

## Essential Oils in the Diet of Crossbred ( $\frac{1}{2}$ Angus vs. $\frac{1}{2}$ Nellore) Bulls Finished in Feedlot on Animal Performance, Feed Efficiency and Carcass Characteristics

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### Abstract

This experiment was carried out to evaluate the animal performance, feed efficiency and carcass characteristics of 27 crossbred bulls ( $\frac{1}{2}$  Angus vs.  $\frac{1}{2}$  Nellore), finished in feedlot for 120 days, with the addition of distinct levels of essential oils in the diets. The experiment was conducted in a completely randomized trial and animals were distributed into three treatments: control (CON), addition of 500 mg/kg of DM/animal/day of a mix of essential oils (E500), and addition of 1000 mg/kg of DM/animal/day of a mix of essential oils (E1000). The essential oils supplemented to the diets were based on a combination of vegetal extracts from: oregano, garlic, lemon, rosemary, thymus, eucalyptus and sweet orange. The bulls had an average age of  $12 \pm 2$  months and weight of  $243.4 \pm 6.8$  kg when the trial started. The roughage and concentrate ratio was 10% and 90%, respectively. The inclusion of essential oils in the diets did not affect final body weight, average daily gain, and feed intake, but there was a tendency for improved feed efficiency for the E500 treatment. The ADG ranged from 1.55 to 1.70. Average DMI and feed conversion were 7.07 kg/day and 4.31, respectively. Essential oil levels did not affect the carcass characteristics. More studies are needed to elucidate the synergism of combinations of essential oils in the rumen.

**Keywords:** cattle, meat quality, natural additives, probiotics, plant extract

### 1. Introduction

Brazilian beef production is characterized by the use of grazing systems (90%), zebu animals and their crosses (80%), increased slaughter age (48 months) and low weight gain throughout life (0.60 kg/day) (Ferraz & Felício, 2010). The use of feedlot, which is based on diets with high levels of grains and cereals, is a tool to improve production efficiency (*e.g.* reduced slaughter age and increased weight gain) and meat quality (Prado et al., 2008). However, feeding management malpractices for ruminants, such as intake of substations amounts of grains, can result in a drastic drop of rumen pH, usually ensuing a metabolic disorder known as acute acidosis (Owens et al., 1998). Thus, ionophores can be added into the diets of cattle to modulate rumen fermentation and buffer the rumen, and the outcome can be improved performance and feed efficiency (Russell & Strobel, 1989). However, the use of antibiotics is facing social criticism due to presence of residues in meat, and the proliferation and transmission of resistant bacteria via the food chain (OJEU, 2003; Russell & Houlihan, 2003). Hence, new alternatives to be used as rumen modulators are in need to cope with the increasing demand for production and safety of livestock products.

Natural products, such as plant extracts and essential oils, are receiving increased attention to be used to improve livestock production. Essential oils are complex mixtures of secondary plant metabolites that can be obtained from plant parts (flowers, buds, seeds, leaves, twigs, barks, fruits, and roots) and they are associated with plant characteristic essences and fragrances (Burt, 2004). Essential oils have long been used in the pharmaceutical and

cosmetic industry (Greathead, 2003). These products have some advantages over commonly used antibiotics, since they can be residue-free and are mostly safe for the food industry (Benchaar et al., 2008).

Active compounds found in essential oils, such as anethol, capsaicin, cardanol, cardol, cinnamaldehyde, eugenol and thymol, are in high concentrations in plant extracts and they can affect the cytoplasmic membrane permeability of microbes (Dorman & Deans, 2000). Moreover, these compounds impact ruminant production by affecting protein and lipid metabolism in the rumen, stimulating of feed digestion, reduction of hyper-producing ammonia bacteria, affecting bacterial colonization of substrates in the rumen, and due to antimicrobial, anti-inflammatory and antioxidant properties (Benchaar et al., 2006; Benchaar et al., 2008; Calsamiglia et al., 2007; Chaves et al., 2008; Cruz et al., 2014; Fugita et al., 2017; Hart et al., 2008; Valero et al., 2016). For example, feeding cinnamaldehyde or essential oils from juniper berry (200 mg/kg of DM) improved average daily gain of lambs (Chaves et al., 2008). Furthermore, synergistic effects between active compounds in essential oils were reported (Valero et al., 2016). Bulls fed 550 mg/kg of DM of essential oils from castor (main active compounds: ricinoleic acid) and cashew nut shell liquid (main active compounds: anacardic acid, cardanol, and cardol) had increased body weight and lower dry matter conversion compared to a control diet (Valero et al., 2016). Nonetheless, there are few studies exploring the synergism of essential oils in beef cattle performance and carcass characteristics. Our hypothesis was that the combination of active compounds in essential oils from various plants would synergize and improve beef cattle production and carcass characteristics. Thus, this study was performed to evaluate the effect of a mix of natural dietary essential oils on animal performance, feed efficiency and carcass characteristics of crossbred young bulls finished in feedlot and fed a high-grain diet.

## 2. Material and Methods

### 2.1 Local, Animals, Diets

This experiment was approved by the São Paulo State University “Julio de Mesquita Filho” (UNESP). This study was conducted at the Rosa & Pedro Sector of the Experimental Station at Iguatemi Farm, Maringá, Paraná, south of Brazil.

Twenty-seven 12±2 months-old crossbred young bulls (½ Angus vs. ½ Nellore) and their half-brothers, with an average weight of 243.5±6.8 kg when the trial started, were randomly assigned to one of three finishing diets (n = 9 per treatment).

The basal diet consisted of 90% concentrate and 10% pelleted sugarcane bagasse. The diets were similar for all animals (Table 1), and were formulated according to the NRC (2000) recommendations for an average daily weight gain of 1.50 kg. The three experimental diets were as follows: diet without the addition of the essential oil mix, or control diet (CON); diet with 500 mg/kg of DM of an essential oil mix (E500); diet with 1000 mg/kg of DM of an essential oil mix (E1000). The oil mix (MixOil®) was manufactured by Animal Wellness Products (A.W.P.™ Oakland-Nebraska-USA), and was incorporated directly into the concentrate. Components of the mix consisted of seven plant extracts: oregano (*Origanum vulgare*), garlic (*Allium sativum*), lemon (*Citrus limonum*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), eucalyptus (*Eucalyptus saligna*) and sweet orange (*Citrus aurantium*). Essential oils from oregano is enriched in carvacrol, garlic in allicin, lemon in limonene, rosemary in pinene, thyme in thymol, eucalyptus in citronellal and citronellol, and sweet orange in limonene.

Table 1. Ingredient and chemical composition and percentage of diets (% DM)

Ingredients	DM <sup>1</sup>	% DM									Diet, %
		CP <sup>2</sup>	OM <sup>3</sup>	Ash	EE <sup>4</sup>	NDF <sup>5</sup>	ADF <sup>6</sup>	TCH <sup>7</sup>	NFC <sup>8</sup>	TDN <sup>9</sup>	
Sugar cane bagasse	94.70	1.83	98.02	1.97	3.60	78.74	49.20	89.37	13.86	42.89	10.00
Cracked corn	88.93	8.99	99.10	0.95	3.50	17.70	4.40	86.60	68.90	90.00	81.95
Soybean meal	88.60	49.00	93.70	6.26	1.30	13.70	5.97	43.50	29.80	84.00	6.51
Limestone	99.30		10.71	89.29							0.46
Yeast	98.00	30.00									0.05
Mineral mixture <sup>10</sup>	99.30		10.70	89.30							0.41
Urea	98.00	260.00	0.56	99.44							0.62
Diet	88.13	12.50	97.34	2.66	2.20	30.30	14.80	84.50	54.20	70.30	100.00

Note. <sup>1</sup>Dry matter, <sup>2</sup>Crude protein, <sup>3</sup>Organic matter, <sup>4</sup>Ether extract, <sup>5</sup>Neutral detergent fiber, <sup>6</sup>Acid detergent fiber, <sup>7</sup>Total carbohydrates, <sup>8</sup>Non-fiber carbohydrates, <sup>9</sup>Total digestible nutrients, <sup>10</sup>By kg: calcium-175 g; phosphorus-100 g; sodium-114 g; selenium-6,004 mg; magnesium-1,250 g; fluoride-1,000 mg.

## 2.2 Nutrients and Diet Analyses

Analytical DM of feed ingredients was determined by drying at 135 °C for 3 h (Table 1), according to the method 930.15 (AOAC, 2005). Nitrogen content in the samples was determined by the method 976.05 (AOAC, 2005). Ash was determined through combustion at 550 °C for 5 h by the method 936 (AOAC, 2005). The OM content was calculated as the difference between DM and ash contents. Ether extract content was determined by the method 920.39 (AOAC, 2005). The NDF content was determined according to Mertens (2002) using  $\alpha$ -amylase. The ADF was determined using the method 973.18 (AOAC, 2005). The total carbohydrates were determined according to Sniffen et al. (1992) using the equation:  $100 - (\% \text{ CP} + \% \text{ EE} + \% \text{ ash})$ . The non-fiber carbohydrate content was determined using the equation:  $100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ EE} + \% \text{ ashes})$ . The total digestible nutrient (TDN) content of diets was estimated using the methodology described by Kears (1982).

## 2.3 Slaughter and Carcass Measurements

Young bulls were finished on their respective diets for 120 days until they reached commercial weights ( $440.3 \pm 10.0$  kg). Bulls were slaughtered at a commercial abattoir 60 km from the feedlot after a fast of solids (14 hours), according Brazilian slaughter practices. The bulls were stunned using a captive-bolt pistol. Animals were bled through exsanguination by cutting the neck vessels, and the head, hide, viscera, tail, legs, diaphragm and excess internal fat was removed. The carcasses were divided medially from the sternum and spine, resulting in two similar halves, which were weighed to calculate the hot carcass weight (HCW). Next, the half-carcasses were identified and stored in a chilling chamber at 4 °C for 24-h period.

The following carcass traits were obtained: HCW was determined after the slaughter and before the carcass was chilled; the percentage of the individual animal dressing was defined as the HCW divided by the live weight 14 hours before slaughter; cold carcass weight was determined after chilling the carcass at 4 °C for 24-h period; cold carcass dressing was defined as the cold carcass weight divided by the live weight 14 hours before slaughter; carcass length was determined by measuring the carcass from the border of the pubis bone until the anterior side of the first rib taken with a ribbon or a tape line; leg length was evaluated using a wooden compass with metallic edges that measures the distance from the anterior border of the pubis bone to a middle point on the tarsus bone; cushion thickness was determined using a wooden compass with metallic edges that measures the distance between the lateral face to the median at the superior part of the cushion (*Biceps femoris*); fat thickness was determined with a caliper averaging three points between the 12<sup>th</sup> and the 13<sup>th</sup> ribs on the *Longissimus* muscle (LM); LM area was measured using a compensating planimeter on a transversal cut between the 12<sup>th</sup> and 13<sup>th</sup> ribs; carcass muscle, fat and bone were physically separated from the *Longissimus* section, which corresponds to the 6<sup>th</sup> rib, and individually weighed according to Robelin and Geay (1975).

## 2.4 Statistical Analysis

The experimental design was completely randomized, with three treatments and nine replications. All studied characteristics were tested for normality (Shapiro-Wilk test). Characteristics that presented normal distribution were analyzed using an analysis of variance using the procedure PROC MIXED of SAS (2004) statistical package (Statistical Analysis System, version 8.1). The model statement contained the fixed effect of the diet. Data were analyzed using bull as random variable. Results are reported as least square means and discriminated using PDIF. Differences between group means were assessed by using the Tukey Test ( $P < 0.05$ ).

# 3. Results and Discussion

## 3.1 Animal Performance and Feed Efficiency

There is limited information on the effects of the supplementation of a mix of essential oils for beef cattle finished in feedlot on feed intake, animal performance and carcass characteristics. Additionally, the results from the existing studies are conflicting. In this study, the addition of 500 or 1000 mg/kg of DM of a mix of essential oils from oregano, garlic, lemon, rosemary, thyme, eucalyptus and sweet orange (main active compounds: carvacrol, allicin, limonene, pinene, thymol, citronellal and citronellol) in the diet of crossbred bulls did not affect ( $P > 0.05$ ) the final body weight (FBW) and average daily gain (ADG) of bulls finished in feedlot (Table 2). Benchaar et al. (2006) fed crossbred steers and heifers (Angus vs. Hereford) with distinct levels (~250 or 500 mg/kg of DM) of a mix of essential oils (clove, vanillin, lemon and thymus), and no differences in FBW and ADG were observed. Likewise, no differences on FBW and ADG were observed when young steers received 80 mg/kg of DM of a mix of thymol, eugenol, vanillin, guaiacol and limonene (Meyer et al., 2009). On the other hand, Valero et al. (2016) observed improved ADG (1.55 vs. 1.26 kg/d) when bulls finished in feedlot were supplemented with 550 mg/kg of DM of cashew and castor oil (main active compounds: ricinoleic acid, anacardic, cardol and cardanol). Also, lambs fed 200 mg/kg of DM of cinnamaldehyde or juniper berry essential

oil (main active compound:  $\alpha$ -pinene) had improved ADG (Chaves et al., 2008). In a trial using supplementation of increasing doses of cinnamaldehyde as single active compound (400, 800, and 1600 mg/animal/d), Yang et al. (2009) observed no differences in FBW and ADG for steers finished in feedlot. Thus, other variables than the quantities of the essential oil supplemented also impact responses of FBW and ADG on animal studies. For example, various active compounds such as carvacrol, allicin, thymol, limonene and pinene, have a broad antibacterial activity, with potential to affect Gram-positive and Gram-negative bacteria (Benchaar & Greathead, 2011). Thus, essential oils can modulate the rumen microbiota, consequently affecting the production volatile organic compounds and protein degradation (Calsamiglia et al., 2007). Also, essential oils can result in decreased total volatile fatty acid production (Cardozo et al., 2005).

The average FBW and ADG was  $440.3 \pm 10.0$  and  $1.64 \pm 0.04$  kg, respectively. In general, crossbred young bulls (*Bos taurus* vs. *Bos indicus*) finished in feedlot are slaughtered with 470 to 500 kg of body weight in Brazil (Ito et al., 2012) and with an ADG between 1.4 and 1.8 kg (Rotta et al., 2009). The high ADG observed in this study (average of 1.64 kg) might be explained due to the use of young bulls slaughtered at 16 months-old. Young animals have hormones that promotes increased growth compared to old animals (Henricks, 1991). Also, young bulls have high levels of testosterone compared to steers, which maintain and promote male secondary sexual characteristics and muscle development (Prado et al., 2009; Marti et al., 2017). Moreover, crossbred cattle (e.g. *Bos taurus* vs. *Bos indicus*) have high ADG due to heterosis expression in the first generation (Ito et al., 2012; Prado et al., 2009; Rotta et al., 2009).

The addition of essential oils in the diets did not alter ( $P > 0.05$ ) dry matter intake (DMI). The DMI was 7.1 kg/day and DMI ratio to body weight was 2.1%. Low DMI ratio to BW can be explained by a diet with high concentrate (90%), which have a greater energetic density compared to roughages. The effect of essential oils and DMI is also conflicting. Benchaar et al. (2006) observed similar DMI on a growth-performance trial, but observed increased DMI on a digestibility trial, both using beef cattle fed similar diets with supplementation of essential oils (~250 or 500 mg/kg of DM) from clove, vanillin, lemon and thymus. Yang et al. (2009) observed a positive short-term effect on DMI (from day 1 to 28) when crossbred steers finished in feedlot (Hereford vs. Angus) were fed increasing doses of cinnamaldehyde (400, 800 and 1600 mg/d). On the other hand, feeding ~500 mg/kg of DM of castor bean and cashew essential oils had no effect on DMI of crossbred bulls (Angus vs. Nellore) finished in feedlot (Fugita et al., 2017). Also, Chaves et al. (2008) did not observed effect on DMI when lambs were fed 200 mg/kg of DM of cinnamaldehyde or juniper berry essential oil. Furthermore, Cardozo et al. (2006) supplemented Holstein heifers with cinnamaldehyde (25 mg/kg of DM) combined to eugenol (12 mg/kg of DM) and observed decreased in DMI. Some essential oils or active compounds in plant extracts can improve, be neutral, or decrease palatability from diets (Calsamiglia et al., 2007; Yang et al., 2009). Thus, palatability or encapsulation of the essential oil need to be considered prior to supplementation.

Table 2. Animal performance, feed intake and feed efficiency of crossbred young bulls ( $\frac{1}{2}$  Angus vs.  $\frac{1}{2}$  Nellore) finished in feedlot supplemented with a mix of essential oils

Parameters	Diets			Mean	SEM <sup>4</sup>	P < F
	CON <sup>1</sup>	E500 <sup>2</sup>	E1000 <sup>3</sup>			
Initial body weight, kg	244.4	245.2	240.9	243.5	6.79	0.94
Final body weight, kg	444.8	431.2	444.9	440.3	10.03	0.82
Average daily gain, kg	1.67	1.55	1.70	1.64	0.04	0.37
Dry matter intake, kg/d	7.08	7.08	7.05	7.07	0.21	0.99
Dry matter intake, %/BW	2.05	2.09	2.06	2.07	0.04	0.95
Dry matter conversion	4.24	4.56	4.14	4.31	0.08	0.08
Crude protein intake, kg/d	0.88	0.88	0.86	0.87	0.02	0.99
Neutral detergent intake, kg/d	1.49	1.48	1.48	1.48	0.04	0.99
Neutral detergent intake, %/BW	0.43	0.44	0.43	0.43	0.08	0.96

Note. <sup>1</sup>CON: basal diet without essential oils, <sup>2</sup>E500: basal diet with addition of 500 mg/kg of DM/animal/day of a mix of essential oils, <sup>3</sup>E1000: basal diet with addition of 1000 mg/kg of DM/animal/day of a mix of essential oils, <sup>4</sup>Standard mean error.

There was a tendency ( $P < 0.08$ ) for improved feed efficiency with addition of 500 mg/kg of DM of a mix of essential oils in the diet when compared to a basal diet (Table 2). Benchaar et al. (2006) observed improved feed

efficiency for beef cattle supplemented with the inclusion of ~250 mg/kg of DM of essential oils. Likewise, Valero et al. (2016) observed improved feed efficiency for bulls finished in feedlot and supplemented with 550 mg/kg of DM of essential oils (cashew and castor). On the other hand, Fugita et al. (2017) did not observe difference on feed efficiency with the addition of ~500 mg/kg of DM of cashew and castor oil in the diet of bulls finished in feedlot. Essential oils can affect methanogens and decrease methane production, thus improving energy efficiency in the rumen (Benchaar & Greathead, 2011). Also, some active compounds in essential oils, such as carvacrol, can increase the production of propionate in the rumen, which is a precursor of glucose in ruminants (Chaves et al., 2008).

The NDF intake ratio to body weight was low (0.43%). Thus, there was no DMI limitation due to physical rumen fill. Mertens (1994) observed that NDF intake of 1.2% of BW or lower would not be a limiting factor for the intake of nutrients. The feed conversion was approximately 4.3 kg of DM for 1 kg of BW gain and may be attributed to the genetic quality of animals ( $\frac{1}{2}$  Angus vs.  $\frac{1}{2}$  Angus), their sexual condition (bulls) and the high-energy density of the diet (70% TDN). Usually, crossbred bulls fed on a high-energy density diets have decreased DMI and feed conversion of 5-6 kg of DM intake for 1 kg of BW gain (Valero et al., 2016; Fugita et al., 2017), resulting in improved feed efficiency (NRC, 2000).

### 3.2 Carcass Characteristics

There is limited information on the effect of essential oils on beef cattle carcass traits. The addition of essential oils in the diets of crossbred bulls finished in feedlot did not affect ( $P > 0.05$ ) carcass weight (hot and cold), carcass dressing (hot and cold), carcass length, leg length or cushion thickness (Table 3). Mean carcass weight, carcass dressing, carcass length, leg length and cushion thickness were 243 kg, 55%, 124 cm, 79 cm and 25.0 cm, respectively. Thus, carcass weight, dressing and conformation and cushion thickness were close to the rates reported for crossbred *Bos taurus taurus* vs. *Bos taurus indicus* finished in feedlot and fed on a high-energy density diet (Prado et al., 2008; Rotta et al., 2009).

Table 3. Carcass characteristics of crossbred young bulls ( $\frac{1}{2}$  Angus vs.  $\frac{1}{2}$  Nellore) finished in feedlot supplemented with a mix of essential oils

Parameters	Diets			Mean	SEM <sup>4</sup>	P < F
	CON <sup>1</sup>	E500 <sup>2</sup>	E1000 <sup>3</sup>			
Hot carcass weight, kg	243.16	239.76	245.68	242.86	6.35	0.93
Hot carcass dressing, %	54.60	55.48	55.14	55.07	0.34	0.58
Cold carcass weight, kg	238.40	235.06	240.86	238.11	6.22	0.93
Cold carcass dressing, %	53.53	54.40	54.06	54.00	0.33	0.58
Carcass length, cm	123.16	124.27	123.66	123.70	0.78	0.85
Leg length, cm	79.94	78.88	78.72	79.18	0.97	0.86
Cushion thickness, cm	24.72	25.00	25.44	25.05	0.39	0.76
Fat thickness, mm	6.66	6.98	6.39	6.68	0.40	0.84
Muscle area, cm <sup>2</sup>	71.11	71.66	74.22	72.33	1.77	0.76
Ratio <sup>5</sup> , cm <sup>2</sup>	0.51	0.52	0.54	0.52	0.01	0.54
Muscle, %	59.89	58.89	62.18	60.32	0.69	0.14
Fat, %	18.08	17.98	16.38	17.48	0.80	0.64
Bone, %	15.10	16.04	15.67	15.60	0.52	0.77

Note. <sup>1</sup>CON: basal diet without essential oils, <sup>2</sup>E500: basal diet with addition of 500 mg/kg of DM/animal/day of a mix of essential oils, <sup>3</sup>E1000: basal diet with addition of 1000 mg/kg of DM/animal/day of a mix of essential oils, <sup>4</sup>Standard mean error, <sup>5</sup>Ratio between muscle length and muscle width.

Fat thickness, muscle area and ratio were similar ( $P > 0.05$ ) between diets (Table 3). Mean fat thickness was 6.7 mm, muscle area was 72.3 cm<sup>2</sup> and the ratio was 0.52 cm<sup>2</sup>, respectively. The fat thickness was high for the requirements of the Brazilian market. In general, fat thickness ranges between 3 to 6 mm (Rotta et al., 2009). The muscle area ranges from 68 to 75 cm<sup>2</sup> and ratio from 0.50 to 56 cm<sup>2</sup>. Despite having FBW lower than 470 kg, carcasses presented good fat thickness and conformation.

Essential oil addition in the diets had no effect ( $P > 0.05$ ) on muscle, fat and bone percentage in the carcass of bulls finished in feedlot (Table 3). The mean percentage of muscle on LM was 60.3%, fat 17.5% and bone

15.6%. Similarly, some authors reported muscle percentages between 58 and 62%, fat between 15 and 20% and bone between 15 and 18% on LM of crossbred bulls (Ito et al., 2012; Prado et al., 2008; Rotta et al., 2009).

In general, the addition of essential oils in the diet of cattle finished in feedlot and fed on a diet with high energy density has no effect in carcass traits (Benchaar et al., 2006; Cruz et al., 2014; Fugita et al., 2017). This is consistent with other studies using beef cattle and lambs (Chaves et al., 2008; Cruz et al., 2014; Yang et al., 2009). Lack of effects of essential oils on carcass traits is in agreement with the absence of the effects of essential oils on ADG and feed efficiency in the present study. Nonetheless, essential oils fed to beef cattle can result in meat with increased shelf life and antioxidants concentration (Monteschio et al., 2017; Rivaroli et al., 2016). Moreover, acceptability of meat from young bulls was improved with the inclusion of a mix of essential oils in the diet (Guerrero et al., 2017).

#### 4. Conclusion

The addition of a mix of essential oils (500 or 1000 mg/kg of DM – oregano (*Origanum vulgare*), garlic (*Allium sativum*), lemon (*Citrus limonium*), rosemary (*Rosmarinus officinalis*), thymol (*Thymus vulgaris*), eucalyptus (*Eucalyptus saligna*) and sweet orange (*Citrus aurantium*) in the diets of crossbred bulls finished in feedlot fed high-concentrate diet did not affect animal performance and carcass characteristics, but there was a tendency for improved feed efficiency when 500 mg/ kg of DM of the mix was added. More studies are needed to elucidate the synergism of mixtures of essential oils in the rumen, and how it affects the rumen microbiome.

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