Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 1, 2020
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

USE OF THE DIETARY SEA BUCKTHORN MEAL AS PHYTOADITIVE IN HEAT - STRESSED BROILER

Mihaela SĂRĂCILĂ[1, 2], Tatiana Dumitra PANAITE[1], Arabela UNTEA[1], Iulia VĂRZARU[1], Dumitru DRĂGOTOIU[2], Rodica Diana CRISTE[1]

[1]National Research and Development Institute for Animal Biology and Nutrition (IBNA) Balotesti, 1, Calea Bucuresti, Balotesti, 077015, Ilfov, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaela.saracila@yahoo.com

Abstract

The paper aimed to characterize sea buckthorn meal (SBTM) and to assess his effect in the diet of hea t- stressed broiler (32˚C). A total of 60 Cobb 500 broiler chicks were assigned in two groups (C, E) which included in the diet corn and soybean meal as main ingredients. Compared with the C diet, the experimental diet (E diet) included the addition of 1% of SBTM. Samples of SBTM (purchased from a local producer) and samples of compound feed were analysed for their proximate composition, minerals, vitamin E and antioxidant activity. A 5-day balance sheet study was conducted on broilers in the grower stage (week 4) and in the finisher stage (week 6). Performance parameters (0-42 days) were recorded. Results showed that SBTM had an important concentration of polyphenols (11.65 mg/g GAE), lutein and zeaxanthin (91.80 mg/kg), expressing a high antioxidant capacity (99.84 mM ascorbic acid equivalent, 92.1 mM vitamin E equivalent). Dietary SBTM is a valuable by-product which can be used in broiler diet without negatively affecting the coefficients of apparent absorption of nutrients and performance, even in heat stress conditions.

Key words: *broiler, heat stress, sea buckthorn meal, chemical characterization, performance.*

INTRODUCTION

The sea buckthorn (*Hyppophae rhamnoides*) is a fruit-bearing shrub occurring naturally in Romanian flora. Sea buckthorn berries are an excellent source of phytochemicals such as ascorbic acid, tocopherols unsaturated fatty acids, and carotenoids (Yang, et al., 2001; Krejcarová et al., 2015). Not only the fruits, but also the meal (obtained as consequence of oil extraction) has a high antioxidant capacity (Püssa et al., 2007; Geetha et al., 2009) because it is rich in carotenoids, xanthophylls and flavonoids (Jung et al., 2012). Research reports show that buckthorn is beneficial for poultry performance (Hu and Guo, 2006; Biswas et al., 2010; Kaushal and Sharma, 2011), without any potential adverse effect on environment (Vlaicu et al., 2017).

Among the oil extraction industry by-products, meals are vegetable raw materials with low cost that can be used in animal feeding (Panaite et al., 2016). The same authors concluded that sea buckthorn meal as by-product meet the feeding requirements for inclusion as dietary ingredients because of their high level of essential nutrients (protein, amino acids, fatty acids and minerals) and to their high antioxidant capacity. Since the leaves, seeds and fruit residues contain high crude protein, they have advantages as basic materials for feed formulations for poultry (Biswas et al., 2010). Heat stress is a significant cause of economic loss in poultry production and is almost inevitable (Hu et al., 2019). Heat stress impairs the nutrient digestibility (de Souza et al., 2016). Nutrient digestibility plays an important role in ensuring the health and productivity of animals. Heat stress mainly leads to a decrease in growth performance and affect the meat quality of poultry by inducing oxidative stress in the body. Researchers (Criste et al., 2017; Hu et al., 2019) reported that improving the antioxidant capacity of poultry may help mitigate the influence of heat stress. The polyphenols are phytocompounds which have a high antioxidant potential, being considered the most efficient active compounds

(Trifunschi et al., 2017). Polyphenols are natural antioxidants that can reduce oxidative stress and widely exist in plants, they therefore have great potential to be used as a novel feed additive for improving productivity in heat-stressed poultry. In recent years, there is a necessity to use low input forages in poultry feeding as food industry by-products (e.g. meals). However, less attention has been paid to the use of sea buckthorn as a poultry and livestock feed, therefore determining the feed value of sea buckthorn will provide scientific information that can be used to promote its use as livestock and poultry feed in heat-stressed broilers. Thus, the paper aimed to characterize sea buckthorn meal (SBTM) and to assess his effect in the diet of heat - stressed broiler (32°C).

MATERIALS AND METHODS

A six-week feeding trial was conducted on 60 Cobb 500 broiler chicks (1 day of age) evaluated in a completely randomized design, with two groups (30 chicks per group). The chicks were housed randomly in three-tiered digestibility cages, allowing the daily recording of the feed intake and excreta. Throughout the experimental period, the environmental temperature of the experimental hall was kept constant at 32ºC. The humidity parameter was 49%, with 0.45% ventilation/broiler, and 850 ppm CO_2 emission. The light regimen was appropriate to the age of the chicks, 23 hours light/1-hour darkness. From 1 day old, broilers received a corn and soybean meal-based control diet (C). Compared with the C diet (Table 1), the experimental diet (E) included the addition of 1% sea buckthorn meal (SBTM). Sea buckthorn meal was purchased from a local producer (E-Prod SRL, Teleorman, Romania), dried, grounded and packed. Feed and water were provided for *ad libitum* consumption. None of the groups (C, E) had monoensin in the premix. Diet formulations were calculated to meet or exceed the minimum requirements for broiler chicks (NRC, 1994). All diets were fed in mash form.
The coefficients of apparent absorption of the nutrients were determined using the balance technique in weeks 4 and 6. In each of these two weeks, the amounts of ingested feed and of excreted droppings were recorded for 5 consecutive days. The droppings were collected daily, for 5 days, at the same hour, weighed and stored in refrigerator (4°C); average weekly samples (6 samples/group) were formed, homogenized and dried in the drying oven, for 48 h, at 65°C. The compound feeds samples and the average weekly samples of droppings/group, were analysed for the dry matter, at 65-103°C (DM); crude protein (CP); crude fat (EE); crude fibre (CF); Ash (ash). The coefficients of apparent absorption of nutrients were calculated as described by Panaite et al. (2017). Throughout the experimental period (0-42 days, broiler age) the performance (average daily gain, average daily feed intake and feed conversion ratio) was monitored. The chemical proximate composition of feed samples was assayed using the chemical methods from Commission of the European Communities (2009) as described by Olteanu et al. (2016). The calcium concentration in samples was determined according to the titrimetric method SR ISO 6490-1/1996 and P by spectrophotometric method. Trace minerals (Cu, Fe, Zn, Mn) concentrations (expressed as mg/kg) were determined by flame atomic absorption spectrometry after microwave digestion.
The total phenol content of SBTM and feed samples was measured spectrophotometrically according to the Folin-Ciocalteu's method, described by Untea et al. (2018). The results were expressed as mg gallic acid equivalent (GAE)/g DW**.**
The total antioxidant capacity of the SBTM and feed samples was evaluated by the phosphor-molybdenum method of Prieto et al. (1999). The results were expressed as Mm ascorbic acid equivalent DW and as Mm vitamin E equivalent DW.
Lutein and zeaxanthin were analysed using the method described by Untea et al. (2020) with a high-performance liquid chromatograph. Vitamin E determination (expressed as mg/kg) was performed according to the method described in EC Regulation no. 152/2009, using a high-performance liquid chromatograph and a PDA-UV detector at a wavelength of 292 nm.

Table 1. Diet formulation

Ingredient	Starter (0-14 days)		Grower (15-28 days)		Finisher (29-42 days)	
	C	E	C	E	C	E
			%			
Corn	32.73	31.73	36.47	35.47	40.45	39.45
Wheat	20	20	20	20	20	20
Corn gluten	2	2	4	4	6	6
Soybean meal	36.17	36.17	30.2	30.2	23.95	23.95
Sea buckthorn meal	-	1	-	1	-	1
Sunflower oil	3.85	3.85	4.31	4.31	4.72	4.72
Monocalcium phosphate	1.68	1.68	1.52	1.52	1.43	1.43
Calcium carbonate	1.5	1.5	1.38	1.38	1.31	1.31
Salt	0.39	0.39	0.38	0.38	0.33	0.33
Methionine	0.33	0.33	0.25	0.25	0.21	0.21
Lysine	0.3	0.3	0.29	0.29	0.36	0.36
Threonine	-	-	0.15	0.15	0.19	0.19
Choline	0.05	0.05	0.05	0.05	0.05	0.05
Premix*	1	1	1	1	1	1
Total	100	100	100	100	100	100
Calculated						
Metabolisable energy, kcal/kg	3.039,79	3.039,79	3.128,99	3.128,99	3.217,72	3.217,72
Lysine	1.44	1.44	1.29	1.29	1.19	1.19
Methionine	0.69	0.69	0.61	0.61	0.57	0.57
Threonine	0.97	0.97	0.88	0.88	0.81	0.81
Tryptophan	0.25	0.25	0.22	0.22	0.19	0.19

*1kg premix contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg Vit. B1; 400 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vit. B6; 4 mg/kg Vit. B7; 100 mg/kg vit. B9; 1.8 mg/kg vit. B12; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.
C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

The complete randomized model was used to analyse the data for growth performance. The effects of treatments were tested by analysis of variance (ANOVA and t test) using Stat view for Windows (SAS, version 6.0). The differences between means were considered statistically significant at P<0.05.

RESULTS AND DISCUSSIONS

Table 2 shows the chemical composition of SBTM, highlighting a rather high level of fibre (16.11%). The high level of fibre had count on choosing the level of inclusion of SBTM in broiler diet. The SBTM also represents a valuable source of antioxidant compounds as polyphenols, lutein and zeaxanthin (Table 2) and vitamin E which contribute to his antioxidant capacity. The meal used in this study was not as rich in minerals as values in the literature for fruit pulp, but as by-product, contains important amount of Mn and Zn (Table 2).

Table 2. Chemical characterization of sea buckthorn meal

Variable	Sea buckthorn meal
Dry matter, %	88.94
Crude protein, %	12.43
Crude fat, %	13.29
Crude fiber, %	16.11
Ash, %	2.89
Cu, mg/kg	6.69
Fe, mg/kg	1.35
Mn, mg/kg	20.26
Zn, mg/kg	31.86
Vitamin E, mg/kg	143.16
Lutein + zeaxanthin, mg/kg	91.80
Total polyphenols, mg GAE /g DW	11.65
mM ascorbic acid equivalent DW	99.84
mM vitamin E equivalent DW	92.10

Compared with the National Research Council (1994), Biswas et al. (2010) reported that the leaves, seeds and fruit residues of sea buckthorn contain enough crude fat and crude protein for poultry growth. Lu et al. (1991) had characterize the sea buckthorn (seeds, leaves and fruit residues) compared with other feed ingredients (alfalfa, green sweet clover, green Symphytum, leaves of sophora, green reserved maize stalk, carrot, powdered soybean stalk, sorghum seed, maize seed, broad bean, soybean, pea). They showed that the crude protein content of sea buckthorn was higher than that of majority, excepted soybean, broad bean and pea. It contains necessary amount of crude protein and fat and stimulates the growth and productivity of livestock and poultry as well, particularly in dry and cold areas (Biswas et al., 2010; Kaushal & Sharma, 2011). Compared with the polyphenols content of SBTM obtained by Panaite et al. (2016), data from Table 2 show a higher content, by 12.13%. Differences may be originating from the method of oil extraction, method of analyse solvent used for extraction, etc. It was reported that sea buckthorn contains large amounts of carotenoids and vitamin E located mainly in membrane and the fleshy mesocarp (Zeb, 2004). The antioxidant vitamin E content of SBTM makes it a valuable contributor in helping the overall health and scavenging free radicals. In this regard, it has been reported that phytochemicals with antioxidant activity offer great hope as a solution for heat stress in poultry (Hu et al., 2019).

Table 3. Chemical composition of the compound feeds depending on the growth stage

Variable	Starter compound feed (0-14 days)		Grower compound feed (15-28 days)		Finisher compound feed (29-42 days)	
	C	E	C	E	C	E
Dry matter, %	88.52	89.18	88.84	89.41	89.18	89.21
Crude protein, %	23.00	22.79	21.50	21.65	20.00	19.89
Crude fat, %	5.48	5.52	6.01	6.07	6.49	6.52
Crude fibre, %	3.77	4.42	3.57	3.67	3.36	3.83
Ash, %	7.08	7.51	7.01	6.70	6.81	6.53
Ca, %	0.96	0.98	0.87	0.89	0.81	0.90
P, %	0.77	0.77	0.70	0.73	0.65	0.66
Cu, ppm	7.09	6.93	8.77	9.44	9.82	9.47
Fe, ppm	523.71	533.60	534.81	536.77	510.25	566.31
Mn, ppm	143.86	152.45	118.80	125.52	111.14	134.31
Zn, ppm	110.17	104.70	109.21	121.83	105.9	109.39
Vitamin E, ppm	44.01	42.89	45.92	47.41	52.16	52.94
Lutein + zeaxanthin, ppm	8.44	7.24	13.32	13.38	16.15	16.71
Total polyphenols, mg GAE/g	1.67	1.63	1.55	1.77	1.87	1.92
Antioxidant capacity mmol ascorbic acid equivalent/kg DW	29.60	27.25	35.36	38.43	30.69	37.99
Antioxidant capacity mmol vitamin E equivalent/kg DW	30.86	28.28	37.13	40.45	31.94	39.96

C- conventional diet; E- conventional diet + 1% sea buckthorn meal

Besides of people feeding, sea buckthorn, especially its leaves, pomace and press cake, can also be used as an ingredient of animal feed (Kaushal and Sharma, 2011). The results of the chemical analysis of the compound feeds (Table 3) shows that in the starter, grower and finishing stage, the compound feeds were balanced as energy and protein content. Notably, once SBTM was added in the experimental diet, an increase in the crude fiber and crude fat content compared with the control diet was observed (Table 3). Regarding the mineral content (Ca, P, Cu, Fe, Mn, Zn) of compound feeds, there were observed a slightly increase in E diet compared with C diet. Vitamin E concentration was slightly higher in experimental diet during grower and finisher stage compared with control diet. The addition of SBTM in broiler diet revealed an increase in polyphenol content of experimental diet in grower and finisher stages, resulting in an increase in the antioxidant capacity (Table 3).

Table 4. Effect of SBTM on the coefficients apparent absorption of the nutrients (grower stage)

Variable	C	E	SEM	p-value
Dry matter (DM)				
Ingested (g/chick/day)	70.14	67.84	2.962	0.7467
Excreted (g/chick/day)	17.42	17.67	1.101	0.9157
Absorbed (g/chick/day)	52.71	50.17	2.032	0.5552
Absorption coefficient (%)	74.79	74.23	0.669	0.6962
Organic matter (OM)				
Ingested (g/chick/day)	62.85	61.02	2.655	0.7487
Excreted (g/chick/day)	13.65	13.63	0.856	0.9210
Absorbed (g/chick/day)	49.20	47.19	1.913	0.6235
Absorption coefficient (%)	77.98	77.58	0.588	0.7450
Crude protein (CP)				
Ingested (g/chick/day)	17.45	16.05	0.745	0.3823
Excreted (g/chick/day)	2.21	2.36	0.181	0.7004
Absorbed (g/chick/day)	15.22	13.70	0.641	0.2533
Absorption coefficient (%)	87.18	85.62	0.722	0.3018
Crude fat (EE)				
Ingested (g/chick/day)	4.65	4.72	0.200	0.8713
Excreted (g/chick/day)	0.39	0.38	0.030	0.7991
Absorbed (g/chick/day)	4.25	4.34	0.176	0.8203
Absorption coefficient (%)	91.16	92.14	0.394	0.5085
Crude fiber (CF)				
Ingested (g/chick/day)	2.95	3.41	0.152	0.1304
Excreted (g/chick/day)	2.12	2.21	0.131	0.7518
Absorbed (g/chick/day)	0.82[a]	1.20[b]	0.074	0.0035
Absorption coefficient (%)	26.55[a]	35.75[b]	2.072	0.0173
Ash				
Ingested (g/chick/day)	5.61	5.21	0.239	0.4290
Excreted (g/chick/day)	3.04	3.01	0.194	0.9506
Absorbed (g/chick/day)	2.57	2.20	0.114	0.1031
Absorption coefficient (%)	44.69	42.81	1.658	0.5944

[a, b]Means in the same column with different superscripts differ significantly (p<0.05).
SEM = standard error of the means; C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

Table 4 data show the coefficient of apparent absorption of nutrients for broilers in the grower stage. Although SBTM was included in the E diet, the coefficient of apparent absorption of dry matter, organic matter, crude protein, crude fat and ash were not recorded significantly differences (P>0.05) compared with C group. Notably is that under heat stress, broilers fed E diet had a significantly (P<0.05) higher coefficient of apparent absorption of crude fibre (Table 4). Under thermoneutral conditions, others (Li et al., 2008) reported an increase in the apparent digestibility of dietary crude protein as consequence of broiler diet supplementation with 0.1% and 0.2% flavones of sea buckthorn.

Table 5. Effect of SBTM on the coefficients of apparent absorption of nutrients (finisher stage)

Variable	C	E	SEM	p-value
Dry matter (DM)				
Ingested (g/chick/day)	82.79	74.20	7.550	0.8025
Excreted (g/chick/day)	18.66	17.79	1.607	0.6697
Absorbed (g/chick/day)	64.13	56.40	6.023	0.5468
Absorption coefficient (%)	76.73	75.68	0.545	0.3576
Organic matter (OM)				
Ingested (g/chick/day)	74.82	67.34	6.831	0.6079
Excreted (g/chick/day)	14.84	14.18	1.284	0.6066
Absorbed (g/chick/day)	59.98	53.16	5.619	0.5691
Absorption coefficient (%)	79.54	78.65	0.458	0.3573
Crude protein (CP)				
Ingested (g/chick/day)	17.42	16.85	1.633	0.8692
Excreted (g/chick/day)	2.42	2.35	0.106	0.7465
Absorbed (g/chick/day)	15.00	14.50	1.544	0.8792
Absorption coefficient (%)	84.57	85.25	0.859	0.7125
Crude fat (EE)				
Ingested (g/chick/day)	5.96	5.52	0.549	0.7081
Excreted (g/chick/day)	0.81	0.69	0.073	0.4175
Absorbed (g/chick/day)	5.15	4.83	0.482	0.596
Absorption coefficient (%)	86.39	87.39	0.390	0.2146
Crude fiber (CF)				
Ingested (g/chick/day)	3.63	3.68	0.349	0.9442
Excreted (g/chick/day)	1.99	1.96	0.190	0.9296
Absorbed (g/chick/day)	1.63	1.72	0.177	0.8162
Absorption coefficient (%)	42.84	46.55	1.430	0.2078
Ash				
Ingested (g/chick/day)	6.44	5.53	0.583	0.4597
Excreted (g/chick/day)	3.01	3.03	0.274	0.9710
Absorbed (g/chick/day)	3.44	2.50	0.349	0.1929
Absorption coefficient (%)	51.54[a]	44.52[b]	1.655	0.0252

[a, b]Means in the same column with different superscripts differ significantly ($p<0.05$). SEM = standard error of the means; C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

The coefficients of apparent absorption of nutrients in the finisher stage, can be visualised in the Table 5. Broiler feeding with diet containing 1% SBTM, did not significantly influence ($p>0.05$) the absorption of DM, OM, CP, EE, CF (Table 5). However, it can be observed that the absorption coefficient of Ash was significantly lower ($p<0.05$) in the group fed diet with 1% SBTM compared with the group fed conventional diet. Fewer results are available on nutrients digestibility in hot environments. Many studies support the idea of heat stress can affects nutrient digestibility (Bonnet et al., 1997; de Souza et al., 2016). This affirmation can be explained by the fact that high environmental temperature alters the morphology of small intestine (decrease in villus height and ratio of villus height to crypt depth) and consequently the absorption of nutrients is affected. It was reported that chronic heat exposure decreases protein digestion, particularly with the summer diet. (Wallis and Balnave, 1984; Zuprizal et al., 1993).

However, in the present study, even if broilers were subjected to heat stress, the absorption of nutrients was not negatively affected. There are studies that have showed the effect of dietary plant materials on digestibility of nutrients in broilers under thermoneutral or heat stress conditions. For example, dietary artichoke extract supplementation improved ($P<0.01$) the digestion coefficients of DM, CP and CF in broilers (Hassan et al., 2015). Significant increases in crude protein (CP) and crude fat (EE) digestibility were achieved by heat-stressed birds fed diets supplemented with cinnamon, turmeric, ginger (0.5 g/kg) or ascorbic acid (200 mg/kg) compared with those of the control group, but those of other nutrients were unaffected (El-Maaty et al., 2014). On the contrary, Cross et al., (2007) showed no effect on the digestibility of nutrients when broilers (7 to 28 days of age) were fed diets with 10 g/kg herb (thyme, oregano, marjoram, rosemary or yarrow) or 1 g/kg of essential oil.

Table 6. Effect of dietary sea buckthorn meal on performance of heat- stressed broiler (0-42 days)

Variable	C	E	SEM	p-value
ADG (g/broiler/day)				
1-14	27.84	27.42	0.519	0.7091
15-28	51.41	48.73	2.205	0.5677
29-42	62.99	57.06	13.063	0.8326
1-42	44.59	40.44	2.632	0.4561
AFI (g feed/broiler/day)				
1-14	37.11	36.53	0.569	0.6352
15-28	77.03	74.04	3.035	0.6452
29-42	97.59	84.36	7.110	0.3769
1-42	72.87	64.98	3.416	0.2671
FCR (g feed/g gain)				
1-14	1.33	1.33	0.014	0.4204
15-28	1.50	1.52	0.147	0.3877
29-42	2.20	1.93	0.119	0.5777
1-42	1.65	1.62	0.029	0.5709

Where: ADG = average daily gain; AFI = average feed intake; FCR = feed conversion ratio; SEM = standard error of the means; C - conventional diet; E - conventional diet + 1% sea buckthorn meal.

The ADG of broilers fed diet with 1% SBTM was not significantly ($p >0.05$) different from that of broilers fed conventional diet (Table 6). Although not statistically significant, the FCR (0-42 days) of broilers from E group was lower compared to those from C group (Table 6). Even if the broilers were exposed to heat stress, there was no effect ($p >0.05$) of diet supplementation with SBTM on AFI (0-42 days). In our study no mortalities were recorded in any of the two experimental groups. Fewer studies were found in the scientific literature on application of sea buckthorn in poultry nutrition. Many researchers reported considerably increase in body weight of livestock and poultry after feeding with leaves, seeds and fruit residues of sea buckthorn (Hu, 2000; Hu and Guo, 2006; Biswas et al., 2010). Zhao et al. (2012) revealed that supplementation of flavones from sea buckthorn leaves significantly decreased AFI without affecting growth performance. Flavones of sea buckthorn play an important role in immunomodulation, antibiosis, and antioxidant reactions (Suryakumar and Gupta, 2011), resulting in the improvement of growth and feed utilization. Under thermoneutral conditions, Ma et al. (2015) showed that the use of 0.05 to 0.10% flavones from sea buckthorn had a positive influence on growth performance of broilers.

CONCLUSIONS

Sea buckthorn meal represents a valuable by product regarding the chemical composition, which can be included in broiler diet. The present findings showed that dietary SBTM, did not negatively affects the coefficients of apparent absorption of nutrients and broiler performance even under heat stress conditions. These results need to be further evaluated in order to assess the effect of SBTM on the antioxidant status of the heat-stressed broiler.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Romanian Ministry of Education and Research and also was financed from Project PN 19 09 0102 and Program 1 - Development National Research-Development, Sub-program 1.2 - Institutional Performance - Projects funding excellence in R & D, Contract no. 17 PFE/17.10.2018.

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