in treatments against eczema, psoriasis and leprosy (Sousa *et al*., 1991).

In short, the positive effects observed *in vitro* motivates further research in this area to determine the ideal dietary inclusion level and the mode of action of these plant products to achieve optimal growth performance.

The purpose of this study was to verify the performance, carcass yield, relative weight of internal organs and intestine microbiology of broiler chickens fed diets containing different inclusion levels of CNSL.

MATERIAL AND METHODS

The experiment was performed using 540 male chicks with one day of age, distributed in a completely randomized design with six treatments and six replications, each replication consisting of fifteen birds. The treatments were: control (T1 – without growth promoter virginiamycin and CNSL); inclusion levels of 0.10mL (T2), 0.20mL (T3), 0.30mL (T4) and 0.40mL (T5) of CNSL/kg of feed; and commercial promoter virginiamycin (T6). These supplements were added to basal diets.

The extraction of CNSL occurred through the thermal-mechanical (hot oil) process, in which very hot CNSL is used as a means to heat the nuts in nature to approximately 190°C; at this temperature, the outer shell breaks down and releases the alkyl phenols present in the porous shell (mesocarp), followed by the removal of the inner bark, which allows the recovery of almonds.

The birds were reared in 30 floor pens of $1.5m^2$ each, with water troughs and hanging feeder, a source of infrared heating lamps of 250W, receiving water and feed ad libitum. The bed of shavings was used at a height of 5cm. The birds were vaccinated against Newcastle and Gumboro diseases at fourteen days of age.

The feeding program consisted of a starter diet (until 21 days of age), a growing diet (22-35 days of age) and a finisher diet (36-40 days of age). The composition of the experimental basal diets is shown in Table 1. All diets for each period were prepared with the same batch of ingredients, and all diets within a period had the same composition. Diets were formulated to meet requirements of the "Brazilian tables for poultry and swine – Feed composition and nutritional requirements" (Rostagno *et al*., 2005) for broilers of those ages. The antibiotic growth promoter virginiamycin was used in the amount of 10ppm.

Table 1. Composition of the experimental diets

Ingredients	Basal diet (%)		
	Starter	Growing	Finisher
Corn (8% CP)	57.279	65.206	66.518
Soybean meal (45% CP)	33.80	26.00	23.90
Meat and bone meal (45% CP)	5.50	5.20	4.80
Limestone (37% Ca)	0.44	0.43	0.45
Soy-oil	2.00	2.20	3.50
Sodium chloride	0.36	0.28	0.31
Sodium bicarbonate	0.09	0.150	0.09
DL-methionine	0.208	0.170	0.159
L-lysine	0.071	0.107	0.123
Vitamin-mineral premix[1]	0.20	0.20	0.15
Senduramicin sodium	0.05	-	-
Salinomicin 12%	-	0.055	-
Virginiamycin 500	0.002	0.002	-
Calculated composition			
Metabolisable Energy (kcal/kg)	3000	3100	3200
Protein	22.45	19.45	18.42
Lysine	1.30	1.12	1.06
Methionine + Cystine	0.92	0.80	0.76
Threonine	0.88	0.76	0.72
Tryptophan	0.27	0.23	0.21
Arginine	1.50	1.27	1.19
Calcium	1.00	0.94	0.88
Available phosphorus	0.48	0.45	0.42
Sodium	0.22	0.20	0.19
Potassium	0.91	0.78	0.73
Chloride	0.30	0.35	0.27
Ether Extract	5.03	5.46	6.66
Fiber	3.59	3.25	3.13
Electrolytic Balance (meq/kg)	243	215	195

[1]The vitamin and trace mineral mix provided the following results per kilogram of diet: retinyl palmitate, 6.000IU; cholecalciferol, 1.000ICU; DL-α-tocopherol acetate, 10IU; menadione sodium bisulfite, 1.0mg; thiamin, 1.8mg; riboflavin, 3.6mg; niacin, 25.0mg; pantothenic acid, 10.0mg; pyridoxine, 3.5mg; folacin, 0.5mg; biotin, 0.15mg; choline, 500mg; copper, 8mg; iron, 80mg; manganese, 60mg; selenium, 0.1mg; and zinc, 40mg.

In the experimental period, body weight, feed intake, feed conversion and viability were evaluated at 21 and 40 days of age. At 40 days old, two birds per replicate (12 birds per treatment) were selected and after a six hour fast they were slaughtered to determine yields of carcass and commercial cuts (breast, thigh and drumstick, back, wings, feet, head and neck), which were expressed as percentage of body weight before slaughter. In addition, proventriculus, gizzard, intestines, pancreas, spleen, heart and liver were removed to determine the relative weight of internal organs (% of body weight).

In the microbiological evaluation one bird per replicate was chosen for the collection of the gastrointestinal tract (small and large intestines). For this purpose, the birds were euthanized through cervical dislocation and a cut in the abdominal cavity was immediately done to expose the intestines. Three ligatures were performed at the beginning of the small intestine, rectum and ileocecal junction with suture material. Respective cuts were performed on both extremities and in the ileocecal junction and immediately placed into zip lock bags (10cm x 14cm x 0.1). Possible residues of cuts on the extremities were removed with distilled water. The samples were identified in the outer and inner part of the zip lock bags and introduced in polystyrene boxes containing ice. Sample collection lasted approximately one hour. After the collections were completed a thermal polystyrene box with a 50 liter capacity containing the samples was completely filled with ice and enveloped to transport via airway to the JFLAB - SP laboratory. The samples were collected according to septic procedures and with sterilized material. The broilers were subjected to a two hour ration fast. The period between the collection and the processing of the samples in the laboratory was about 20 hours.

Samples of 25g of intestinal contents were placed in vials containing 225mL of saline solution at 0.85% resulting in a 10^{-1} dilution. From this dilution consecutive decimal dilutions were performed using the same proportion. The presumptive test was done from 10^{-1} to 10^{-7} dilutions of the contents from intestinal samples. For each dilution a inoculum of 1.0mL was used in the petri dish for each microorganism under evaluation. *Escherichia coli* was enumerated on Mac-Conkey Agar (Difco) after aerobic incubation at 37°C for 24h. For the growth of *Clostridium perfringens*, each dilution was inoculated into fluid Thioglycolate media. For assessment of *Salmonella* intestinal contents of birds were inoculated in test tubes containing enrichment media Rappaport Vassiliadis, Selenite-Novobiocin and Tetrationate and incubated for 24h. The colony count data was expressed as the number of colony forming unit (CFU) per gram of intestinal content. These values were transformed into $\log_{10}$ for analysis and interpretation of results (APHA, 2001).

This study was composed of a randomized complete design with six treatments. The number of replicates varied according to the data assessed, including six for performance and microbiological count data and 12 for the slaughter yield and relative weight of internal organs. All data was analyzed through the GLM SAS procedure (Statistical..., 2000). Treatment means were compared through the Tukey test at 5%. The regression analysis was used to determine linear and quadratic responses to 5%. This assessment left out the treatment with growth promoter. The microbiological count data (*Escherichia coli*) was transformed into the square root of (X+1).

RESULTS

The results of broiler performance at 21 days of age are presented in Table 2. The results of body weight, feed intake, feed conversion and viability of birds were not significantly affected by treatments (P>0.05).

Table 2. The effects of cashew nut shell liquid (CNSL) on performance of broilers at 21days of age

Treatments	Variables			
	Body weight (g)	Feed Intake (g)	Feed: gain (g:g)	Viability (%)
T1	994	1083	1.136	96.7
T2	1016	1107	1.134	95.6
T3	995	1079	1.130	100
T4	1011	1099	1.133	97.8
T5	1008	1093	1.129	97.8
T6	1024	1111	1.129	95.6
CV, %	3.69	4.63	3.51	4.49

Means within a column did not differ (P>0.05) by Tukey test.

The data corresponding to five levels of inclusion of cashew nut shell liquid (CNSL) were subjected to regression analysis. The results showed no linear and quadratic effects in the variables under study.

In the present study there were no significant differences (P>0.05) for the body weight, feed intake, feed conversion and viability of birds variables at 40 days of age (Table 3).

Carcass yield was significantly influenced (P<0.05) by treatments (Table 4). Birds fed the growth promoter had higher carcass yield when compared to the treatment without the inclusion of additives (P<0.05).

Table 3. The effects of cashew nut shell liquid (CNSL) on the performance of broilers at 40 days of age

Treatments	Variables			
	Body weight (g)	Feed Intake (g)	Feed: gain (g:g)	Viability (%)
T1	2817	4639	1.672	93.3
T2	2796	4675	1.697	90.0
T3	2768	4560	1.674	87.5
T4	2807	4642	1.680	89.8
T5	2730	4592	1.709	87.6
T6	2841	4653	1.664	92.0
CV, %	4.58	3.16	3.72	7.82

Means within a column did not differ (P>0.05) by Tukey test.

Table 4. Carcass yield of broilers fed diet cashew nut shell liquid (CNSL)

Treatment	Variables							
	Carcass*	Breast	Thighs	Back	Wings	Feet	Head	Neck†
T1	79.8b	24.6	21.3	16.2	7.1	3.63ab	2.28	3.88
T2	81.5ab	24.2	21.8	17.4	7.1	3.64ab	2.37	3.86
T3	82.0ab	24.8	21.2	17.1	7.0	3.63ab	2.25	3.73
T4	81.9ab	24.2	21.3	17.9	7.8	3.86b	2.51	3.59
T5	82.3ab	24.8	21.5	17.3	7.2	3.69ab	2.39	3.65
T6	82.1a	24.7	21.9	18.1	7.4	3.53a	2.49	3.88
CV, %	1.74	6.54	7.14	11.8	10.7	6.84	14.4	18.8

Means followed by distinct letters in the same column are different (P<0.05) by Tukey test.
*Linear Effect ($Y = 79.8 + 17.8X$ $R^2 = 0.65$)
†Linear Effect ($Y = 3.89 – 0.73X$ $R^2 = 0.82$)

The data subjected to regression analysis showed a linear effect (P<0.05) for carcass yield ($Y = 79.8 + 17.8X$ $R^2 = 0.65$). According to the data we can infer that the increase in the level of inclusion of CNSL increases carcass yield. Feet yield was greater for treatment with virginiamycin in relation to the group that received the CNSL level of 0.30mL/kg (P<0.05). Neck yield showed a linear effect (P<0.05). The results showed that the relative weight of the neck decreased with increasing levels of CNSL inclusion ($Y = 3.89 - 0.73X$ $R^2 = 0.82$). The regression analysis for the other slaughter yield parameters showed no significant effect (P>0.05).

The results on the relative weight of internal organs are shown in Table 5. Results showed statistical differences in relative weight of the intestines between treatments with the growth promoter and control (P<0.05). Broilers fed the growth promoter had a lower relative intestine weight when compared to broilers fed the control diet.

Table 5. Relative weight of internal organs (% of body weight) of birds at 40 days of age fed diet with cashew nut shell liquid (CNSL).

Treatment	Variables						
	Proventriculus	Gizzard	Intestines*	Pancreas	Spleen	Heart	Liver
T1	0.31	1.23	3.59a	0.17	0.098	0.43	2.03
T2	0.32	1.15	3.35ab	0.16	0.088	0.43	1.97
T3	0.33	1.20	3.17ab	0.14	0.083	0.39	1.80
T4	0.34	1.20	3.04ab	0.18	0.091	0.43	1.86
T5	0.30	1.17	2.91ab	0.17	0.085	0.41	1.90
T6	0.30	1.11	3.03b	0.15	0.073	0.40	1.92
CV, %	20.1	13.6	13.1	21.9	34.0	15.8	19.1

Means followed by distinct letters in the same column are different (P<0.05) by Tukey test.
*Linear Effect ($Y = 3.55 – 1.67X$ $R^2 = 0.98$)

The data on the relative weight of internal organs was submitted to regression analysis and the results showed that only the relative weight of the intestines had a linear effect ($P<0.05$) ($Y=3.546 - 1.67X$ $R^2 = 0.98$), while the other parameters showed no significant effect ($P>0.05$).

The concentration of *Escherichia coli* in the intestines of birds was not statistically affected by treatments (Table 6). However, there was a gradual reduction of the concentration of bacteria in the intestinal contents to the level of 0.30mL/kg (quadratic effect $P<0.05$). *Salmonella* spp. and *Clostridium perfringens* were not identified.

Table 6. Results of microbiological analyses of the intestinal content of broiler chickens fed cashew nut shell liquid (CNSL) at 40 days of age.

Treatment	Variables		
	Escherichia coli[*1] (CFU/g)	*Clostridium perfringens*	*Salmonella* spp.
T1	1.007199	Negative	Absent
T2	1.000958	Negative	Absent
T3	1.000340	Negative	Absent
T4	1.000002	Negative	Absent
T5	1.006058	Negative	Absent
T6	1.001249	Negative	Absent
CV, %	0.09	-	-

Means within a column did not differ ($P>0.05$) by Tukey test. [1]Transformed data $\sqrt{(x+1)}$
[*]Quadratic effect ($Y = 1.00711 - 0.0743X + 0.178X^2$ $R^2 = 0.92$)

DISCUSSION

The vegetal extracts are a relatively young class of feed additives and in recent years these feed additives have gained considerable attention in the feed industry. They are a wide variety of herb, spices and products derived thereof and are mainly essential oils. Theses additives have been developed for use as alternatives to antibiotics in the animal industry partly due to their biological properties such as antimicrobial and antiseptic activities. However, limited studies are available to assess the possible application of theses extracts as alternatives to antibiotics in broiler chicken production (Langhout, 2000).

This study evaluated the inclusion of cashew nut shell liquid (CNSL) as a possible replacement for growth promoters in broiler chicken diets. The results showed that there was no improvement in body weight, feed intake, feed conversion and viability of the birds when growth promoter virginiamycin and different levels of inclusion of CNSL were added to the diet.

Presumably, the current study conducted with well nourished animals and disinfected environments might have not induced the improvement of growth-related parameters in birds fed the diet supplemented with antibiotics and the cashew nut shell liquid (0 to 0.40mL of CNSL/kg of diet). It has also been well documented that dietary antibiotics such as penicillin do not promote growth performance when animals are raised in germ-free conditions (Coates *et al*., 1963). Similar to antibiotics, dietary CNSL as a growth promoter could not give beneficial results when birds are kept at optimal conditions such as highly digestible diets and clean conditions (Jang *et al*., 2004).

Some experiments with broiler chickens also resulted in no statistical difference in the performance of animals supplemented with different species, concentrations and combinations of plant extracts in the diet. According to Lee *et al*. (2003), the absence of effect on broiler performance may be related to the composition of the basal diet provided and/or environmental conditions in which the experiment was conducted. Rations with ingredients with high digestibility limit the proliferation of bacteria in the intestinal tract, because there is no substrate available for bacterial growth, thereby reducing the antimicrobial potential of plant extracts. The same can be observed if the birds have been raised in places with low immune challenges and strict sanitary control (Simsek *et al*., 2007).

This is the first study conducted to evaluate the inclusion of CNSL in the diet of birds and there are no parameters to compare these results to in the current literature. However, the results show that bird performance was normal for the experimental conditions. This is an important indicator showing that the health, management and nutrition were adequate for animal growth, thus preventing the observation of possible benefits of tested treatments.

In this study, the carcass yield was higher in the treatment containing virginiamycin compared with the control. In addition, there was a linear upward effect with increasing inclusion levels of CNSL in the diet. The results are consistent with those observed by Manoj *et al*. (2008) who observed a higher carcass yield in broiler chickens fed virginiamycin when compared to control. In addition, Alçiçek *et al*. (2004) observed higher carcass yield when they added a mixture of essential oils in the diet. However, the results of the present study are in disagreement with the results of Garcia *et al*. (2007) who showed that the carcass, right breast, and right thigh yields of birds at 49 days of age were unaffected when broilers were fed avilamycin or 200ppm of blended oregano, cinnamon and pepper essential oils. Furthermore, Leeson *et al*. (2005) observed no effect on carcass weight when broilers were fed virginiamycin or 0.2 or 0.4% butyric acid. In addition, Isabel and Santos (2009) showed that the breast weight as a percentage of the carcass was significantly higher in birds fed supplements of 100 ppm of clove and cinnamon oils than in control.

Relative weights of the proventriculus, gizzard, pancreas, spleen, heart and liver were not affected by dietary treatment. The results of the present study are in agreement with the results of Hernandez *et al*. (2004) who found no differences in gizzard, liver and pancreas weights of broiler chickens fed diet containing an essential oil extract from oregano, cinnamon and pepper and a labiatae extract from sage, thyme and rosemary. Similar results were observed by Jamroz *et al*. (2005) who used essential oils in broiler diets based on maize and locally grown cereals.

Relative weight of intestines was lower in the treatment with a growth promoter when compared to control, and there was a reduction in this organ's weight with increasing inclusion levels of CNSL. Taking both statistical analyses into account, the inclusion of virginiamycin and CNSL reduced the relative weight of the intestines. Visek (1978) demonstrated that the inclusion of antibiotics reduces the weight and length of the intestines in poultry. An outstanding feature of germ-free animals are the greatly enlarged ceca which to date have not been explained. They are believed to contain biologically active substances based upon the behavior of the cardiovascular system after cecectomy (Gordon and Pesti, 1971).

It is known that some feed additives originating from plant products have a profound impact on gut microflora either directly or indirectly (Cowan, 1999), although birds have little nutritional advantage from intestinal microflora compared with other species of animals. In the current study, birds fed diets containing CNSL showed a similar magnitude of *Escherichia coli* when compared to those fed antibiotic-supplemented diet. However, there is a reduction in the concentration of bacteria when the level of inclusion of CNSL increased, reaching a minimum concentration to the level of 0.30mL/kg.

Almost all the CNSL commercially available is submitted to a heating process. In the thermal processing, the anacardic acids are easily decarboxylated to cardanols. As a consequence of this process, the strongest antibacterial activity of anacardic acid in CNSL is reduced because cardanols are less active. Thus, the antibacterial activity of CNSL is weakened by thermal processing verifying that the activity against *Staphylococcus aureus* and *Bacillus ammoniagenes* is reduced 32 times (Himejima and Kubo, 1991). In this study, probably because the CNSL had been subjected to heat, the lowest concentrations of CNSL (0 to 0.20mL/kg) failed to reduce the concentration of *Escherichia coli* in intestinal contents.

In addition, the tested Gram negative pathogen, *Escherichia coli*, was not particularly sensitive to virginiamycin. It is known to be active mainly against Gram positive microbes (Proudfoot *et al*., 1990) and this is therefore in agreement with the current findings. Moreover, the presence of *Clostridium perfringens* was not observed, and strains of *Salmonella* spp. were absent.

Antimicrobial activity has been recognized as the major beneficial effect of vegetal extract on animal production, although the exact antimicrobial mechanism has not been fully revealed. Numerous *in vitro* studies demonstrated that essential oils including thymol, carvacrol, etc., displayed antimicrobial activity against intestinal microbes such as *Clostridium perfringens, Salmonella* Typhimurium and *Escherichia coli* (Hammer *et al*., 1999). On the other hand, in an *in vivo* study, it seems that the effect of vegetal extracts on gastrointestinal microflora is not consistent, even though essential oils have been generally recognized as an antimicrobial agent. Therefore, it is speculated that the *in vivo* antimicrobial property of vegetal extracts in birds can be influenced by basal diet and environment conditions.

The present results clearly show that CNSL can control the proliferation of *Escherichia coli* in the broiler intestine. Moreover, it appears that CNSL in the current study prevents the presence of *Clostridium perfringens* and *Salmonella* spp. in intestinal contents of broilers.

CONCLUSIONS

The cashew nut shell liquid (CNSL) can be included in the diet of broiler chickens in the levels studied at different phases of growth without harming the performance and carcass yield of birds. *Escherichia coli* concentration in intestinal contents is reduced by the inclusion of CNSL in the diet.

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