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Estimation of fecal production, digesta flow and digestibility in dairy heifers with different indicator

Diego Azevedo Mota[1*], Telma Teresinha Berchielli[2], Roberta Carrilho Canesin[3], Ana Paula de Oliveira Sader[4], Juliana Duarte Mesana[3] and Marcos Vinicius Biehl[5]

*[1]Colegiado de Zootecnia, Universidade Federal do Oeste do Pará, Av. Marechal Rondon, s/n, 68135-110, Bairro Caranazal, Santarém, Pará, Brazil. [2]Departamento de Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. [3]Programa de Pós-graduação em Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. [4]Laboratório de Nutrição Animal, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. [5]Departamento de Zootecnia, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo, Brazil. *Author for correspondence. E-mail: diegomota@zootecnista.com.br*

ABSTRACT. This study evaluated fecal excretion by means of the internal indicator indigestible neutral detergent fiber (iNDF) and external indicators such as chromium complexed with ethylenediaminetetraacetic acid (Cr-EDTA) and ytterbium chloride ($YbCl_3$), in addition to estimating the duodenal flow of dry matter and the total, ruminal and post-ruminal apparent digestibility coefficients. Eight crossbred Holstein x Zebu heifers were distributed into a 4 x 4 Latin square. Indicators Cr-EDTA, $YbCl_3$ and iNDF did not efficiently estimate the fecal production ($p < 0.05$), with results of 1.64, 1.71 and 2.71 kg day^{-1}, respectively, compared with the total feces collection, which resulted in 1.39 kg day^{-1}. The estimated values of dry matter flow, both for the methodologies of single and double indicator, can be considered biologically acceptable. However, the value obtained by the Cr-EDTA/$YbCl_3$ association, used as a double indicator, was the most reliable, due to better recovery of the external indicators (Cr-EDTA and $YbCl_3$), which have had averages of 89 and 85%, respectively, in comparison with the internal one (iNDF), which averaged 67%. The ruminal and post-ruminal digestibility coefficients, estimated by the Cr-EDTA/$YbCl_3$ association, were considered the best indicators owing the dry matter flow estimated by this association.

Keywords: Cr-EDTA, indigestible fiber, sugarcane, ytterbium chloride.

Estimativa de produção fecal, fluxo de digesta e digestibilidade em novilhas leiteiras com utilização de diferentes indicadores

RESUMO. Objetivou-se com este trabalho avaliar a produção fecal por meio do indicador interno, fibra em detergente neutro indigestível (FDNi), e externos, cromo complexado com ácido etilenodiaminotetracético (Cr-EDTA) e o cloreto de itérbio ($YbCl_3$), além de estimar o fluxo duodenal da matéria seca e os coeficientes de digestibilidades aparentes total, ruminal e pós-ruminal, de diferentes nutrientes. Foram utilizadas oito novilhas mestiças Holandês/Zebu, distribuídas em duplo quadrado latino 4 x 4. Os indicadores Cr-EDTA, $YbCl_3$ e o FDNi não estimaram produção fecal de forma eficiente ($p < 0,05$), obtendo resultado de 1,64; 1,71 e 2,71 kg dia^{-1}, respectivamente, quando comparado à coleta total de fezes, que obteve resultado de 1,39 kg dia^{-1}. Os valores estimados de fluxo de matéria seca, tanto para a metodologia de único, quanto para de duplo indicador, podem ser considerados biologicamente aceitáveis. Contudo, o valor obtido pela associação Cr-EDTA/$YbCl_3$, utilizada na forma de duplo indicador, foi o mais confiável, pela melhor recuperação dos indicadores externos (Cr-EDTA e $YbCl_3$), que obtiveram médias de 89 e 85%, respectivamente, em comparação ao interno (FDNi), que obteve média 67%. Os coeficientes de digestibilidade ruminal e pós ruminal, estimados pela associação Cr-EDTA/$YbCl_3$, foram considerados melhores, em consequência do valor de fluxo de matéria seca estimado por esta associação.

Palavras-chave: Cr-EDTA, fibra indigestível, cana-de-açúcar, cloreto de itérbio.

Introduction

The knowledge of the chemical composition and the obtainment of digestibility values estimates are admittedly essential to determine the nutritional value of feeds. However, strict control of intake and/or fecal production is not always possible (BERCHIELLI et al., 2005a and b). In light of this

need, a wide number of substances denominated indicators have been used as experimental tool for the study of the digestible function of ruminants (MAEDA et al., 2011). According to Detmann et al. (2004) the use of indicators is characterized by a basic parameter of indigestibility of the diet consumed, and after, of indirect methods for the digestibility of the diet, avoiding the total collection of feces.

This concept is also applied when the objective is to determine the partial digestibility based on the digesta flow. However, at these estimations, when utilizing only one indicator, it is usually assumed that the digesta flow is independent of its phases, and one sample is representative. However, to avoid non-representative sampling of the digesta, the infusion of the indicator can and must be separated into different phases and, then, mathematically reassembled (AHVENJÄRVI et al., 2003).

According to France and Siddons (1986), the digesta presents a liquid phase (liquid and small particles) and a solid phase (medium and large particles), which enables the use of two different indicators considered ideal for each phase. For the liquid phase, Cr-EDTA has been widely employed (NOGUEIRA FILHO et al., 2001). In the solid phase, indicators such as indigestible fibers, e.g., iNDF are employed (DIAS et al., 2007; MAEDA
et al., 2011). As a single indicator, iNDF or $YbCl_3$ are recommended (MAEDA et al., 2011), although further research is necessary on the choice of indicators and the establishment of one or more indicators in the study of the duodenal flow estimate for the obtaining accurate results for the ruminal and post-ruminal digestibility coefficients.

This study evaluated the chromium complexed with ethylenediaminetetraacetic acid (Cr-EDTA), ytterbium chloride ($YbCl_3$) and indigestible neutral detergent fiber (iNDF) in the estimation of fecal production; and analyzed the duodenal flow of dry matter, through the methods of single and double indicators and total, ruminal and post-ruminal apparent digestibility coefficients of different nutrients in dairy heifers in the post-weaning period.

Material and methods

Eight Holstein x Zebu heifers with average body weight (BW) of 202.1 ± 11.5 kg and at 18 months of age fistulated in the rumen and duodenum were utilized. Heifers were housed in individual 21 mm pens during the adaptation period and in cages for metabolism assays with trays adapted to total feces collection during the experimental period. Pens and cages had individual drinkers and feeders.

Experimental diets had the same amount of protein and were supplied at a forage:concentrate ratio of 60:40 on a dry matter basis. The forage supplied exclusively was sugarcane, harvested manually at every two days and chopped daily to be supplied to animals. Chopping was performed with the aid of stationary chopper adjusted so that the particle size did not exceed 2.0 cm. Concentrate was composed of ground corn grain, mineral supplement, urea and different protein sources (soybean, cottonseed, peanut and sunflower meals). The percentage of ingredients and chemical composition of experimental diets are presented in Table 1.

In the morning feeding (7:00 a.m) animals received all the forage and approximately 50% of the total concentrate, whereas in the afternoon feeding (3:30 p.m), the rest of the concentrate was supplied and mixed with the feed present in the feeders. In the period of adaptation of animals to the diet, which lasted nine days, the adjustment of intake was done, so that leftovers would not exceed 10% of the total feed supplied. In the digestibility and nutrient flow assay, leftovers were measured daily for determination of dry matter intake.

Table 1. Percentage of ingredients and chemical composition of experimental diets.

Ingredients	Diets[1] (%DM)			
	SBM	CTM	PNM	SFM
Sugar cane	60.00	60.00	60.00	60.00
Soybean Meal	15.00	-	-	-
Cottonseed Meal	-	15.00	-	-
Peanut Meal	-	-	14.80	-
Sunflower Meal	-	-	-	18.00
Corn	23.35	23.50	23.30	20.00
Mineral supplement[2]	1.00	1.00	1.00	1.00
Urea	0.65	0.50	0.90	1.00
	Chemical composition			
Dry matter (%)	53.52	53.66	53.54	53.77
Organic matter (%DM)	96.58	96.55	96.75	96.84
Crude protein (%DM)	13.49	13.31	13.32	13.12
Neutral detergent fiber (%DM)	38.80	35.10	35.90	36.22
Acid detergent fiber (%DM)	18.87	18.92	19.49	19.98
Lignin (%DM)	2.01	2.78	2.23	2.17

[1]SBM – diet containing 60% sugar cane and 40% concentrate based on soybean meal; CTM - diet containing 60% sugar cane and 40% concentrate cottonseed meal; PNM - diet containing 60% sugar cane and 40% concentrate peanut meal; SFM - diet containing 60% sugar cane and 40% concentrate sunflower meal. [2]Product Composition (Calcium: 146 g; Phosphorus: 40 g; Magnesium: 20 g; Sulfur: 40 g; Sodium: 56 g; Copper: 350 g; Manganese: 900 mg; Zinc: 1300 mg; Iodine: 24 mg; Cobalt: 10 mg; Selenium: 10 mg; Fluorine (max.): 400 mg; Monensin: 670 mg.

The indicators used at the estimation of fecal production and apparent total and partial digestibility in the digestive tract were the complexed chrome (Cr-EDTA) and ytterbium chloride ($YbCl_3$) as external indicators, and indigestible neutral detergent fiber (iNDF) naturally present in the feed, as internal indicator. The adaptation to external indicators occurred during six days after the adjustment to diets through the supply of 150 mL Cr-EDTA and 2 mg Yb kg^{-1} BW $YbCl_3$, directly into the rumen of animals, diluted in 0.5 L water day^{-1} and manually homogenized. During the adaptation period until the end of the experimental periods, indicators were infused into the rumen in two daily doses before each animal feeding session (6:30 a.m and 3:00 p.m).

Total feces collection was performed for five days. Feces were daily removed from trays at 8h, weighed, homogenized and sampled separately per animal at every period. Samples of feces were subjected to nitric-perchloric digestion according to De Vega and Poppi (1997). The determination of chromium and ytterbium concentrations was undertaken by readings in atomic absorption spectrophotometer according to Willians et al. (1962).

For the determination of iNDF, 0.5 g samples of diets, leftovers, duodenal digesta and feces were placed in TNT bags (non-woven textile), with a weight of 100 g m m^{-2}, made with the dimensions 5 x 5 cm. The samples were stored, from the relationship of DM 20 mg per square centimeter of surface (NOCEK, 1997) and incubated in triplicate for 264 hours in the rumen of three heifers, as described by Casali et al. (2008). After this period, bags were removed, washed in running water until total clearing of the water and subsequently dried in a forced-ventilation oven. After this procedure, the remaining material of each incubated sample was accumulated, forming a composite sample, which was subjected to neutral detergent solution, with an autoclave (40 minutes, 111ºC and 0.5 atm), according to Senger et al. (2008).

The estimation of the duodenal dry matter flow was performed for two days, at the established collection times: at 5:00; 8:00 a.m., 2:00 and 8:00 p.m on the first day, at 2:00, 11:00 a.m., 5:00 and 11:00 p.m on the second day, aiming to obtain a representative sample over the 24 hours. Samples were collected at each time straight from the cannula of the duodenum of animals, at the amount of two samples of 500 mL $animal^{-1}$ $collection^{-1}$, for further determination of the duodenal dry matter flow through methodologies of single and double indicators.

The samples used in the single indicator method were collected and immediately dried in forced-ventilation oven at 55ºC for 72 hours, so there was no separation of solid and liquid phases, keeping the original duodenal content. From these samples, a composite sample was taken per animal, in each experimental period, based on the dry matter of each sample/time. The calculation at the determination of the flow, with the methodology of single indicator, was performed according to the suggestion of France and Siddons (1986), and the indicators employed in this estimation were Cr-EDTA, $YbCl_3$ and iNDF.

The flow was estimated by the double indicator method, as described by Faichney (1975). In this method, Cr-EDTA was used as a marker of the liquid phase and its concentration was estimated in the original sample, in which there was no separation of liquid and solid phases of the rumen content. Indicators $YbCl_3$ and iNDF, in turn, were employed as markers of the solid phase, and were determined in the partial sample, which only contained the solid phase of the duodenal content, once the liquid phase was disregarded. For this separation, samples were frozen at -15ºC after collections, to be then thawed and filtered through twice-folded cotton fabric for separation of liquid and solid phases. The phase considered solid was the one which was retained in the fabric, whereas the phase considered fluid was through the fabric and was disregarded. After drying and milling, a composite sample based on the dry matter of each sample/time and per experimental period was formed, for further analysis of indicators. The combinations utilized at the determination of the flow estimated by the double-indicator method were Cr-EDTA + $YbCL_3$ and Cr-EDTA + iNDF, for subsequent calculations of reconstitution of the sample and estimation of the duodenal digesta flow.

The apparent digestibility coefficients of DM and nutrients were determined through the quantification of observed DM intake and fecal excretion obtained by the daily total feces collection, during five collection days. In order to prevent contamination with urine, Folley probes no. 22 and/or 22 were inserted in heifers. At the end of each experimental period, composite samples of feces per animal and per period were

[U1] Comentário: 100 g/m^2 usando as medidas internacionais!

constituted according to the amount of DM excreted daily per animal.

Fecal production, fecal dry matter retrieval and retrieval of indicators were estimated according to methodology described by Schneider and Flatt (1975) and the digestibility coefficients, according to Ahvenjärvi et al. (2003).

Composite samples of feeds, leftovers and feces, which were stored at -15ºC, after thawed, were dried in forced-ventilation oven at 55ºC and ground in knife mill with 1 mm mesh sieve (AOAC, 1990). Contents of dry matter (DM), mineral matter (MM) and nitrogen (N) were determined according to the AOAC (1990), and for conversion into crude protein, the correction factor of 6.25 was utilized.

The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined with samples subjected to digestion in solutions of neutral and acid detergents, respectively, according to Van Soest et al. (1991), in non-woven-fabric (TNT) bags, of 100 g $m.m^{-1}$, made with dimensions 5 x 5 cm with fiber digester device. At the determination of lignin, 72% sulfur acid was utilized (VAN SOEST, 1994).

The experimental design adopted was a double 4 x 4 Latin square, with eight animals, four treatments and four experimental periods. Fecal production responses were compared with the fecal production observed by the total collection and analyzed by the Dunnett's test, with significance level of 5%. The retrieval of dry matter and indicators, the dry matter flow and the total and partial apparent digestibility values were analyzed by the Tukey's test at 5% significance using the statistical package (SAS INSTITUTE, 2003), where the effect of the diet was not considered in the data analysis, once it had no significance, so that mean values were obtained for each studied variable of the eight animals estimated according to each indicator utilized.

Results and discussion

The results for fecal dry matter production (FDMP), both in kg day^{-1} and % of BW, estimated by indicators Cr-EDTA, $YbCl_3$ and iNDF have overestimated the fecal production in comparison with the total collection ($p < 0.05$) (Table 2). The result obtained by Cr-EDTA demonstrated that this indicator cannot be used at the estimation of fecal dry matter production. Such fact can be attributed to the characteristic of each indicator; Cr-EDTA presents a faster rate of passage through the digestive tract, which is typical of markers of the liquid phase (BERCHIELLI et al., 2005b), besides presenting difficulty of association with the solid phase of the digesta (ELLIS et al., 1982).

Table 2. Fecal dry matter production (FDMP) observed and estimated by different indicators.

	Indicator					
Variables	TC[1]	Cr-EDTA	$YbCl_3$	iNDF	P	CV
FDMP, kg day^{-1}	1.39	1.64*	1.71*	2.17*	<0.001	12.86
FDMP, %BW	0.64	0.75*	0.75*	1.00*	<0.001	15.67

*Different from the control by Dunnett's test at 5% probability; p = probability; CV = coefficient of variation; [1]TC = total feces collection.

For $YbCl_3$, in turn, it was expected different results, since it can be employed to mark the solid phase of the digesta (BÜRGER et al., 2000), in addition to the link of this marker with the cell wall of plants (ELLIS et al., 2002).

Studies that have used iNDF as an indicator to estimate fecal production presented diverse results. When analyzing iNDF as an indicator to estimate the fecal production of cattle fed different forages obtained by means of two incubation methods (*in vitro* and in situ). Berchielli et al. (2005a) verified different behavior according to forage studied, in which the estimates of fecal production utilizing this indicator differed and did not differ from the total collection, for sugarcane and corn silage, respectively. Among the reasons for these differences, the authors stressed the key role of the fiber of each forage which can affect its degradation rate and extension.

Casali et al. (2008) attributed the wide variation in the results to the *in situ* incubation time, which can be found at values ranging from 98 to 288 hours. Thus, the abovementioned authors suggested the time of 264 hours to obtain iNDF, which was the incubation time adopted in this study. However, despite of adopting the incubation time of 264 hours recommended by Casali et al. (2008), the results obtained in this study through iNDF were not appropriate, probably because the choice of the methodology and the method of conduction of the analytical motion for the laboratory determination of NDF might have presented problems that have been determinant on the results.

At the retrieval of fecal dry matter (RFDM), no statistical difference was observed between the results obtained with Cr-EDTA and $YbCl_3$ ($p > 0.05$), of 119.96% and 125.37%, respectively. These values were lower ($p < 0.05$) than calculated through iNDF (162.78%). The iNDF presented inferior value ($p < 0.05$) at the retrieval of

indicators (RIND), when compared with the values obtained by Cr-EDTA and $YbCl_3$, which were similar to each other ($p < 0.05$) (Table 3).

Table 3. Retrieval of fecal dry matter (RFDM), retrieval of indicators (RIND), and dry matter flow (DMF), estimated by single and double indicator methods.

Variables	Indicator					*P*	CV
	Cr-EDTA	$YbCl_3$	iNDF	Cr-EDTA $YbCl_3$	Cr-EDTA iNDF		
RFDM, %	119.96 b	125.37 b	162.78 a	-	-	<0.001	22.44
RIND, %	89.00 a	85.00 a	67.00 b	-	-	<0.001	16.73
DMF, kg day^{-1}	2.44 a	2.55 a	2.10 b	1.94 c	1.58 c	<0.001	24.59

Means followed by different letters in the row differ by Tukey's test (α = 5%); p = probability; CV = coefficient of variation.

The dry matter flow (DMF) values estimated by Cr-EDTA and $YbCl_3$ were similar ($p > 0.05$), with averages of 2.44 and 2.55 kg day^{-1}, respectively; these results were superior ($p < 0.05$) both to iNDF (2.10 kg day^{-1}) and to the values estimated by Cr-EDTA/$YbCl_3$ and Cr-EDTA/iNDF associations, used as a single indicator, of 1.94 and 1.58 kg day^{-1}, respectively. The averages observed for the two associations in the double-indicator methodology were similar ($p > 0.05$).

The results obtained can be considered biologically adequate, once considering a dry matter flow average as pertinent, the indicators utilized at the estimation of the flow should present a good retrieval in the feces and the value of the estimated flow should be lower than the amount of feed ingested by the animal. Thus, the dry matter flow values estimated by the indicators, both in the methodology of single and double indicator were lower than the average dry matter intake observed, which was 5.34 kg day^{-1}.

Concerning the retrieval of indicators in the feces, the values obtained by Cr-EDTA and $YbCl_3$ were 89.0 and 85.0%, respectively; these values were higher ($p < 0.05$) than obtained by iNDF, which was 67.0%. Therefore, the averages of FDM obtained by Cr-EDTA and $YbCl_3$ (in the methodology of single indicator) met the considerations described above with more reliability. However, considering that the digesta should preferably be separated into different phases, to be then mathematically reconstituted, the utilization of the double-indicator system results in average FDM values with higher reliability (FAICHNEY, 1975; OWENS; HANSON, 1992). However, among the associations studied, the Cr-EDTA/$YbCl_3$ presented the best estimate of dry matter flow, since the two indicators Cr-EDTA and $YbCl_3$ were used for the liquid and solid phases, respectively, thus resulting in the greatest RIND values (Table 3).

Ahvenjärvi et al. (2003) studied samples of flow obtained in the omasum and observed a greater accuracy of the double indicators. The authors also suggested the use of three indicators to mark the different phases of the digesta, improving thus the result of the calculation of sample reconstitution.

The total apparent digestibility values of dry matter, organic matter, crude protein and neutral detergent fiber obtained by means of total feces collection were 67.62, 65.23, 67.20 and 50.23%, respectively; they were similar ($p > 0.05$) to the coefficients estimated by means of indicators Cr-EDTA and $YbCl_3$ (Table 4), and superior ($p < 0.05$) to the values obtained by iNDF, which were 63.62, 61.30, 62.57 and 44.43% for DM; OM; CP and NDF, respectively. The results were coherent with the values observed for the retrieval of the indicators in the feces, in which those with the highest coefficients of retrieval in the feces have estimated the best averages of total apparent digestibility of dry matter and other nutrients.

Table 4. Total apparent digestibility values of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF), observed and estimated by different indicators.

Total Apparent Digestibility	Indicator				*P*	CV
	TC[1]	Cr-EDTA	$YbCl_3$	iNDF		
DM	67.62 a	66.86 a	67.01 a	63.62 b	<0.001	11.64
OM	65.23 a	64.80 a	65.54 a	61.30 b	<0.001	11.80
CP	67.20 a	65.11 a	66.29 a	62.57 b	<0.001	17.69
NDF	50.53 a	49.07 a	48.83 a	44.43 b	<0.001	13.46

Means followed by different letters in the row differ by Tukey's test (α = 5%); p = probability; CV = coefficient of variation. [1]TC = total feces collection.

The ruminal and post-ruminal digestibility coefficients (Table 5) are a direct consequence of the values obtained at the estimations of fecal production and dry matter flow. In the fecal production, external indicators Cr-EDTA and $YbCl_3$ were those which best estimated this variable in comparison with the data obtained through total feces collection. The most accurate values of flow were obtained by the Cr-EDTA/$YbCl_3$ association, which resulted in ruminal and post-ruminal digestibility coefficients of dry matter, organic matter, crude protein and neutral detergent fiber of 74.47, 24.41, 76.20 and 21.82, 30.24, 69.75, 93.62 and 7.19%, respectively.

Table 5. Ruminal and post-ruminal digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF), estimated by single and double indicator methods.

Variables	Indicador					*P*	CV
	Cr-EDTA	$YbCl_3$	iNDF	Cr-EDTA $YbCl_3$	Cr-EDTA iNDF		
Ruminal Apparent Digestibility[1] (%)							
DM	66.02 c	60.45 c	69.74 b	74.47 a	74.83 a	0.015	10.34
DO	66.94 c	61.95 c	71.55 b	76.20 a	77.05 a	0.036	11.84
CP	26.39 b	21.21 c	31.32 a	30.24 a	25.73 b	>0.001	10.18
NDF	83.60 b	78.15 b	92.11 a	93.62 a	95.01 a	>0.001	9.14
Post-Ruminal Apparent Digestibility[2] (%)							
DM	33.98 a	39.55 a	31.66 b	24.41 c	24.86 c	>0.001	10.50
DO	33.06 a	38.05 a	30.86 b	21.82 c	22.55 c	>0.001	13.73
CP	73.61b	78.79 a	68.68 c	69.75 c	74.27 b	0.012	16.62
NDF	16.40 a	21.85 a	6.80 b	7.19 b	5.27b	>0.001	11.83

Means followed by different letters in the row differ by Tukey's test (α = 5%); p = probability; CV = coefficient of variation; [1]TC = total feces collection. [2]Obtained depending on the total digestibility of nutrients in 100%.

It is worth emphasizing that an ideal indicator is the one which predicts the digestibility in the entire tract and, above all, provides exact information on the extension and direction of the effects induced by diets without modifying their direction.

Conclusion

The indicators examined were not effective in estimating fecal production, through comparison of the fecal production obtained by the total feces collection. External indicators (Cr-EDTA and $YbCl_3$) presented a better retrieval when compared with the results obtained with internal indicator (iNDF). The Cr-EDTA/$YbCl_3$ association, used in the form of double indicator, promoted the greatest estimate of the dry matter flow, and consequently the best ruminal and post-ruminal digestibility coefficients.

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