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REGULAR ARTICLES

# Influence of *S. babylonica* extract on feed intake, growth performance and diet in vitro gas production profile in young lambs

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**Abstract** An experiment was completed to determine the effect of *Salix babylonica* (SB) extract supplementation to the diet of growing lambs. Eighteen Katahdin × Pelibuey male lambs (14±2 kg live body weight) were divided randomly in individual cages into three groups and fed three diets varying in SB: a control group was fed on total mixed ration (TMR) without SB (SB0), an SB25 group was fed on TMR plus SB extract at 25 mL/lamb/day, and an SB50 group was fed on TMR plus SB extract at 50 mL/lamb/day on dry matter intake (DMI), average daily gain (ADG), feed efficiency, and in vitro gas production (GP) in lambs fed on TMR. In vitro GP of the TMR fed to lambs was recorded at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h of incubation with 0, 0.6, 1.2, and 1.8 mL extract per gram of DM. Addition of SB extract at low and high doses improved the DMI of lambs by 59.9 and 33.2 %, respectively. Relative to the control, low and high extract doses achieved greater lamb ADG during the experimental period. The asymptotic GP increased ($P<0.05$) with increasing dose of SB extract without affecting the rate of GP or the initial delay before GP begins. Linear increases for in vitro GP with advancing time with different SB extract doses were observed. It is suggested that the use of *S. babylonica* extract with the rate of 25 mL/lamb/day is beneficial to young lamb's performance growth and thus can be safely used as a feed additive in diets without any negative effects on animal health.

**Keywords** Average daily gain · In vitro gas production · Lambs · *Salix babylonica*

**Abbreviations**

| | |
|---|---|
| ADG | Average daily gain |
| CP | Crude protein |
| DM | Dry matter |
| DMD | In vitro dry matter digestibility |
| DMI | Dry matter intake |
| $GY_{24}$ | Gas yield at 24 h of incubation |
| ME | Metabolizable energy |
| MP | Microbial protein production |
| OMD | In vitro organic matter digestibility |
| $PF_{24}$ | Partitioning factor at 24 h of incubation |
| SB | *Salix babylonica* |
| SCFA | Short-chain fatty acids |
| TMR | Total mixed ration |

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## Introduction

Plant secondary metabolites of *Salix babylonica* (SB) extract seem to be alternatives to replace chemical additives (Jimenez-Peralta et al. 2011). Extracts of SB had secondary metabolites that could modulate ruminal fermentation and improve nutrient utilization in ruminants (Salem et al. 2011; Salem 2012). The antimicrobial activity of SB extracts

attributed to the concentration of secondary metabolites such as alkaloids, saponins, and phenolics (Jimenez-Peralta et al. 2011).

Secondary metabolites have the ability to suppress or stimulate microbial growth, reduce nutritional stress such as bloat, and/or improve animal health and productivity resulting in positive effects on daily gain, voluntary feed intake, and milk production (Salem et al. 2011). Besides having a protective effect on the protein in the rumen to promote duodenal absorption, they minimize the excretion of nitrogen, modify acetate-to-propionate ratio in the rumen fluid, and decrease parasitic load (Jimenez-Peralta et al. 2011).

The use of plant extract could be limited by their secondary compound concentrations; consumption of large amounts of tannins or saponins may have a direct hemolytic effect and may even cause death (Athanasiadou and Kyriazakis 2004). Moreover, long-term feeding of plants rich in secondary compounds may have detrimental effects on animal health (Mahgoub et al. 2008). Rumen microorganisms have the ability to degrade low concentrations of these secondary metabolites without any negative effects on rumen fermentation. Rumen microorganisms can also degrade alkaloids (Wachenheim et al. 1992), saponins (Hart et al. 2008), and phenolics (Varel et al. 1991) and utilize them as an energy source. Gürbüz et al. (2008) and Gürbüz and Davies (2010) illustrated that low condensed tannin content increases digestibility and gas production. They also stated that the absence of condensed tannin had improved rumen fermentation kinetics. Our objectives were to evaluate the addition of different doses of *Salix babylonica* extract on feed intake and average the daily gain of young lambs fed on total mixed ration.

## Materials and methods

### Animals, treatments, and housing

Eighteen Katahdin × Pelibuey male lambs, 2 to 3 months of age with 14±2 kg body weight, were used in a completely randomized design experiment. After 2 weeks of adaptation on total mixed ration (TMR; Table 1), lambs were weighed and distributed in individual cages into three experimental groups of six lambs per group as follows: a control group was fed on TMR without *S. babylonica* extract (SB0), an SB25 group was fed on TMR plus 25 mL/lamb/day of SB extract, and an SB50 group was fed on TMR plus 50 mL/lamb/day of SB extract. Lambs in the three groups were fed ad libitum a TMR that was formulated to meet the nutrient requirements of growing lambs (NRC 1985). Extract was orally administered daily before morning feeding to each lamb. Feed intake was recorded daily, and body weight was recorded at 20, 40, and 60 days of the experiment for average daily gain (ADG) calculation.

**Table 1** Ingredients and chemical composition of total mixed ration

| Total mixed ration | g/kg DM |
|---|---|
| Ingredients | |
| Soya bean meal | 220 |
| Alfalfa hay | 150 |
| Sorghum grain | 550 |
| Fish Meal | 35 |
| Mineral/vitamin premix[a] | 25 |
| Salt | 20 |
| Chemical composition | |
| Organic matter | 973.0 |
| Crude protein | 208.0 |
| Ether extract | 11.9 |
| Neutral detergent fiber | 364.2 |
| Acid detergent fiber | 66.0 |
| Acid detergent lignin | 41.4 |

Adapted from Salem et al. (2011)

[a] Mineral/vitamin premix (25) (vitamin A (12,000,000 IU), vitamin D3 (2,500,000 IU), vitamin E (15,000 IU), vitamin K (2.0 g), vitamin B1 (2.25 g), vitamin B2 (7.5 g), vitamin B6 (3.5 g), vitamin B12 (20 mg), pantothenic acid (12.5 g), folic acid (1.5 g), biotin (125 mg), niacin (45 g), Fe (50 g), Zn (50 g), Mn (110 g), Cu (12 g), I (0.30 g), Se (200 mg), and Co (0.20 g))

Extracts of SB contained in g/kg DM of leafs: 12.8 total phenolics, 4.8 saponins, 72.5 aqueous fraction of lectins, polypeptides, starch (Cowan 1999).

A weekly stock volume (2 L) of the extract was prepared for supplementation. Lambs were received the extract orally to ensure that the animals received its extract daily dose. Fresh water was always available.

### Preparation of extracts

*S. babylonica* was prepared weekly as described previously in Salem (2012). Briefly, leaves were collected randomly from several young and mature trees from several locations in the south of the Estado de México. Leaves were freshly chopped into 1- to 2-cm lengths and immediately extracted at 1 g of leaf per 8 mL of water. Plant materials were individually soaked and incubated in water in the laboratory at 25 to 30 °C for 48 to 72 h in closed jars of 20 L. After incubation, jars were heated at 30 °C for 1 h, and then immediately filtered and the filtrates were collected and stored at 4 °C for further use.

### In vitro experiment

#### *Treatments*

Four extract doses (i.e., 0, 0.6, 1.2, and 1.8 mL/g dry matter (DM) of diets fed to lambs) were administered in three

replicates for each treatment on the resultant in vitro fermentation kinetic profile of the substrate. The diet composition which was used to feed the rumen fluid donor lambs is presented in Table 1.

*In vitro incubations*

Rumen fluid was collected from the same four growing Katahdin × Pelibuey lambs of Jimenez-Peralta et al. (2011), with a live body weight of 24±0.3 kg that were fed on TMR ad libitum. Samples (1 g) of substrate were weighed into 120-mL serum bottles. Extract doses (i.e., 0, 0.6, 1.2, and 1.8 mL/g DM) were applied directly onto the substrate inside the bottles immediately before adding the buffer medium and rumen fluid.

Ruminal contents of each lamb were obtained immediately before the morning feeding, mixed, and strained through four layers of cheesecloth into a flask with an $O_2$-free headspace. Ten milliliters of particle-free ruminal fluid was added to each bottle, and 40 mL of the buffer solution (Goering and Van Soest 1970) with no trypticase was immediately added in a proportion of 1:4 (*v*/*v*).

A total of 36 bottles (three bottles for each extract dose in three different runs with three bottles as blanks (rumen fluid only)) were incubated for 72 h. Once all bottles were filled, they were immediately closed with rubber stoppers, shaken, and placed in the incubator at 39 °C. Volume of gas produced was recorded at incubation times of 2, 4, 6, 8, 10, 12, 24, 48, and 72 h after inoculation using the reading pressure technique (RPT; DELTA OHM, Italy; Theodorou et al. 1994). At the end of incubation (72 h), bottles were uncapped; pH was measured immediately with a pH meter (GLP 22, Crison Instruments, Barcelona, Spain). Contents of each bottle were then transferred to filtered fermentation residue for determination of apparent degraded substrate.

*Apparent degraded substrate*

At 72 h of incubation, the contents of each serum bottle were filtered through sintered glass crucibles under vacuum. Fermentation residues were dried at 65 °C overnight to estimate potential DM disappearance. Loss in weight after drying was the measure of nondegradable DM. DM degradability (DMD; mg/g DM) at 72 h of incubation was calculated as the difference between DM content of substrate and its nondegradable DM (Ørskov and McDonald 1979).

Chemical analyses and secondary metabolite assay

Samples of TMR were collected twice weekly along the experiment to calculate DM intake (DMI). Samples of TMR were ground to pass a 1-mm screen on a 4 Wiley Mill model and analyzed according to AOAC (1997) for dry matter (DM, #934.01), ash (#942.05), and ether extract (#920.39). The neutral detergent fiber (Van Soest et al. 1991), acid detergent fiber, and lignin (AOAC 1997; #973.18) analyses used an ANKOM$^{200}$ Fibre Analyzer unit (ANKOM Technology Corporation, Fairport, NY, USA). Neutral detergent fiber was assayed without use of an alpha amylase but with sodium sulfite in the neutral detergent fiber. Both neutral detergent fiber and acid detergent fiber are expressed without residual ash. Total N was determined with a N gas analyzer utilizing an induction furnace and thermal conductivity (LECO FP-528; AOAC 1997; method #990.03).

Secondary metabolites of SB extract were determined in triplicate according to the method descried in Salem (2012).

Calculations

Results of kinetic parameters of GP (mL/ g DM) were fitted using the NLIN option of SAS (2002) to the France et al. (2000) model as

$$A = b \times \left(1 - e^{-c(t-L)}\right)$$

where $A$ is the volume of GP at time $t$, $b$ is the asymptotic GP (mL/g DM), $c$ is the rate of GP(/h) from the slowly fermentable feed fraction $b$, and $L$ is the discrete lag time prior to GP.

Metabolizable energy (ME, MJ/kg DM) and in vitro organic matter digestibility (OMD, %) were estimated according to Menke et al. (1979) as

$$\mathrm{ME(MJ/kg\ DM)} = 2.20 + 0.136\ GP + 0.057\ \mathrm{CP}$$

$$\mathrm{OMD\ (\%)} = 14.88 + 0.889\ \mathrm{GP} + 0.45\ \mathrm{CP} + 0.0651\ \mathrm{XA}$$

where DM is dry matter, CP is crude protein in percent, XA is ash in percent, and GP is the net GP in milliliters from 200 mg of dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation ($PF_{24}$ is a measure of fermentation efficiency) was calculated as the ratio of in vitro DM digestibility (DMD, mg) to the volume of gas (mL) produced at 24 h (i.e., DMD/total gas production ($GP_{24}$); Blümmel et al. 1997).

Gas yields ($GY_{24}$) were calculated as the volume of gas produced after 24 h (mL gas/g DM) of incubation divided by the amount of DMD (g) as

$$\mathrm{Gas\ yields\ (GY_{24})} = \mathrm{Milliliter\ gas\ per\ gram\ DM/grams\ DMD}$$

Short-chain fatty acids (SCFA) were calculated (Getachew et al. 2002) as

$$\mathrm{SCFA\ (mmol/200\ mg\ DM)} = 0.0222\ \mathrm{GP} - 0.00425$$

where GP is the 24-h net GP (mL/200 mg DM).

Microbial biomass production (MP) was calculated (Blümmel et al. 1997) as

$$\mathrm{MP\ (mg/g\ DM)} = \mathrm{Milligrams\ DMD} - (\mathrm{Milliliter\ gas} \times 2.2\ \mathrm{mg/mL}),$$

where 2.2 mg/mL is a stoichiometric factor that expresses the milligrams of C, H, and O required for SCFA gas associated with the production of 1 mL of gas (Blümmel et al. 1997).

### Statistical analyses

The experimental design was completely randomized, where lambs were the experimental units. Measured parameters (ADG and feed intake) of this experiment were analyzed using the following statistical model:

$$y_{ijk} = \mu + d_i + a(d)_{j(i)} + \varepsilon_{ijk},$$

where $y_{ijk}$ is the value measured at period $k$ on the $j$th lamb assigned to the $i$th diet (extract dose), $\mu$ is the overall mean effect, $d_i$ is the $i$th fixed diet (extract) effect, $a$ (d)$_{j(i)}$ is the random effect of the $j$th lamb within the $i$th diet, and $\varepsilon_{ijk}$ is the random error associated with the $j$th lamb assigned to the $i$th diet.

The SB extract effects were determined as linear contrasts within SAS as defined by Steel and Torrie (1980). Data of in vitro ruminal GP and fermentation parameters were analyzed as a randomized design using the PROC MIXED procedure of SAS (2002). Data of each of the three runs within the same sample of the substrate were averaged prior to statistical analysis. Mean values of each individual run (three runs) were used as the experimental unit. The statistical model was

$$Y_{ij} = \mu + D_j + \varepsilon_{ij},$$

where $Y_{ij}$ represents every observation of the $i$th lamb diet when incubated in the $j$th extract doses, $D_j$ is the extract doses, and $\varepsilon_{ij}$ is the experimental error. Tukey's test was used for the multiple comparisons of mean values for each run, and linear effects were calculated at $P<0.05$.

## Results

The addition of SB extract improved the DMI (SB25 > SB50, $P=0.0457$) of lambs by about 60 and 33 % for SB25 and SB50, respectively (Table 1 and Fig. 1). The lower dose of SB (i.e., 25 mL/lamb/day) extract showed higher lamb ADG (g/day; SB25>SB50=SB0, $P<0.05$) during the experiment. The highest ($P=0.04$) ADG was found during the last 20 days of the experiment, while the lowest ($P=0.03$) was observed during the first 20 days. Relative to the control, the mean ADG during the experimental period showed that SB25 and SB50 achieved higher ADG values (176 and 144 %). Feed efficiency (g DMI/kg ADG) was not affected ($P=0.527$) by SB extract administration (Table 2).

The asymptotic GP ($b$, mL/g DM) increased ($P=0.001$) with the increasing dose of SB extract. SB extract levels did not affect the rate of GP ($c$, $P=0.117$) or the initial delay before GP begins ($L$, $P=0.66$). Linear increases for in vitro GP (mL/g DM) with advancing time with different SB extract doses were observed. Relative to the control, the addition of SB extract resulted in an increase ($P<0.05$) in gas production after 24, 48, and 72 h of incubation. GP was not affected during the first 12 h (i.e., $GP_6$ and $GP_{12}$) of incubation in all SB extract doses. The production rate started to increase after 24 h of incubation (i.e., $GP_{24}$, $GP_{48}$, and $GP_{72}$). Some fermentation parameters (pH, $PF_{24}$, and $GY_{24}$), were not affected by the addition of different SB concentrations, while others (DMD, OMD, ME, SCFA, and MP) increased ($P<0.05$) with the increasing SB extract doses (i.e., 0.6 and 1.2 mL/g DM). The highest dose of SB extract (1.8 mL/g DM) decreased all rumen fermentation parameters compared to the other doses (Table 3).

## Discussion

### Feed intake

The addition of SB extract increased the lambs' DMI in both low and moderate doses which may be related to improved rumen fermentation kinetics. The administration of low doses of SB extract encouraged some rumen bacterial species to metabolize phenolic compounds (Wachenheim et al. 1992;

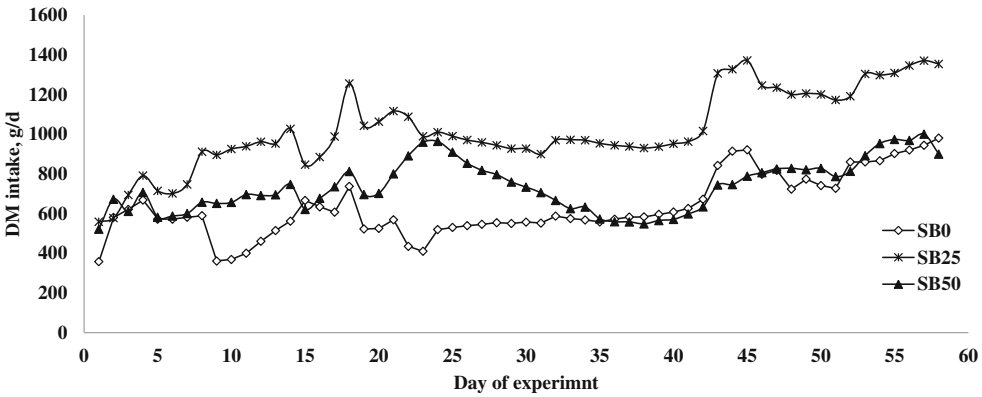


**Fig. 1** Daily dry matter intake (g/day) during the 60 days of experiment

**Table 2** DMI, ADG, and feed efficiency in growing lambs fed with the total mixed ration with different doses of *S. babylonica* extract

| | Doses of *S. babylonica* (mL/lamb/day) | | | SEM | *P* linear |
|---|---|---|---|---|---|
| | SB0 | SB25 | SB50 | | |
| DMI (g/day) | 634.1 c | 1,013.7 a | 844.4 b | 15.56 | 0.0457 |
| ADG (g/day) | | | | | |
| 20 days | 109.6 b | 227.0 a | 130.8 b | 12.61 | 0.0325 |
| 40 days | 119.5 c | 203.4 a | 154.6 b | 11.68 | 0.0424 |
| 60 days | 141.0 b | 222.0 a | 157.5 ab | 10.83 | 0.0473 |
| Grams of DMI per kilogram of ADG | 4.8 | 4.7 | 5.9 | 0.76 | 0.5274 |

Means in rows with different letters differ at $P<0.05$

*DMI* dry matter intake, *ADG* average daily gain, *SB S. babylonica*. $n=6$ lambs per group

Hart et al. 2008; Varel et al. 1991) and may have acted as catalysts for fiber degradation by increasing the access of fibrolitic bacteria to cell wall polysaccharides in the diet. This action will lead to the increase rates of disappearance in the rumen and of the passage, increasing feed intake as a result (Conrad 1966). However, the high SB extract dose (i.e., high secondary metabolite administration) with antimicrobial activity will decrease microbial activity and diet fermentability and digestibility which will negatively affect feed intake (Jimenez-Peralta et al. 2011; Salem et al. 2011). DMI is also related to the degradability of protein (Tomlinson et al. 1997). It is well known that some tree extracts reduce microbial protein degradability (Mueller-Harvey 2006). The observed low DMI with high SB doses apparently decreased microbial activity and diet fermentability and digestibility which apparently negatively affected feed intake (Jimenez-Peralta et al. 2011; Salem et al. 2011).

## Growth performance

The addition of SB25 increased the lambs' ADG compared with SB50 and control. Many reasons can explain this

**Table 3** In vitro gas production parameters, gas volume accumulated after different hours of incubation, and rumen fermentation profile of the total mixed ration with different doses of *S. babylonica* extract

| | Doses of *S. babylonica* (mL/g DM) | | | | SEM | *P* linear |
|---|---|---|---|---|---|---|
| | 0 | 0.6 | 1.2 | 1.8 | | |
| Gas production parameters[a] | | | | | | |
| *b* | 230.6 c | 305.5 b | 375.7 ab | 389.1 a | 11.83 | 0.0001 |
| *c* | 0.059 | 0.058 | 0.050 | 0.042 | 0.0036 | 0.1166 |
| *L* | 3.14 | 2.1 | 2.09 | 1.76 | 0.587 | 0.659 |
| In vitro gas production (mL/g DM) | | | | | | |
| $GP_6$ | 70.9 | 90.2 | 98 | 86.1 | 5.52 | 0.8169 |
| $GP_{12}$ | 119.4 | 153.7 | 170.4 | 153.2 | 8.62 | 0.65 |
| $GP_{24}$ | 175.6 d | 230.1 c | 263.4 a | 246.0 b | 11.07 | 0.0422 |
| $GP_{48}$ | 216.1 e | 286.8 c | 342.1 a | 336.4 a | 11.32 | 0.0015 |
| $GP_{72}$ | 226.4 f | 300.9 d | 365.6 a | 369.7 a | 11.04 | 0.0001 |
| Rumen fermentation profile[b] | | | | | | |
| pH | 6.28 | 6.26 | 6.27 | 6.07 | 0.071 | 0.5086 |
| DMD | 991 e | 1,212 c | 1,348 a | 1,277 b | 45 | 0.0322 |
| OMD | 556 e | 653 c | 713 a | 682 b | 19.7 | 0.1722 |
| ME | 8. 2 d | 9.6 c | 10.6 a | 10.1 b | 0.3 | 0.0421 |
| $PF_{24}$ | 5.82 | 5.27 | 5.12 | 5.19 | 0.125 | 0.3199 |
| $GY_{24}$ | 173.4 | 189.6 | 195.4 | 192.6 | 3.58 | 0.3009 |
| SCFA | 3.88 e | 5.09 c | 5.83 a | 5.44 b | 0.246 | 0.0432 |
| MP | 604.4 e | 706.2 c | 768.6 a | 736.1 b | 20.7 | 0.0442 |

Different letters following means in the same row indicate differences at $P<0.05$

*pH* ruminal pH, *DMD* dry matter degradability (mg/g DM), *SCFA* short-chain fatty acids (mmol/g DM), $GY_{24}$ gas yield at 24 h (mL gas/g DMD), $PF_{24}$ partitioning factor at 24 h of incubation (mg DMD/mL gas), *OMD* in vitro organic matter digestibility (g/kg DM), *ME* metabolizable energy (MJ/kg DM), *MP* microbial protein production (mg/g DM)

[a] Adapted from Jimenez-Peralta et al. (2011)

[b] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (per h); *L* is the initial delay before gas production begins (h)

response. The addition of SB extract improved ruminal fermentation kinetics through the reduction of methane proportion of gas produced during fermentation, leading to more energy available for growth, increasing the short-chain fatty acid and ME density of the diet (Jimenez-Peralta et al. 2011). Preventing protein microbial degradation, increasing amino acid flow to the duodenum, and subsequently increasing absorption of amino acids also are promoted by secondary metabolites (Mueller-Harvey 2006) of SB extract.

In vitro ruminal gas production

The addition of SB extract expected to be beneficial to rumen function on the basis of their stimulating effect on fermentation, increasing degradability of CP and cell wall constituents as well as increasing MP. Ruminal GP and rumen fermentation activities were increased with low and moderate doses of SB extract (i.e., 0.6 and 1.2 mL/g DM), but the highest dose (1.8 mL/g DM) decreased them. These low doses of secondary metabolites can increase CP degradability and cell wall constituents as well as increase MP production. The increase in MP production may possibly have been due to improved synchronization between energy and N release in the rumen as a consequence of some chemical constituents of the plant extracts. Some of these phenolic compounds may interact with biosynthesis of aromatic amino acids, as both biosynthesis pathways are linked through cinnamic acid. Increasing the concentrations of SB extract to the levels that rumen microorganisms can tolerate (i.e., up to 1.2 mL/g DM) will cause a reduction on methane production proportion of the produced gas during fermentation, resulting in more energy available for increasing SCFA, ME, and OMD of the diet (Table 3).

## Conclusion

Daily administration of *S. babylonica* extract increased dry matter intake with a concomitant improvement of young lambs daily gain, in addition to improved ruminal fermentation kinetic and in vitro GP with doses up to 1.2 mL/g DM of diet than higher doses (1.8 mL/g DM of diet). The addition of *S. babylonica* extract with the rate of 25 mL/lamb/day is beneficial to young lambs and thus can be safely used as a feed additive in diets without any negative effects on animals.

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