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THE BIOMASS QUALITY OF COMMON NETTLE, *Urtica dioica* L., AND ITS POTENTIAL APPLICATION IN MOLDOVA

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Abstract

Common nettle, Urtica dioica L., is an herbaceous perennial forb belonging to the Urticaceae family, distributed in temperate and tropical region in many parts of the world, and it has been reported to have multiple uses. We investigated the quality of the biomass of the local ecotype of Urtica dioica grown in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova. The results revealed that the dry matter of the common nettle whole plant harvested in flowering period contained 20.7% CP, 10.6% ash, 26.8% CF, 32.9% ADF, 57.4% NDF, 6.6 % ADL, 26.3% Cel, 24.5% HC, with forage value 633 g/kg DDM, RFV= 103, 12.47 MJ/kg DE, 10.23 MJ/kg ME and 6.25 MJ/kg NEl. The ensiled mass contained 22.1% CP, 14.5% ash, 28.3% CF, 33.8% ADF, 53.0% NDF, 6.6 % ADL, 27.3% Cel, 19.2% HC, with forage value 626 g/kg DDM, RFV= 110, 12.34 MJ/kg DE, 10.13 MJ/kg ME and 6.14 MJ/kg NEl. It has been determined that studied common nettle substrates have C/N=13.4-15.0 and the biochemical methane potential reaches 319-321 l/kg ODM. The local ecotype of Urtica dioica can be used as an alternative forage source for farm animals or as co-substrate in biogas generators for renewable energy production.

Key words: *biomass, biomethane, green mass, silage, Urtica dioica.*

INTRODUCTION

The genus *Urtica* L. belongs to the family *Urticaceae* Juss. which comprises 46 accepted species names, 3 species occur in Bessarabia. The most prominent members of the genus are stinging nettle *Urtica dioica* L., which is distributed in temperate region in many parts of the world: Europe, Africa, Asia and North America. It isan herbaceous perennial plant, growing about 2 m in height, with square, green and erect stem, covered with stinging hairs with hooked protrusions. The soft green leaves are 3 to 15 cm long, opposite. The leaves have a strongly serrated margin, with rounded or cordate base and acute or acuminate leaf apex. The inflorescences are axillary, spiked, by four per node, with many small, green, unisexual flowers. The flowering period lasts from June to October; it is a wind pollinated plant. The fruits are oval, about 1.5 mm long and 1 mm wide, bilaterally compressed, with thin, grey-green or brown pericarp, with remaining Perigonia lacinia. The root system reaches depths of 30 cm in the soil, it consist of yellowish and cylindrical rhizomes and stolons. Stinging nettle can reproduce vegetatively and by seeds. It produces abundant seeds, 5000-20000 seeds per shoot, the seeds can germinate since the first days after reaching maturity. The stinging nettle is a nitrophilous plant that grows in a wide range of habitats, as a common species of riparian habitats, swamps, meadows, riverbanks, wastelands, floodplains, and disturbed areas, is present around the margins of arable fields and gardens (Kavalali, 2004; Mirza, 2010; Bisht et al., 2012; Di Virgilio et al., 2014; Gînju, 2020).

Nettle has been used as a folk medicine and as a food and forage sources from a long time. All morphological parts of nettle - stem, leaves, roots and seeds are utilized to produce many added-value natural products. It has been used frequently for its medicinal properties since the Bronze Age (3000-2000 B.C.) as a traditional herbal remedy in treatment of variety of diseases as gout, nephritis, haematuria, jaundice, menorrhagia, anemia, eczema, arthritis. Other research into nettle indicates that it has very high antioxidant potential, but is also known for a wide range of other activities such as antimicrobial, antiulcerogenic, analgesic, diuretic, antidiabetic, anti-inflammatory and anti-rheumatic (Teleuță et al., 2008; Biesiada et al., 2010; Bisht et al., 2012; Di Virgilio et al., 2014; Janet al., 2017; De Vico et al., 2018; Kregiel et al., 2018; Devkota et al., 2022; Tarasevičienė et al., 2023).

Stinging nettle has gained both commercial and scientific interest due to its multipurpose character. *Urtica dioica* is a valuable herbaceous plant and can be used for feed purposes as a feed additive for livestock, especially poultry (Egorov, 2014; Adhikari et al., 2015; Alieva et al., 2016; Milosevic et al., 2021; Kosolapov et al., 2022). According to Totev (1964) the chemical composition of *Urtica* hays was 2l-23% CP, 3-5% EE, 35-39% NFE, 9-2l% CF, l9-29% ash.

Producing industrial biomass on marginal lands, which are unsuitable for food production, might help mitigate potential conflicts between food and non-food production. Stinging nettle has a further card to play in this context, as it grows vigorously everywhere, without intensive inputs such as pesticides, herbicides, or irrigation, even in fairly poor soil, which maintains soil structure, can improve soils that have been overfertilized with nitrogen and phosphate. The nettle yields range from 6-15 t/ha, which depends on the level of fertilization, agronomic treatments, soil type, and nettle plant can be used to produce biofertilizer and new high-quality agricultural raw materials for the production of various dyes, for the textile industry and the energy sector (Dreyer, 1996; Hartl & Vogl, 2002; Lehtomäki, 2006; Guil-Guerrero et al., 2014; Dubrovskis et al., 2018; Garmendia et al., 2018).The chemical composition of extracted nettle fibres was 65-85% cellulose, 5-12% hemicellulose, 2-4% lignin (Agus Suryawan et al., 2017; Viotti et al., 2022). The stinging nettle dry biomass contained 45.76% C, 5.60% H, 0.87 % N, 0.10% S, 6.93% ash and 17.48 MJ/ kg NCV, the specific density of pellets reached a maximum of 1068 kg/m^3 (Jankauskiene et al., 2016).

Nettle could be a promising candidate contributing to the reduction of atmospheric GHG emissions, it consumes significantly larger quantities of atmospheric CO_2 (18.8 t/ha), in relation to biomass, than mature forests (Butkute et al., 2015).

The aim of the current study was to evaluate some biological peculiarities, the quality of fresh and ensiled biomass of stinging nettle *Urtica dioica* L., as feed for ruminant animals, as well as substrate for the production of biomethane by anaerobic digestion.

MATERIALS AND METHODS

The localecotype of stinging nettle *Urtica dioica*, which growsin the experimental plot of the National Botanical Garden (Institute) of Moldova, Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″, served as subject of research, andthe common sainfoin, *Onobrychis viciifolia* ‘*Anamaria*’ and the low-coumarin local ecotype of yellow sweet clover, *Melilotus officinalis*, were used as control variants. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. The plant growth, development and productivity were assessed according to methodical indications. The stinging nettle and yellow clover green mass were harvested in the flowering period, common sainfoin - in the budding-flowering stage. The green mass yield was measured by weighing. The dry matter content was determined by drying the samples up to constant weight at a temperature of 105°C. For ensiling, the harvested green mass was chopped with a stationary forage chopping unit, shredded and compressed in well-sealed containers. After 45 days, the containers were opened and the sensorial and chemical characteristics of the prepared silages were determined in accordance with standard laboratory procedures and the Moldavian standard SM 108 for forage quality analysis. For chemical analysis, plant samples were dried in a forced air oven at a temperature of 60°C, then they were milled in a beater mill equipped with a sieve with diameter of openings of 1 mm and some of the main biochemical parameters were assessed: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were determined by the near infrared spectroscopy (NIRS) technique PERTEN DA 7200. The concentration of cellulose (Cel), hemicellulose (HC), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl), dry matter digestibility (DMD) and relative feed value (RFV) were calculated according to standard procedures.

The carbon content of the substrates was determined using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations indicated by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

In the second year, the local ecotype of *Urtica dioica* started active growth in the first days of March, common sainfoin, *Onobrychis viciifolia* - at the end of March, but yellow sweet clover, *Melilotus officinalis*– in the first days of April. At the time when the green mass was harvested, the *Urtica dioica* plants reached 138 cm in height, *Melilotus officinalis* - 112 cm, but *Onobrychis viciifolia* plants - 99 cm. The productivity of *Urtica dioica* was 4.01 kg/m^2 green mass or 0.83 kg/m^2dry matter, *Melilotus officinalis* reached respectively 3.78 kg/m^2green mass or 1.17 kg/m^2 dry matter, but the yield of *Onobrychis viciifolia* was 4.23 kg/m^2 green mass or 1.01 kg/m^2 dry matter.

The biochemical composition, nutritive and energy value of the green mass harvested from the tested species are presented in Table 1. Analysing the results of the biochemical composition of green mass, we found that the dry matter of the studied species differs essentially in the concentration of crude protein, crude fibre, cell wall fractions and ash. We found that *Urtica dioica* whole plantswere characterised by very high content of crude protein and optimal content of crude fibre as compared with *Onobrychis viciifolia* and *Melilotus officinalis*. The content of minerals in *Urtica dioica* is higher than in *Onobrychis viciifolia* and lower than in *Melilotus officinalis.* The concentration of neutral detergent fibre and acid detergent lignin is higher than in the control legume species. *Urtica dioica* harvested green mass contained very high amounts of hemicellulose. The dry matter digestibility and energy concentration is lower in *Urtica dioica* mass than in *Onobrychis viciifolia*, but does not differ much from *Melilotus officinalis* green mass.

Table 1. The biochemical composition and the nutritive value of the harvested green mass of the studied species

Indices	*Urtica dioica*	*Onobrychis viciifolia*	*Melilotus officinalis*
Crude protein, g/kg DM	207	177	179
Crude fibre, g/kg DM	268	293	330
Minerals, g/kgDM	106	96	118
Acid detergent fibre, g/kg DM	329	309	331
Neutral detergent fibre, g/kg DM	574	447	473
Acid detergent lignin, g/kg DM	66	49	44
Cellulose, g/kg DM	263	260	287
Hemicellulose, g/kg DM	245	138	142
Dry matter digestibility, g/kg DM	633	648	631
Relative feed value	103	135	124
Digestible energy, MJ/ kg	12.47	12.73	12.42
Metabolizable energy, MJ/ kg	10.23	10.45	10.20
Net energy for lactation, MJ/ kg	6.25	6.48	6.22

The results of the estimation of the quality of green mass from *Urticaceae* species are given in the specialized literature. According to Larin et al. (1952) *Urtica dioica* plants contained in dry matter 15.8-23.3% CP, 2.5-5.0% EE, 13.0-27.1 % CF, 35.0-44.5% NFE. Medvedev & Smetannikova (1981) showed that nettle dry matter contained 23.8% CP, 3.7 % EE, 24.5% CF, 33.1% NFE, 16.4% ash, the nutritive value was 0.19 feed unit/kg green fodder. Bogachkov & Morozov (1990) found that nettles contained in the dry matter 19.7% CP, 2.27% EE, 40.03% NFE, 24.4% CF, 14.39% ash, 3.32% Ca and 0.31% P.Nielsen & Soegaard (2000) remarked that the forage quality of *Urtica dioica* plants grown in semi-natural grassland, cut in June-July, was 318-364 g/kg NDF and 674-748 g/kg IVOMD, but in *Trifolium pratense* forage, it was 361-440.4 g/kg NDF and 570-673 g/kg IVOMD. Kshnikatkina et al. (2005) reported that the dry matter from *Urtica dioica* contained 20.73% CP, 3.72% EE, 14.18% CF, 40.30% NFE, 14.19% ash, 1.70% Ca, 0.80% P. Biesiada et al. (2010) remarked that the content of crude protein in nettle leaves was 17.31-24.12% in first cut mass, 19.37-24.50% in second cut mass and 21.43-25.68% in third cut mass. Egorov (2014) reported the chemical composition of flour from *Urtica dioica* was 908 g/kg DM, 24.8% CP, 5% EE, 13% CF, 32.91% NFE, 11% sugars, 4.5% starch, 19.1% ash, 1.4% Ca and 0.5% P. Guil-Guerrero et al. (2014) compared the quality of green mass from *Urtica* species and found that the chemical composition and energy value of *Urtica dioica* was 33.3% CP, 1.1% EE, 11.1% CF, 21.1% ash, 33.3% carbohydrates and 15.67 MJ/kg GE, but *Urtica urens* - 20.0% CP, 1.7% EE, 15.3% CF, 14.6% ash, 51.7% carbohydrates and 16.20 MJ/kg GE, respectively. Adhikari et al. (2015) showed that *Urtica dioica* flour contained 33.8% CP, 9.1% CF, 3.6% EE, 16.2% ash, 37.4% carbohydrates and 3070 kcal/kg energy value. Ciopata et al. (2015) reported that the chemical content of *Urtica dioica* was 27.8% CP, 15.3% CF, 28.4% NDF, 19.5% ADF, 20.72% ash and 73.8% ODM, but *Trifolium repens* 28.7% CP, 17.9% CF, 30.9% NDF, 22.8% ADF, 10.99% ash, 72.8% ODM. Kulivand & Kafilzadeh (2015) remarked that *Urtica dioica* contained 11.95% CP, 1.45%EE, 49.52% NDF, 39.06% ADF, 18.2% ash. Alieva et al. (2016) showed that the nutrient content in *Urtica dioica* plants was 22.88-23.15% CP, 2.12-2.81% EE, 12.47-13.00% CF, 32.91-35.99% NFE and 17.01-19.10% ash. Andualem et al. (2016) studied the biochemical composition of *Urtica simensis* whole plants and found that the fresh mass contained 167 g/kg dry matter, 25.4% CP, 39.3% NDFom, 21.4% ADFom, 4.29% ADL, 21.7% ash. Yakovchik & Yakovchik, (2017) reported that *Urtica dioica* contained 22-23% CP, 18-21% CF,75-85% dry matter digestibility, 70-75 g/kg carotene, 0.18-0.19 nutritive units/kg green mass and 190-210 g DP/nutritive units, but *Urtica cannabina* 21-22 % CP, 2.5-3.0% EE, 20-22% CF, 7-9% ash. Arros et al. (2019) mentionedthat the chemical and mineral composition of *Urticaurens* leaf powder was 24% CP, 2.5-5.0% EE, 8.7% CF, 31.6% NFE, 29.1% ash, 1.65% Ca, 0.51% P, 0.44% Mg. Zhang et al. (2020, 2022) remarked that the composition and nutritive value of *Urtica cannabina* was 16.5% CP, 2.9%EE, 38.2% NDF, 30.1% ADF, 29.5% NDC, 3.81% lignin, 18.9% ash, 3.44% Ca, 1.6% P and 58.4% IVDMD. Kosolapov et al. (2022) noted that *Urtica dioica* fodder contained 21.1-24.2% CP, 2.5-4.2% EE, 12.0-14.8% CF, 15.0% - 17.6% ash. Huang et al. (2023) indicated that whole plants of *Urtica cannabina* contained 310.9 g/kg DM, 12.57% CP and 6.18% WSC.

Table 2. The biochemical composition and the nutritive value of the ensiled mass from the studied species

Indices	*Urtica dioica*	*Onobrychis viciifolia*	*Melilotus officinalis*
Crude protein, g/kg DM	221	142	178
Crude fibre, g/kg DM	283	312	348
Minerals, g/kg DM	145	118	103
Acid detergent fibre, g/kg DM	338	317	333
Neutral detergent fibre, g/kg DM	530	470	462
Acid detergent lignin, g/kg DM	65	40	38
Cellulose, g/kg DM	273	277	285
Hemicellulose, g/kg DM	192	153	129
Dry matter digestibility, g/kg DM	626	642	630
Relative feed value	110	127	129
Digestible energy, MJ/kg DM	12.34	12.63	12.41
Metabolizable energy, MJ/kg DM	10.13	10.37	10.19
Net energy for lactation, MJ/kg DM	6.14	6.38	6.20

The proportion of conserved forages significantly increased in relation to the total yearly feed production, and the feed quality has markedly improved during the last 50 years. During times of plentiful growth, fodders can be stored as silage or hay. Currently, silage is the most common source of preserved feed for ruminant animals.

When opening the glass containers with prepared silage from the studied species, there was no gas or juice leakage from the preserved mass. The analysed stinging nettle *Urtica* *dioica* silage was characterized by dark green leaves and yellow stems, specific smell, the consistency was preserved in comparison with the initial green mass, without mould and mucus. As a result of the performed analysis, it was determined that the fermentation profile of stinging nettle silage was characterized by: pH=7.60, 32.2 g/kg total organic acids, 7.5 g/kg free lactic acid, 0.6 g/kg free acetic acid, 6.8 g/kg fixed lactic acid, 5.9 g/kg fixed acetic acid, 12.4 g/kg fixed butyric acid.

Analysing the biochemical composition of ensiled mass from the studied species, Table 2, it has been determined that the concentrations of nutrients in the dry matter varied as follows: 142-221 g/kg CP, 283-348 g/kg CF, 317-338 g/kg ADF, 462-530 g/kg NDF, 38-65 g/kg ADL, 273-285 g/kg Cel, 129-192g/kg HC and 103-145 g/kg ash. The nutritive and energy values of the ensiled mass were 62.6-64.2 % DMD, RFV=110-129, 12.34-12.63 MJ/kg DE, 10.13-10.19 MJ/kg ME and 6.14-6.20 MJ/kg NEl. As compared with the harvested mass, the silage from *Urtica dioica* had high concentration of crude protein, crude fibre, minerals, and low content of neutral detergent fibre and hemicellulose. We would like to mention that the ensiled mass from stinging nettle, as compared with the studied leguminous species, is characterized by higher content of de crude protein, crude fibre,cell wall fractions (NDF, ADF, ADL), hemicellulose and low cellulose nutritional value and energy supply of the feed. The nutritional value and energy supply of the ensiled stinging nettle feed is similar to that of sweet clover silage, but lower than common sainfoin haylage.

In the literature sources, there is little information regarding the chemical composition and nutritional value of ensiled mass from *Urtica* species. According to Medvedev & Smetannikova (1981), *Urtica cannabina* silage contained 33% DM, including 5.1% CP, 0.1% EE, 4.8% CF, 17.5% NFE, 5.5% ash, 22 g/kg DP and 0.175feed unit/kg, but rapeseed silage - 12.7% DM, including 2.4% CP, 0.1% EE, 1.3% CF, 5.0% NFE, 2.7% ash, 37 g/kg DP and 0.1feed unit/kg silage. Zhang et al. (2014) reported that *Urtica cannabina* silage provides more than 200 g/kg DM crude protein and had 74 % dry matter digestibility. A study conducted by Zhang and co-workers (2015) showed that the quality of pure *Urtica cannabina* silage was 209 g/kg DM, 19.0 % CP, 34.7% NDF, 28.0% ADF, 1.6% EE, 1.85% WSC, 57.0% IVDMD, 34.5% IVNDF, 32.9% IVADF, mixed silage with corn flour 316 g/kg DM, 16.8% CP, 27.6% NDF, 24.9% ADF, 1.45% EE, 2.15% WSC, 77.8% IVDMD, 46.8% IVNDF, 42.4% IVADF. Huang et al. (2023) found that *Urtica cannabina* silage is characterized by 294.7-303.6 g/kg DM, 11.52-11.65% CP, 4.96-4.81% WSC, pH=6.29-6.34, 28.0-29.7 g/kg lactic acid, 6.2-7.4 g/kg acetic acid, 4.5-5.7 g/kg propionic acid, 5.49-5.67% ammonium-nitrogen.

The depletion of fossil fuels, environmental pollution and energy insecurity have become global challenges in recent years. Renewable energy sources coming from biomass could play an important role in terms of energy supply and positive environmental effects. Biogas has become important as a renewable source of energy because of its decentralized approach, and it can be used to obtain heat and electrical power in special installations, but also as fuel in internal combustion engines. Many bacteria affect anaerobic digestion, including acetic acid-forming bacteria (acetogens) and methane-forming bacteria (methanogens). These microorganisms are very sensitive to environmental variations since they are obligatory anaerobic. These organisms promote a number of chemical processes in converting the biomass to biogas. The carbon to nitrogen ratio constitutes a basic factor governing the correct course of methane fermentation.

The results regarding the biochemical biomethane production potential of investigated substrates are shown in Table 3. The nitrogen concentration in the tested *Urtica dioica* substrates ranged from 33.12 g/kg to 35.36 g/kg, the estimated content of carbon - from 475.00 g/kg to 496.67 g/kg, the C/N = 13-15, but the substrates from the studied leguminous species contained 22.72-28.64 g/kg nitrogen, 490.00-502.22 g/kg carbon and C/N = 18-22. Essential differences were observed between concentrations of hemicellulose and acid detergent lignin. The *Urtica dioica* substrates contained high concentration of these substances. The biochemical methane potential of the tested stinging nettle substrates did not vary essentially - 319-321 l/kg VS, but was lower in comparison with the substrates of yellow sweet clover and common sainfoin.

Table 3. The biochemical biomethane production potential of the researched substrates

Indices	*Urtica dioica*		*Onobrychis viciifolia*		*Melilotus officinalis*	
	green mass	silage	green mass	haylage	green mass	silage
Crude protein, g/kg DM	207.00	221.00	177.00	142.00	179.00	178.00
Minerals, g/kg DM	106.00	145.00	96.00	118.00	118.00	103.00
Nitrogen, g/kg DM	33.12	35.36	28.32	22.72	28.64	28.40
Carbon, g/kg DM	496.67	475.00	502.22	490.00	490.00	498.33
Ratio carbon/nitrogen	15	13	18	22	17	18
Hemicellulose, g/kg DM	245.00	192.00	138.00	153.00	142.00	129.00
Acid detergent lignin, g/kg DM	66.00	65.00	49.00	40.00	44.00	38.00
Biomethane potential, L/kg VS	319	321	340	343	344	353

Several publications have documented the biomethane production potential of substrates from *Urtica* species. According to Lehtomaki (2006) the methane potential of common nettle achieved3000-5000 m^3/ha/year or 30-50 MWh/ha/year. Lehtomäki et al., 2008, remarked that harvested nettle biomass contained 150-303 g/kg DM, 123-283 g/kg OM, 12.0-42.0 g/kg nitrogen, 410-472 g/kg carbon, C/N=10-41, 189-280 g/kg lignin, the specific methane yield varied from 210 to 420 l/kg VS, but red cloverbiomass contained 153-399 g/kg DM, 138-387 g/kg OM, 19.0-52.0 g/kg nitrogen, 449-478 g/kg carbon, C/N = 9-25, 185-224 g/kg lign in with specific methane yield 280-300 l/kg VS. Wellinger et al. (2013) reported that the dry matter yield of nettles was 6-10 t/ha, the methane yield 2200-3600 m^3/ha/year and energy 21-35 MWh/ha/year. Dubrovskis et al. (2018) found that the average specific biogas or methane production per unit of dry organic matter added (DOM) from common nettle was 0.709 L/g or 0.324 L/g, but from common nettle with biocatalyst Metaferm - 0.752 L/g or 0.328 L/g, respectively. Cepo (2021) mentioned that the biogas potential of *Urtica dioica* substrate was 0.43 m^3/kg of dry organic matter and methane potential 0.25 m^3/kg of dry organic matter.

CONCLUSIONS

The dry matter of the local ecotype of common nettle, *Urtica dioica* whole plants harvested in the flowering period contained 20.7% CP, 10.6% ash, 26.8% CF, 32.9% ADF, 57.4% NDF, 6.6% ADL, 26.3% Cel, 24.5% HC, with forage value 633 g/kg DDM, RFV = 103, 12.47 MJ/kg DE, 10.23 MJ/kg ME and 6.25 MJ/kg NEl.

The ensiled mass contained 22.1% CP, 14.5% ash, 28.3%CF, 33.8% ADF, 53.0% NDF, 6.6% ADL, 27.3% Cel, 19.2% HC, with forage value 626 g/kg DDM, RFV = 110, 12.34 MJ/kg DE, 10.13 MJ/kg ME and 6.14 MJ/kg NEl.

The studied common nettle fresh and ensiled mass substrates have C/N = 13.4-15.0 and the biochemical methane potential reaches 319-321 l/kg ODM.

The local ecotype of common nettle, *Urtica dioica*, can be used as an alternative forage source for farm animals or as co-substrate in biogas generators for renewable energy production.

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