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### REFERÊNCIA

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## Effect of sorghum tannins in sheep fed with high-concentrate diets

[Efeito do tanino do sorgo em dietas de ovinos com alto concentrado]

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### ABSTRACT

The aim of this study was to evaluate the nutritional value of three sorghum cultivars with different concentrations of condensed tannins in sheep diets. Six adult sheep (LW=56kg) with rumen and duodenal fistulas were assigned to experimental groups using two 3x3 Latin Square designs. The diets were formulated using three sorghum cultivars: LTC (low-tannin cultivar), MTC (medium-tannin cultivar) and HTC (high-tannin cultivar). Microbial nitrogen (MN) concentration in the duodenum was measured using a <sup>15</sup>N trace technique. LTC, MTC and HTC diets presented values of 788, 722 and 747 (SE=20.6) g kg<sup>-1</sup> for dry matter digestibility and 633, 535 and 530 (SE=35.8) g/kg for crude protein digestibility. The LTC diet was significantly different from the other diets (P<0.05). The nitrogen balance was 145.5, 94.8 and 83.8g kg<sup>-1</sup> (SE=13.0) for LTC, MTC and HTC, respectively, with LTC showing greater nitrogen retention (P<0.05). Values obtained for MN in the digesta were 301, 364 and 469 (SE=30.6) g kg<sup>-1</sup> for LTC, MTC and HTC, respectively, and there was no statistical difference (P>0.05) between the diets. The presence of condensed tannins in the sorghum interfered with the sheep's nitrogen retention; however, the microbial protein supply to the duodenum of the animals was not inhibited.

Keywords: feed evaluation; microbial protein; nitrogen balance; rumen

### RESUMO

O objetivo do presente estudo foi avaliar o valor nutricional de três cultivares de sorgo com diferentes concentrações de tanino condensado em dietas de ovinos. Seis carneiros machos, adultos, castrados, canulados no rúmen e duodeno foram divididos em três grupos experimentais, em um delineamento em quadrado latino 3x3. Os tratamentos foram representados por três dietas experimentais contendo diferentes cultivares de grão de sorgo: LTC (cultivar com baixa concentração de tanino), MTC (cultivar com concentração média de tanino) e HTC (cultivar com concentração alta de tanino). As concentrações de nitrogênio de origem microbiana no duodeno (MN) foram mensuradas pela técnica de marcador isótopo estável <sup>15</sup>N. As dietas LTC, MTC e HTC apresentaram valores de 788, 722 e 747g.kg<sup>-1</sup> (EP=20,6) para a digestibilidade aparente da matéria seca e 633, 535 e 530g.kg<sup>-1</sup> (EP=35,8) para a digestibilidade aparente da proteína bruta. A dieta LTC apresentou diferença significativa quando comparada às demais dietas para as avaliações de digestibilidade (P<0,05). Os valores encontrados para o balanço de nitrogênio foram de 145,5; 94,8 e 83,8g.kg<sup>-1</sup> (EP=13,0) para LTC, MCT e LTC, respectivamente, sendo a dieta LTC a que apresentou maior retenção de nitrogênio (P<0,05). Os valores obtidos para MN na digesta foram de 301, 364 e 469 para LTC, MTC e HTC, respectivamente, e não apresentaram diferenças estatísticas (P>0,05). As diferentes concentrações de taninos condensados nos cultivares de sorgo resultaram em melhorias na digestibilidade e retenção de nitrogênio das dietas, entretanto o fluxo de proteína microbiana não foi inibido.

Palavras-chave: avaliação de alimentos, balanço de nitrogênio, proteína microbiana rúmen

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## INTRODUCTION

The sorghum (*Sorghum bicolor*) grain is an important energetic resource in feeding ruminants. Despite the ruminant advantage of having an efficient fibre utilisation basis, the inclusion of greater amounts of starch in the finished sheep diet implies a larger accumulation of fat in the carcass and, therefore, better meat quality.

High concentrations of grains in ruminants' diets may result in negative effects on microbial fermentation (Mould, 1988). The high availability of fermentable carbohydrates and rapid microbial growth result in low pH values in the rumen, which inhibit the growth of cellulolytic microorganisms.

Some sorghum cultivars contain high concentrations of tannins in the kernels. The presence of these molecules in animal diets is associated with certain negative effects, such as a decrease in dry matter (DM) intake, a decrease in protein digestibility, a greater loss of endogenous protein and the inhibition of microbial activity (Makkar *et al.*, 1995; Ben Salem *et al.*, 1997, Godoy *et al.*, 2004). On the other hand, studies have shown the positive effects of condensed tannins, including higher absorption of amino acids in the intestine, decreased nitrogen loss through urine and decreased endoparasites (Aerts *et al.*, 1999; Athanasiadou *et al.*, 2001).

It is the purpose of the present paper to evaluate diets formulated with different sorghum cultivars containing different concentrations of tannins in sheep nutrition.

## MATERIALS AND METHODS

This experiment was performed in the Laboratório de Nutrição Animal do Centro de Energia Nuclear na Agricultura (CENA) of the Universidade de São Paulo (USP). The experiment was conducted using two simultaneous Latin Square designs with three treatments and six animals (Mead *et al.*, 1999).

The animals used were six adult castrated sheep, predominantly of the Santa Inês breed. The animals had duodenum and rumen fistulas and initiated the experiment with an average weight of 56kg.

Three experimental diets were formulated: a low-tannin diet (LTC, with Agromen 8050), a medium-tannin diet (MTC, with SEMEALI hybrid) and a high-tannin diet (HTC with BR700); each diet used one of three different sorghum cultivars with increasing levels of condensed tannins (Tab. 1). The experimental diets were formulated according to the nutritional requirements and recommendations of the National Research Council (1985) for sheep. The recommendation for adult sheep was followed (>50kg of live weight) using a 20:80 forage:concentrate ratio (% DM).

Table 1. Composition of the experimental diets: LTC (Agromen 8050), MTC (Semeali commercial hybrid) and HTC (BR700) formulated with three sorghum cultivars with different concentrations of condensed tannins (CT)

Ingredients/ Diets	LTC (g kg <sup>-1</sup> DM)	MTC (g kg <sup>-1</sup> DM)	HTC (g kg <sup>-1</sup> DM)
Hay	200	200	200
Sorghum	730	690	700
Soybean meal	50	90	80
Mineral mixture	20	20	20
CP (g/kgMS)	120	120	120
ME (Mca/kg)*	2.57	2.58	2.58
CT (%)	0	1.30	1.70

\* estimated according to the NRC for sheep (1985)

The concentrations of condensed tannins in the kernels of the three cultivars chosen for the experimental diets were determined. The concentrations of condensed tannins (CT) found were 0, 10.9 and 20.4g kg<sup>-1</sup> of DM for the three cultivars, respectively. They were analysed according to the Butanol/HCl method described by Porter *et al.* (1986).

Coast-cross hay (*Cynodon dactylon*) with 10% crude protein (PB) was used as roughage. A commercial mineral supplement containing macro- and microminerals recommended for sheep (ovinoFós-Tortuga) was included in the diet (2% of the DM).

The experimental diets were offered in a restricted form, fixed at 3% of the live weight of each animal per day. Food was given twice per

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day, at 8:30 and 16:30; the refusals, when there were any, were collected and weighed.

For each period, the animals were kept in metabolism cages during 14 days, including eight days for diet adaptation and six for the collection of the refusals, total faeces, urine, ruminal liquid (RL) and duodenal content (DC). Rumen infusions were also made using a  $^{15}\text{N}$ -labelled solution in order to study microbial synthesis. Samples of RL were also collected for analysis of the ammonia nitrogen ( $\text{N-NH}_3$ ) content and rumen pH monitoring.

The refusals were collected daily, weighed and taken to an oven ( $55^\circ\text{C}$  for 48h). The material was dried and ground (1mm), avoiding the separation of roughage and concentrate. The samples were then analysed for the concentrations of the following: DM, organic matter (OM), nitrogen (N), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). The analyses of N, CP and ADF were performed as established by the A.O.A.C (Association..., 1990) and the NDF analysis was performed as established by Van Soest *et al.* (1991).

All the faeces were collected and weighed. Afterwards, 10% aliquots of the total weight were taken from each sample. They were then dried ( $55^\circ\text{C}$  during 48h), ground (1mm) and submitted to the same analysis as the refusals.

The total urine was collected in a container with 100 mL of 10% sulphuric acid ( $\text{H}_2\text{SO}_4$ ). The total volume was measured on a daily basis and diluted in water until a final volume of three litres was reached. An aliquot of 20mL was collected and frozen for eventual nitrogen analysis.

For the study of microbial synthesis and of the flux of microorganisms between the rumen and the duodenum,  $^{15}\text{N}$  was used as a marker. A solution of radiolabeled ammonium sulphate [ $(^{15}\text{NH}_4)_2\text{SO}_4$ ] was prepared with a 90% enrichment. The solution was diluted in 60 mL of distilled water and injected into the rumen of the animals through a syringe.

Every two hours, 2mL of the solution was injected, such that the whole content of the syringes was applied over a 24-h period. The

animals received the solution for 72h until the radiolabeled nitrogen was incorporated by the rumen microorganisms (steady-state). Each animal received a dose of  $1\text{mg}$  of  $^{15}\text{N}$   $\text{kg}^{-1}$  of live weight per day during the three days of infusion, as established by McAllan *et al.* (1994).

The collection of the DC started 36h after the first injection of the marked salt solution. A minimum of 200mL of DC was collected at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68 and 72h. At each time point, 20mL of the total quantity collected was separated and frozen ( $-20^\circ\text{C}$ ).

After the conclusion of the experiment, the samples for all time points were homogenised every 24h, yielding three samples (pooled) representing DC 24, 48 and 72h after the administration of the marker.

All the DC samples were lyophilised, weighed and analysed by mass spectrometry (ANCA, Europa Scientific). The levels of N and  $\delta^{15}\text{N}$  (enriched with  $^{15}\text{N}$ ) in the samples were then determined.

RL samples were also taken at the end of the 72h in order to determine the  $\delta^{15}\text{N}$  content of the rumen microorganisms. The microorganisms were isolated from the ruminal content by centrifuging twice. Approximately 100mL of RL was collected from each animal and centrifuged at  $500 \times g$  for 10 minutes. From the first centrifugation, only the supernatant was collected in order to separate the coarse particles of the RL. The supernatant portion was then centrifuged at  $2000 \times g$  for 20 minutes. After the second centrifugation the supernatant was discarded, yielding a pellet composed of bacteria in the liquid phase (LAB). The pellets were then lyophilised, weighed and subjected to mass spectrometry analysis.

The enrichment of the rumen microorganisms with  $^{15}\text{N}$  was calculated based on the  $\delta^{15}\text{N}$  values obtained in the pellet after 72 h minus the value of the natural enrichment of the isotope (0.3663%), as provided by the International Atomic Energy Agency (IAEA). The same method was used to calculate the DC enrichment.

The amount of microbial nitrogen (MN) contained by the DC was calculated as

established in studies by Brandt *et al.* (1980), McAllan *et al.* (1994), and Pérez *et al.* (1997), based on the following equation:

$$MN\% = \frac{{}^{15}N_{\text{digesta}}}{{}^{15}N_{\text{bacteria}}} \quad (1)$$

To determine the pH and the concentration of nitrogen in ammonia form in the RL, samples of RL were collected using a probe inserted directly into the rumen of the animals. Approximately 60mL of RL were collected with the probe. The collections were undertaken at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9.5, 14 and 22h after the first meal.

The RL was immediately transferred to a beaker, after which the pH was measured and an aliquot of approximately 10mL was collected in glass containers. A few drops of concentrated H<sub>2</sub>SO<sub>4</sub> (18N) were added to the containers in order to reduce the loss of nitrogen through volatilisation. The tubes were then shaken and frozen. The concentrations of N-NH<sub>3</sub> in the RL were determined using a micro-Kjeldahl apparatus, following the methodology described by Preston (1995).

The Latin Square was the statistical design used for the experiment, as follows:  $Y_{ijk} = \mu + t_i + a_j + p_k + e_{ijk}$ , where  $t_i$  is the effect of treatment  $i$

(diets),  $a_j$  is the effect of animal  $j$ ,  $p_k$  is the effect of the period  $k$  and  $e_{ijk}$  is the associated error.

The model was analysed using the SAS program, Statistical... (2000), which was also used for comparisons of averages using Tukey's test at a 5% probability.

## RESULTS

Table 2 demonstrates the results of consumption and apparent digestibility of dry matter (DMD), crude protein (CPD) and neutral detergent fibre (NDFD) as well as the nitrogen balance (NB) and microbial nitrogen (MN).

No significant differences ( $P > 0.05$ ) in DM intake were found. The DMD was different between the cultivars with high and low concentrations of tannins ( $P < 0.05$ ): 788g kg<sup>-1</sup> was the highest value, obtained with the LTD diet. The CP digestibility was higher ( $P < 0.05$ ) for the LTC diet, and no significant differences were found between the MTC and HTC diets. For the NDFD, the highest values obtained were for LTC and HTC, with an average of 582 g kg<sup>-1</sup> for the MTC diet, which was different only from the LTC diet ( $P < 0.05$ ).

Table 2. Voluntary intake, dry matter digestibility (DMD), crude protein digestibility (CPD), nitrogen balance (NB), and microbial nitrogen (MN) in non-wool sheep consuming diets (LTC, MTC, and HTC) containing three sorghum cultivars with different concentrations of condensed tannins

Diets	Consumption (g day <sup>-1</sup> )	DMD (g kg <sup>-1</sup> )	CPD (g kg <sup>-1</sup> )	NDFD (g kg <sup>-1</sup> )	NB (%)	MN (g kg <sup>-1</sup> )
LTC	1449	788a	633a	692a	14.55a	301
MTC	1430	722b	535b	582b	9.48b	364
HTC	1445	747b	530b	641ab	8.38b	469
P>F <sup>1</sup>	0.9644	0.0325	0.0094	0.0041	0.0051	0.1011
CV <sup>2</sup> (%)	9.1	4.7	10.8	6.2	20.9	37.1

<sup>1</sup> P<F probability of significance, <sup>2</sup> Coefficient of variation.

As for the NB, the LTC diet showed a larger ( $P < 0.05$ ) retention of nitrogen (14%) when compared to the 9% and 8% values obtained for MTC and HTC, respectively.

The MN content was expressed based on the total value of MN obtained in the digesta (g kg<sup>-1</sup>); there were no significant differences between the treatments ( $P > 0.05$ ).

A decline in ruminal pH values was observed after meals in all three treatment groups. The lowest averages were obtained at 3 h and 14 h after the first meal.

The ruminal pH values remained between 6.0 and 5.6 throughout the day. No significant differences were observed between the treatment groups ( $P > 0.05$ ), with all three diets displaying similar pH curves (Figure 1).

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The ammonia nitrogen (N-NH<sub>3</sub>) values obtained in the RL remained between 40 and 80 mg L<sup>-1</sup> for all the treatment groups during the first 24 h. Significant differences were observed between the treatment groups just 9h after the first meal

(Figure 2). The LTC diet showed a concentration of 48 mg L<sup>-1</sup>, which was lower than the concentrations obtained for MTC and HTC (76 and 67 mg L<sup>-1</sup>, respectively).

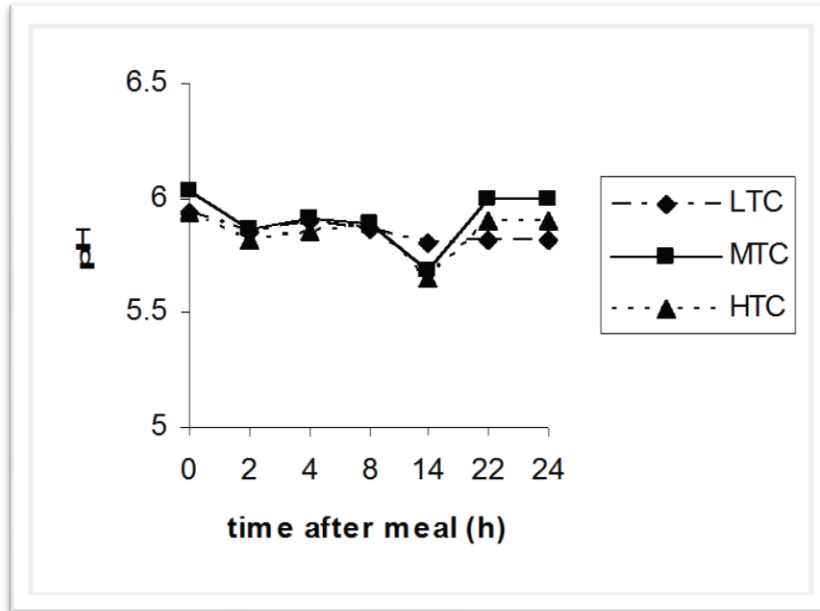


Figure 1. Ruminal pH variations in sheep fed with three different sorghum cultivars with different condensed tannin concentrations (LTC, MTC and HTC).

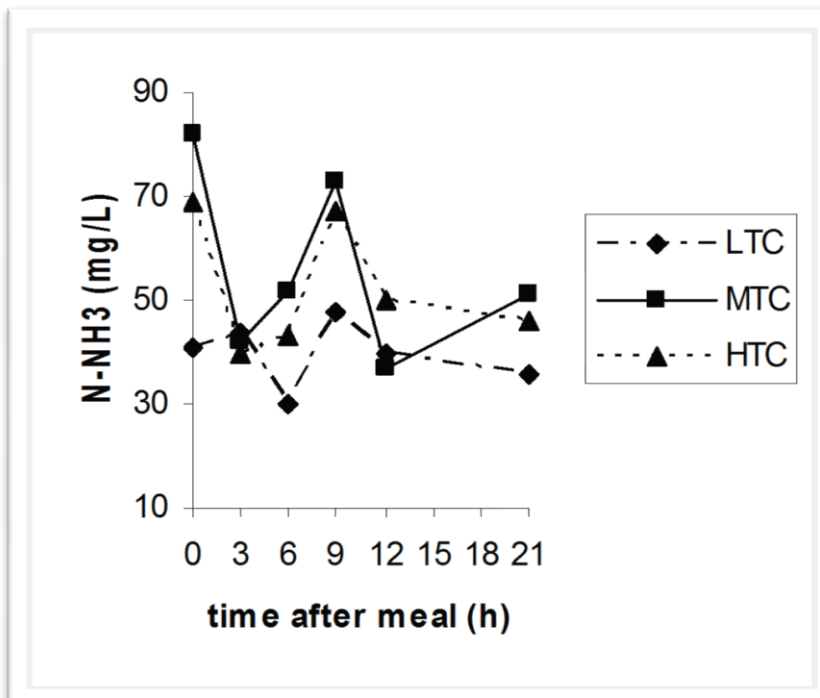


Figure 2. Variations in the ruminal N-NH<sub>3</sub> of ovines fed with three sorghum cultivars with different condensed tannin concentrations (LTC, MTC and HTC)

## DISCUSSION

There was no difference in the consumption of DM between the different diets, indicating that the cultivars with higher tannin concentrations did not present an astringent effect. A decrease in the palatability of rations due to the astringent effect of tannins was described by Butler and Rogler (1985). Some authors, such as McNaughton (1987), have observed negative effects on the voluntary intake of ruminants with tannin concentrations of 5% in the diet.

In studies conducted in Brazil with arboreal species from the Northeast (Silva *et al.*, 1998; Beelen *et al.*, 2003; Godoy *et al.*, 2004), a decrease was observed in the sheep's voluntary intake, showing that consumption may be related not only to tannin concentration but also to the adaptation of the animals and the rumen microorganisms to the inhibitory effects of these molecules.

The values obtained for the apparent DMD indicate an efficient utilisation of these diets by the animals. Values between 72% and 77% for the organic matter digestibility (OMD) were found by Sherrod and Furr (1970) in sheep receiving 81% sorghum from different cultivars in their diets. Defoor *et al.* (2001), when comparing different plantation densities and processing methods for sorghum, obtained a value of 74% for the DMD in sheep fed with 60% grain.

The LTC diet presented the best DMD, indicating a possible effect of CT in the MTC and HTC diets. Such effects are associated with a larger loss of DM in the faeces due to its unavailability for fermentation and digestion. A decrease in DMD was described in *in situ* and *in vitro* experiments that used cultivars with high- and low-tannin concentration grains (Campos *et al.* 2002; Cabral *et al.*, 2003; Souza *et al.* 2003).

The CP digestibility also showed differences between the diets, with LTC showing a higher digestibility than MTC and HTC. The decrease in CPD is characteristic of the effect of CT in animal digestion. These molecules have a high affinity with proteins, causing the formation of bonds that can make this nutrient unavailable for absorption. Butler (1989) associated the reduction in protein digestibility by tannins with

the reduction of enzymatic activity associated with protein digestion. Possible connections between the tannins and such enzymes would thus be responsible for such inactivity. Komolong *et al.* (2001) worked with increasing levels of quebracho extract (73% CT in the DM) in sheep diets and noticed a 10% reduction in the nitrogen apparent digestibility ( $R^2=0.97$ ) associated with an increase of N in the faeces ( $R^2=0.98$ ).

The decrease in fibre digestibility was also described as an anti-nutritional effect of the CT (Reed, 2001). The present study found a significant decrease in NDFD for the MTC diet. Although this diet had 1.3% CT in the DM, no decrease in NDFD was observed for the HTC diet (1.7% CT in DM).

The nitrogen balance indicated a larger difference between the diets with and without tannins. Nitrogen retention was greater for the LTC diet (14.5%), providing evidence of greater nitrogen use by the animals.

Some studies that evaluated nitrogen balance and retention in sheep fed with forage containing different CT concentrations noticed a greater nitrogen loss through the faeces and a decrease of such losses through urine (Ramirez *et al.*, 1998; Ngwa, 2002). Such results indicate greater nitrogen retention for diets with moderate levels of CT (1-3% of DM).

The nitrogen balance and digestibility results indicate a greater nitrogen loss by the animals that received CT in their diet, the microbial nitrogen (MN) levels in the DC did not show significant differences between the treatments ( $P>0.05$ ), indicating that the CT did not decrease the microbial activity of the rumen.

Experiments such as the one undertaken by Pérez *et al.* (1997) have yielded values of 11.9 and 11.5g MN per day for the LAB and the SAB measured using  $^{15}\text{N}$  in sheep receiving soybean meal diets with a high concentrate proportion.

Other studies that determined the MN concentrations in sheep fed with 80% concentrate in a restricted form and *ad libitum* obtained values of 14.5 and 17.4g MN per day, respectively (Carro *et al.*, 2000; Valdés *et al.*, 2000).

The low values of the rumen pH reflected the increase of lactic acid concentrations due to elevated proportions of concentrate in the diet. Decreases in the sheep's ruminal pH when they were fed diets with elevated proportions of concentrate have been described by Carro *et al.* (2000). The authors evaluated diets with different proportions of concentrate (80:20, 60:40, 40:60 and 20:80). The rumen pH values decreased linearly as concentrate levels increased (6.45; 6.40; 6.20 and 5.96; respectively).

The elevated proportions of concentrate and the low pH values may have been responsible for the low N-NH<sub>3</sub> concentrations found in the RL. Changes in the ruminal microbial population due to the extended digestion of starches may result in decreased protein digestion. Studies by Sultan and Loerch (1992) showed maximum values of 80 and 200 mg L<sup>-1</sup> for sheep fed with 9.5 and 20% of CP, respectively, whereas the highest values found by Valadares *et al.* (1997) in bovines were 90 and 200 mg L<sup>-1</sup> for diets containing 7% and 14.5% of CP, respectively.

### CONCLUSIONS

The diets formulated from sorghum cultivars with higher concentrations of condensed tannins resulted in lower nitrogen use by the sheep. However, no decrease in the microbial nitrogen supply for intestinal absorption was identified. The study did not identify any anti-microbial action of the sorghum condensed tannins, but the hybrids that presented high levels of CT contributed to an important reduction in nitrogen retention.

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