FEED UTILIZATION EFFICIENCY AND NITROGEN BALANCE IN DUAL PURPOSE COWS FED LOCAL DIETS IN THE SUBHUMID TROPIC OF MEXICO

Dixan Pozo-Leyva1a, Fernando Casanova-Lugo1b*, Felipe López-González2 , Alvar A. Cruz-Tamayo3 , Ricardo L. D. Costa4 , and Alfonso J. Chay-Canú**l5**

- ^{1a} Tecnológico Nacional de México/I.T. de la Zona Maya. Carretera Chetumal-Escárcega km 21.5, Ejido Juan Sarabia, 77960, Othón P. Blanco, Quintana Roo, Mexico https://orcid.org/0000-0002-3139-8512
- 1b Tecnológico Nacional de México/I.T. de la Zona Maya. Carretera Chetumal-Escárcega km 21.5, Ejido Juan Sarabia, 77960, Othón P. Blanco, Quintana Roo, Mexico https://orcid.org/0000-0003-2485-9170
- 2 Instituto de Ciencias Agropecuarias y Rurales (ICAR), Universidad Autónoma del Estado de México, Campus UAEM El Cerrillo, El Cerrillo Piedras Blancas, 50090 Toluca, Estado de México, Mexico https://orcid.org/0000-0002-5518-5458
- 3 Facultad de Ciencias Agropecuarias, Universidad Autónoma de Campeche. Escárcega, Campeche. C.P. 24350, Mexico
- https://orcid.org/0000-0002-5509-3430
- ⁴Instituto de Zootecnia. Rua Heitor Penteado, 56, Centro, CEP 13380-011, Nova Odessa, São Paulo, Brazil
- https://orcid.org/0000-0001-8888-6915
- 5 División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco. Carretera Villahermosa-Teapa, km 25, R/a. La Huasteca 2ª. Sección, C.P. 86280. Villahermosa, Mexico https://orcid.org/0000-0003-4412-4972
- * Corresponding author: fkzanov@gmail.com

ABSTRACT

The objective of the study was to evaluate the effect of different levels of maize silage on feed utilization efficiency and nitrogen (N) balance in dual-purpose cows during the dry season. Nine crossbred cows with live weight of 423±12 kg were used. The treatments were: T1= 2 kg DM of maize silage + 3.9 kg DM of sorghum stubble; T2= 4 kg DM of maize silage + 1.8 kg DM of sorghum stubble; T3= 6 kg DM of maize silage. Additionally, all cows consumed Panicum maximum cv. Mombasa grass, supplemented with poultry manure and commercial concentrate in doses of 2.0, 2.9 and 2.3 kg DM, respectively. Data comparison was performed by ANOVA, and the experiment was conducted using a completely randomised mathematical model with three replicates. No significant differences (*p* **>0.05) were observed for any of the variables evaluated. N intake reached 279.8 g day–1 of which** 8.5% was excreted in milk, 42.1% in faeces and 41.2% in urine. Milk production was 4.4 kg cow⁻¹ **day–1, with 33.1 g kg–1 fat and 34.4 g kg–1 crude protein (CP). N balance was 27.5 g cow–1 day–1, while utilization efficiency reached 8.6%. A higher inclusion of maize silage increased CP and N contents of the diet supplied to the cows, but it did not result in higher milk production. Feed utilization efficiency was limited even in the treatment with the lowest N content in the ration (T1). Further research is required to evaluate the economic and productive viability of the dietary inclusion of different silages, as a feeding strategy to cope with the low availability of forages during dry periods in low-scale milk production systems.**

Keywords: maize silage; dry season; family livestock; milk production; supplementation.

INTRODUCTION

Nitrogen (N) is a fundamental element for the maintenance of agricultural systems, not only from an environmental perspective, but also directly involved in production processes. N surpluses in agricultural systems arise from the limited utilization efficiency of crops to transform the nutrients present in slurry and the inefficient capacity of animals to convert the N consumed into meat and milk (Akert et al., 2020).

N surpluses have become a constraint, with economic repercussions for agricultural production systems. Although pasture grazing is implemented to reduce production costs, animal stabling is required to reduce pasture deterioration during the dry season, increasing food dependence on external products such as commercial concentrates. Therefore, the acquisition of purchased feed has become one of the limitations for producers, added to the environmental impact when inputs come from other regions or are imported (Aarons et al., 2017).

N surpluses are linked to environmental pollution by volatilization, denitrification and leaching, with slurry being the main contributor of N losses, which contributes to climate change (Akert et al., 2020; Gutiérrez-León et al., 2023). Urinary excretion is considered the main source of nitrate $(NO₃^-)$ leaching, while manure is the main emitter of ammonia (NH_3) and nitrous oxide (N_2O) , being directly proportional to dietary N concentrations (Dijkstra et al., 2013; Aarons et al., 2017), since 72% of the N consumed is excreted through slurry (Pozo-Leyva et al., 2021a).

Nutrient balance is a management tool used internationally for the evaluation of sustainability of agricultural systems and the identification of improvement strategies (Dijkstra et al., 2013). Therefore, the objective of the study was to evaluate the effect of different levels of maize silage on feed utilization efficiency and nitrogen (N) balance in dual-purpose cows during the dry season.

MATERIALS AND METHODS

Location of the experimental site

The experimental site was located in the municipality of Othón P. Blanco, Quintana Roo, Mexico ($18^\circ 30'$ N and $88^\circ 29'$ W) in the border area between Mexico and Belize. In the locality, the climate is warm sub-humid, with temperatures ranging from 12 to 36 °C, and an average rainfall of 1,260 mm (Adame-Castro et al., 2020).

Experimental site

The experiment was carried out in two family production systems, under a participatory research scheme. Given that management, housing and feeding strategies for both family systems were the same, both herds were considered as one production system (Pozo-Leyva et al., 2021b).

Bioassay

The experiment had a duration of 36 days, from December 11, 2020 to January 16, 2021. Nine multiparous cows from different crosses of local zebu breeds were used (Brahman, Gyr and Guzerat). On average, lactation was 152 days, with a live weight of 423±12 kg at the beginning of the experiment. The cows had, on average, 4.3 calvings, while milk production reached 4.9 kg cow−1 day−1, with fat content of 3.1%, density of 31.9%, crude protein (CP) of 3.4%, and non-fat solids of 9.5%. During the experiment, the cows were housed in pens, with access to water, and feed supply was independent. Milking was done once a day, mechanically, and after milking the calves were housed with the cows for an average of seven hours a day.

Feeding strategies (treatments)

Three treatments were formulated considering the availability of local inputs, giving preference to feeds produced within the production system and the inclusion of by-products from the local agricultural industry (Carey et al., 2023).

The treatments included different levels of maize silage and sorghum stubble, as follows: T1= 2 kg DM of maize silage + 3.9 kg DM of sorghum stubble; T2= 4 kg DM of maize silage + 1.8 kg DM of sorghum stubble; T3= 6 kg DM of maize silage. Additionally, all animals consumed *Panicum maximum* cv. Mombasa grass, supplemented with poultry manure and commercial concentrate in doses of 2.0, 2.9 and 2.3 kg DM, respectively. The forage of the grass *P. maximum* cv. Mombasa was cut and transported from a pasture that was far from the production system facilities. The treatments were formulated to include different levels of CP, considering live weight, days in lactation and level of milk production according to NRC (2001); a dry matter (DM) consumption of 3.2% of the live weight (from each animal) was considered (Pozo-Leyva et al., 2021a and 2024). Three experimental periods were established with eight days of adaptation to each treatment and four days for sample collection (Miguel et al., 2014).

When changes in diet are slight, short experimental periods can be used, up to 36 days, which allows a reduction in experimental costs and a decrease in work time with the animals, which is convenient in research with participating producers (Pozo-Leyva et al., 2021b; 2024). It is worth mentioning that it was not possible to measure food rejection due to the lack of infrastructure and complexity of the smallscale production system, since it frequently ends up contaminated with faeces and urine.

Quantification of N intake and excretion by cows in milk production

To determine the chemical composition of the ration ingredients, a 1.5 kg sample of each ingredient was taken separately, while one sample per treatment was also collected. To

determine total N, a sample was taken from each treatment prior to the beginning of the experimental period and analysed in triplicate. Dry matter (DM) content was determined by forced air oven drying at 65 °C for 48 h and \check{N} content was determined by the dry combustion technique using a CHNS/O PerkinElmer 2400 Series II elemental analyzer (PerkinElmer Inc., Massachusetts, USA), then converted to CP using the conversion factor 6.25 (NRC, 2001). In addition, organic matter (OM) and ash contents were determined by muffle ashing at 550 °C for 3 h. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin contents were determined using the ANKOM A200 fibre analyser (ANKOM Technology, Macedon, NY, USA) according to the procedure described by Van Soest (1991). *In vitro* digestibility of dry matter (DIVMS) was determined by incubation in rumen liquid (Gómez-Miranda et al., 2020).

To determine N excretion in milk, individual milk production was measured after milking using a digital scale with a capacity of 30 kg, during the last four days of each experimental period. Subsequently, individual milk samples were taken to determine fat and CP content using an ultrasonic milk analyzer (Lacticheck TM , Model LC-01, Page & Pedersen International, Hopkinton, M.A).

To obtain N content in milk, CP content was divided by a factor of 6.38 according to NRC (2001). Additionally, milk urea nitrogen (MUN) was determined by colorimetry following the method described by Chaney and Marbach (1962). To determine fat- and protein-corrected milk production (FPCM), the formula described by Battini et al. (2015) and Pozo-Leyva et al. (2022) as used:

FPCM = Milk yield (kg day⁻¹) × [0.1226 × Fat (%) + $0.0776 \times CP(\%) + 0.2534$

For estimation of urinary N content, the formula described by Jonker et al.(1998) was used, where urinary N (g row^{-1} day⁻¹) is equal to 12.54 times MUN $(g \, dL^{-1})$. To determine N outputs in manure, individual samples were taken directly from the rectum of the animal; the samples were air-dried, then ground and sieved in a sieve with a mesh opening of 1.0 mm, and finally total N content was determined by means of a CHNS/O PerkinElmer 2400 Series II Elemental Analyzer (PerkinElmer Inc., Massachusetts, USA). To determine DM content, samples were dried in a forced air oven at 60°C for 72 h, recording the weight of the sample before and after drying. The volume of manure was determined by total collection for 24 h (Ding et al., 2019).

Additionally, individual blood samples were taken on the last day of each experimental period. The samples were obtained from the coccygeal vein with the use of a vacutainer (Becton Dickinson Vacutainer System), collecting approximately 10 mL of blood, and centrifuged

(10 min, $2810 \times g$), 2-3 h after collection to isolate serum. Serum and plasma samples were stored at -20°C before analysis for biochemical indices. To determine serum urea concentration, creatinine and blood glucose content were determined according to Arjona-Alcocer et al. (2020). Urea values were multiplied by 0.467 to convert to blood urea nitrogen (BUN) according to the procedure described by Ding et al. (2019) and Arjona-Alcocer et al. (2020).

Balance N and feed utilization efficiency

To determine N balance, feed intake and excretion in milk and slurry were considered, following the procedure described by Orlandi et al. (2020). For the determination of feed utilization efficiency (FUE), N excretion in milk and N consumption were considered.

Experimental design

Cows were assigned to three groups (squares) of three cows each, based on milk yield, in a 3 × 3 Latin square design repeated three times. Treatment sequences were randomised in squares 1 and 3, and the sequence of treatments in square 2 mirrored square 1 to minimise carry-over effects. Cows were randomly assigned to the treatment sequence. Latin square designs are appropriate when it is necessary to control two sources of variability. In these designs, the number of levels of the main factor has to coincide with the number of levels of the two block variables or secondary factors. This design is used to conduct experiments in heterogeneous conditions where properties change in two directions as occurs in participatory rural research, where conditions are often very changeable (Pozo-Leyva et al., 2024).

Statistical analysis

Data comparison was performed by ANOVA using Minitab® version 19.0 software. For the chemical composition of treatments and blood values, a completely randomised mathematical model with three replicates was used:

$$
Y_{ij} = \mu + t_i + e_{ij}
$$

where: μ = Overall mean; t= effect due to treatment, eij= Effect due to experimental error.

For animal response variables, the statistical analysis was conducted as described by López-González et al. (2020):

$$
Y_{ijkl} = \mu + S_i + V_{j(i)} + P_k + t_1 + e_{ijkl}
$$

where μ = general mean; s = effect due to squares; $i = 1, 2, 3; c$ = effect due to cows within squares $j =$ 1, 2, 3; $p =$ effect due to experimental periods $k = 1$, 2, 3; *T* = effect due to treatments; *l* = 1, 2, 3; and *e* = residual error term.

Tukey's test was applied when significant differences were detected by ANOVA. Data were

analysed with Minitab 14.0 statistical software (Minitab, State College, PA, USA).

RESULTS AND DISCUSSION

Chemical composition of the rations

The bromatological characteristics of the rations were similar among treatments (Table 1). The average DM intake was 532.7 g kg^{-1} , which was inversely proportional to the inclusion of maize silage, while OM content was shared for all treatments 912.8 g kg–1. NDF and ADF increased in the treatment with the lowest maize silage inclusion.

The rations fed to the animals can be considered as average quality, since CP content exceeds 150 g kg^{-1} in good quality feed. On the other hand, NDF concentrations exceed 500 g kg– 1 , which could limit the digestibility of the rations (Partida-Hernández et al., 2019). These results agree with Piñeiro-Vázquez et al. (2017), who documented that N utilization in tropical areas is limited by low CP concentrations of pastures and high NDF, ADF and lignin content, which is more severe in the dry season, where rations supplied do not exceed a CP concentration of 60.6 g kg⁻¹ DM and NDF of 670.6 g kg⁻¹ DM.

Milk production and animal response variables by treatment

response variables and milk composition were similar among the treatments evaluated (p >0.05), even when maize silage supply was increased (Table 2). The average live weight was 424.2 kg with a milk production of 4.4 kg cow⁻¹ day⁻¹, with a fat content of 33.1 g kg⁻¹, CP of 31.5 g kg⁻¹, N 5.4 g kg⁻¹ and MUN of 9.0 mg dL⁻¹ (Table 2). These results agree with those of Arjona-Alcocer et al. (2020), who evaluated the use of mixed rations in Yucatan, Mexico. The authors used Holstein × Zebu cows with an average live weight of 450 kg in the second third of lactation, with a milk production ranging between 3.3 and 4.9 kg cow⁻¹ day⁻¹ during the experiment and a CP content of 32.4 g kg⁻¹. However, a study conducted by Bakyusa-Katongole et al. (2016) reported milk yields ranging from 6 to 7 kg row^{-1} day⁻¹ in cows from multiple crosses with local breeds. In this sense, the differences found in milk production with respect to this previous research may be due to the fact that the cows were in the second third of lactation and animal genetics (Arjona-Alcocer et al., 2020). Since the animals use a large part of the nutrients ingested in the maintenance of gestation, instead of depositing them in the mammary glands with respect to cows of high genetic merit (Arjona-Alcocer et al., 2020).

In the present study, MUN values ranged from 8.4 to 9.7 mg dL $^{-1}$, which is considered a moderate concentration. This indicates a correct nutrient supply since MUN concentrations <8.00 mg dL^{-1} is considered as insufficient in CP supply, while concentrations > 12 mg dL⁻¹ indicate an excess in nutrient supply (Siachos et al., 2019).

Table 1. Bromatological characteristics of rations used for animal feeding.

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Parameters	T1	T ₂	T3	Mean	SE	<i>p</i> -value
Live weight ($kg \text{ cow}^{-1}$)	423.30a	427.50a	421.80a	424.20	25.59	0.581
Milk production ($kg \text{ cow}^{-1} \text{ day}^{-1}$)	4.91a	4.16a	4.22a	4.43	0.56	0.833
Milk production FPCM (kg cow ⁻¹ day ⁻¹)	4.24a	3.71a	3.95a	3.97	0.43	0.598
Milk production (kg cow ⁻¹ treatment ⁻¹)	176.76a	149.76a	151.92a	159.48	0.52	0.729
Fat $(g \text{ kg}^{-1})$	30.54a	32.22a	36.57a	33.11	4.01	0.906
$CP (g kg^{-1})$	34.41a	34.04a	34.91a	34.45	0.87	0.127
$N(g kg^{-1})$	5.39a	5.34a	5.47a	5.40	0.14	0.124
$MUN (mg dL-1)$	8.37a	9.00a	9.68a	9.02	0.73	0.676

Table 2. Milk production, chemical composition and animal response variables by treatment.

CP, crude protein; N, nitrogen; MUN, milk urea nitrogen; SE, standard error.

Mean values followed by the same letters in the same row do not differ according to Tukey's test ($p > 0.05$).

These concentrations can be influenced by breed, lactation period, parity and CP intake. In addition, it influences N utilization and excretion by animals (Siachos et al., 2019).

Mass balance and nitrogen utilization

N intake, excretion in milk, urine and faeces showed no significant differences (p >0.05). Consequently, N balance and feed utilization efficiency, showed the same behaviour (Table 3).

DM intake was similar for the three treatments, but CP content increased numerically with the greater inclusion of maize silage, which was reflected in the 45.6 g of N with respect to T1. This agrees with Pozo-Leyva et al. (2021a; 2024), who found that an increase in the N concentration of the ration is not reflected in higher milk production or N content, but in the excretion of N from the slurry as reported in the present experiment.

The feeding strategy with the lowest N content was T1, which presented the lowest N concentration in slurry and the highest N output in milk. In addition, the treatment recorded lower N balance and higher FUE with respect to T2 and T3, coinciding with Niu et al. (2016). This demonstrates that quantifying N consumption and excretion in low-scale production systems helps to improve resource management on farms, increasing production, decreasing production costs due to N surplus and mitigating the environmental burden of the systems.

Aarons et al. (2017) have indicated that slurry from cattle production is considered the largest contributor to atmospheric N emissions regardless of housing strategy, whether confined or grazing. In addition, the same authors report that slurry N concentrations are directly proportional to ration N concentrations and are directly proportional to atmospheric ammonia emissions. In addition, urinary excretion is considered the main source of nitrate leaching to groundwater (Orlandi et al., 2020).

Overall, of the total N consumed, 41.3% was excreted in faeces (Pozo-Leyva et al., 2021a), 40.4%

in urine, only 8.5% in milk and 9.8% in animal maintenance, called N balance. However, Xie et al. (2019) found that 34.1% of N was excreted in faeces, 33.2% in urine, 28.1% in milk and 4.6% as N retained by the animal for maintenance. However, Aarons et al. (2017) reported that the N content of slurry was four times higher than that of milk, while Niu et al. (2016) reported that only 25% of the ingested N is converted into milk or meat. These differences may be explained by the higher live weight of animals, which implies higher feed intake, ration N content, animal genetics and higher milk production (Aarons et al., 2017). In fact, one of the strategies to dissipate N loads is increased milk production (Pozo-Leyva et al., 2019).

FUE behaved similarly among treatments, with values that fluctuated between 8 and 10%, which differs from Pozo-Leyva et al. (2021a) and Barros et al. (2017), who obtained FUE values of 19 and 31%, respectively. This could be interpreted as a low efficiency of the evaluated system, while productive strategies, feeding strategies, and environmental, ecosystemic and cultural conditions can account for the differences observed with respect to previous research. According to Akert et al. (2020), FUE is a very complex indicator, since even systems with low N supply in the feed can present a limited FUE, as is the case of T1.

Blood parameters

Regarding the blood parameters evaluated, no significant statistical differences were observed (p >0.05). On average, blood urea concentrations were 29.13 mg dL⁻¹, BUN 13.61 mg dL⁻¹, creatinine 0.83 mg dL⁻¹ and glucose 34.17 mg dL⁻¹ (Table 4).

The results obtained in the present study differ from those previously reported in the literature. For instance, Naveed-ul-Haque et al. (2018) reported BUN values ranging from 21 to 27 mg $d\bar{L}$ ⁻¹ and glucose values between 17.8 and 73.0 mg dL–1; Arjona-Alcocer et al. (2020) reported BUN concentrations from 13 to 19 mg dL^{-1} ; and Shekhar et al. (2010) obtained BUN concentrations from 23

Parameters	Τ1	T2	T ₃	Mean	SЕ	p -value
DM consumption ($kg \, day^{-1}$)	12.69a	12.83a	12.67a	12.73	0.76	0.559
CP intake (g day ⁻¹)	1607.82a	1746.16a	1892.90a	1748.96	107.71	0.601
N consumption (g day ⁻¹)	257.25a	279.39a	302.86a	279.83	17.23	0.601
Milk N output $(g \text{ cow}^{-1} \text{ day}^{-1})$	26.28a	22.09a	23.00a	23.79	2.85	0.712
Manure production ($kg \text{ cow}^{-1} \text{day}^{-1}$)	32.01a	31.51a	31.45a	31.66	0.48	0.669
DM in manure $(\%)$	18.70a	19.73a	21.18a	19.87	0.35	0.358
N in manure $(g \, kg^{-1})$	17.78a	18.12a	18.37a	18.09	0.69	0.569
Manure production (kg DM ⁻¹ cow ⁻¹ day ⁻¹)	6.02a	6.25a	6.69a	6.32	0.12	0.800
N of manure (g cow ⁻¹ day ⁻¹)	105.33a	113.69a	127.92a	115.65	6.07	0.800
Urine N $(g \text{ cow}^{-1} \text{day}^{-1})$	104.71a	112.59a	121.29a	112.86	9.15	0.658
Slurry N $(g \text{ cow}^{-1} \text{day}^{-1})$	210.04a	226.28a	249.21a	228.51	13.80	0.732
N balance (g cow ⁻¹ day ⁻¹)	20.93a	31.02a	30.65a	27.53	9.06	0.171
FUE $(\%)$	10.22a	7.91a	7.59a	8.57	0.71	0.212

Table 3. Nitrogen balance and feed utilization efficiency according to treatment.

DM, dry matter; CP, crude protein; N, nitrogen; FUE, feed utilization efficiency; SE, standard error. Mean values followed by the same letters in the same row do not differ according to Tukey's test ($p > 0.05$).

SU, serum urea; BUN, blood urea nitrogen; SE, standard error.

Mean values followed by the same letters in the same row do not differ according to Tukey's test $(p > 0.05)$.

to 26 mg dL⁻¹ and glucose levels between 50 and 53 mg $\text{d}L^{-1}$. These differences may be explained by the chemical composition of the ration ingredients, since blood indicators are closely related to the nutritional components supplied to the rumen, and their main variations are determined by the protein content of the ration (Arjona-Alcocer et al., 2020).

Naveed-ul-Haque et al. (2018) pointed out that, increasing ration CP concentrations increased BUN, while glucose concentrations were not affected by increasing dietary CP concentrations, which agrees with the results obtained in the present study.

The similarities in blood glucose concentrations among the different treatments could be due to a high rate of glucose utilization, and thus the homeostatic processes carried out in the animal's organism do not reflect considerable changes in glucose level (Shekhar et al., 2010). In fact, Arjona-Alcocer et al. (2020) have mentioned that blood glucose concentrations are closely related to milk yields, since lactose intervenes in the osmotic regulation of milk volume by using glucose in its synthesis. In addition, glucose provides carbon for lactose synthesis, which could explain the results found in the present study.

CONCLUSIONS

The increase in the inclusion of corn silage increased the CP and N contents of the diet supplied to the cows, but it did not result in a higher milk production. In general, 8.5% of the N consumed is excreted in milk, 41.3% in faeces and 40.4% in urine. Feed utilization efficiency was limited even in the treatment with the lowest N content in the ration (T1). A viable way to favourably influence these parameters is to increase milk production and maintain or reduce N intake to minimize possible detrimental effects on the environment. Finally, further research is required to evaluate the economic and productive viability of the dietary inclusion of different silages, based on local resources, as a feeding strategy to cope with the low availability of forages during dry periods in low-scale milk production systems.

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Author contributions

Dixan Pozo-Leyva, Fernando Casanova-Lugo, Felipe López-González, Ricardo L. D. Costa, and Alfonso J. Chay-Canúl had active participation in the bibliographic review. Dixan Pozo-Leyva, Fernando Casanova-Lugo, and Alvar A. Cruz-Tamayo, had active participation in the development of the methodology. Dixan Pozo-Leyva, Felipe López-González, Alvar A. Cruz-Tamayo, Ricardo L. D. Costa, and Alfonso J. Chay-Canúl had active participation in the discussion of the results. All authors reviewed and approved the final version of the article.

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