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Effect of lairage duration on some blood constituents and beef quality in bulls after long journey

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ABSTRACT

With the aim to contribute to the determination of an adequate resting time for cattle after long transportation, the effects of different lairage times on some haematic parameters and meat quality of bulls subjected to long commercial journeys were investigated. Thirty-nine Limousine bulls supplied by one farm located near to Saragoza (Spain) were examined after 5 consignments at the final destination, following a journey of 2,550 km to the "San Giorgio" abattoir (Palermo, Italy). Transport time was of 53.6±10.9h; lairage duration for bulls of the 1st, 3rd and 5th consignments was of 31h on average ("Short Lairage" group), whereas, for those of the 2nd and 4th consignments, was of 59 and 57h, respectively ("Long Lairage" group). As regards the blood cell counts, data showed a significant effect ($P<0.001$) of the lairage duration on leukocyte and platelet counts. No significant effect was observed for erythrocyte count, haemoglobin and hematocrit in relation to the lairage duration, although the repeated measure analysis of variance showed that, irrespective of lairage duration, the hematocrit increased significantly between unloading and slaughter. Haematological parameters showed a significant ($P<0.05$) effect of the lairage time only on CK and Cortisol. CK enzyme showed an increase in the "Short Lairage" group (33.2% vs. 14.3%) whereas, Cortisol showed a decrease in the "Long Lairage" group (36.3% vs. 3.8%). The different lairage duration did not significantly ($P>0.05$) affect the incidence of slight and severe carcass bruises. As regards meat quality, lairage duration significantly influenced the pH_u which was higher ($P<0.01$) in the muscle of the "Long Lairage" group, the luminosity at 24h post mortem which was significantly higher ($P<0.05$) in animals of the "Short Lairage" group, and the red and yellow indices which were higher in the "Long Lairage" group. The "Short Lairage" group showed a lower ($P<0.01$) value of cooking loss and higher ($P<0.01$) value of tenderness. Data show that pre-slaughter lairage duration after a long transport may influence the blood parameters as well as meat quality. On the whole, the increase in lairage duration over 36h does not determine any benefit for the animal's well-being whereas it can cause a reduction in beef quality. For very long transports, it would be better to have an adequate organisation of the facilities in order to diminish the pre-slaughter lairage duration.

Key words: Lairage time, Long transport, Blood parameters, Meat quality.

RIASSUNTO

EFFETTO DELLA DURATA DELLA SOSTA PREMACELLAZIONE SUI PARAMETRI EMATICI E SULLA QUALITÀ DELLA CARNE DI VITELLONI SOTTOPOSTI A LUNGO TRASPORTO.

Al fine di contribuire alla individuazione di un tempo adeguato di sosta al termine di un lungo trasporto, sono stati esaminati gli effetti di diverse durate della sosta al macello su alcuni parametri ematici e sulla qualità della carne in bovini maschi interi sottoposti a viaggi commerciali di lunga durata. Lo studio è stato condotto su 39 vitelloni Limousine allevati nelle medesime condizioni presso un unica azienda situata nelle vicinanze di Saragoza (Spagna). Gli animali sono stati esaminati al termine di 5 viaggi commerciali dopo un tragitto di 2500 km, presso lo stabilimento di macellazione "San Giorgio" di Gangi (Palermo). Il tempo di trasporto è stato pari a ore 53,6±10,9. Per i soggetti della prima, della terza e della quinta consegna, la durata della sosta è risultata compresa tra 24 e 36 ore, con una media pari a 31 ore (gruppo "Short Lairage"), mentre nella terza e nella quinta consegna la sosta è stata pari, rispettivamente, a 57 e 59 ore (gruppo "Long Lairage"). I risultati relativi all'esame emocromo-citometrico, hanno evidenziato un effetto significativo (P<0,001) della durata della sosta pre-macellazione sia sul numero dei leucociti che sulle piastrine. La durata della sosta non ha mostrato avere nessun effetto significativo su globuli rossi, emoglobina ed ematocrito anche se l'analisi della varianza per misure ripetute ha mostrato che, indipendentemente dalla durata della sosta, l'ematocrito è variato significativamente dal momento dello scarico a quello della macellazione, aumentando durante tale periodo. L'analisi statistica effettuata sui parametri ematochimici ha evidenziato un effetto significativo (P<0,05) della durata della sosta solo sull'enzima CK e sul cortisolo. L'enzima CK ha mostrato un incremento nel gruppo "Short Lairage" (33,2% vs 14,3%) mentre il Cortisolo ha mostrato una diminuzione nel gruppo "Long Lairage" (36,3% vs 3,8%). La durata della sosta non ha influenzato significativamente (P>0,05) l'incidenza di lesioni lievi e gravi registrate sulle carcasse. Per quanto concerne la qualità della carne, la durata della sosta ha influenzato significativamente il pH_u, risultato più elevato (P<0,01) nel muscolo dei soggetti del gruppo "Long Lairage"; la luminosità a 24h post mortem è risultata significativamente più elevata (P<0,05) nei soggetti del gruppo "Short Lairage" rispetto a quelli del gruppo "Long Lairage", mentre gli indici del rosso e del giallo sono risultati essere maggiori in quest'ultimo gruppo. Il calo peso dopo cottura è risultato significativamente minore (P<0,01) nel gruppo "Short Lairage", lo stesso gruppo "Short Lairage" ha fatto registrare carni significativamente più tenere (P<0,01). Dai risultati ottenuti emerge come la durata della sosta pre-macellazione dopo un trasporto di lunga durata può influenzare il quadro ematologico e la qualità della carne. Nel complesso è emerso che prolungare la sosta oltre le 36 ore non provoca alcun beneficio per il benessere dell'animale e rischia di peggiorare la qualità della carne. Nel caso di trasporti così lunghi come quelli esaminati sarebbe opportuno una migliore organizzazione della logistica al fine di ridurre il tempo di attesa degli animali prima della macellazione.

Parole chiave: *Sosta pre-macellazione, Lungo trasporto, Profilo ematico, Qualità carne.*

Introduction

Lairage before slaughtering has three important functions: it permits animals sanitary control *ante mortem*, it allows recovery from physical and nervous stress caused by previous transportation, and it guarantees a constant supply to the slaughtering chain. The presence of an adequate facility assigned for these functions is an essential requisite for slaughterhouses according to the European and Italian legislations (EC, 1991; Italian LD, 1994). It is not easy to identify optimum lairage duration at the abattoir to permit recovery from physical and psychological stress caused by previous transportation and to optimise the meat quality. For short transportation of less than 8 h, a recent study in Italy has shown that lairage duration increase is related to reduction in carcass value and to the decrease in the num-

ber of slaughtered heads per year (Nanni Costa *et al.*, 2001). This resting period is about 30 min for calves and 90 min for cows, whereas it becomes 33 min in abattoirs that handle 100,000 heads per year and 87 min for abattoirs that handle 10,000 heads per year.

In general, as indicated by Knowles (1999), there is the tendency to kill animals as soon as they leave the lorry to avoid dark cutting beef and carcass bruising. Nevertheless, after a long transport, an adequate lairage time is necessary for recovery from the stress of journey. Knowles *et al.* (1999) report that a lairage of 24h, with feed and water available, permits recovery from the stress caused by a journey lasting from 14 to 36h. Cockram and Corley (1991) observed that cattle kept overnight in the lairage had a greater concentration of free fatty acids at the time of slaughter than those slaughtered the same day of arrival, whereas no significant differences were observed in either blood composition or handling and behaviour between the two cattle groups. Gallo *et al.* (2003) reported a deterioration of meat quality in relation to the increase in duration of pre-slaughter period in steers that rested at the abattoir for 3, 6, 12 or 24h after a journey of 3 and 16h. Wythes *et al.* (1988) observed that steers kept in lairage for 26.5h had a higher mean bruise score than those that rested for only 2.5h while they did not find any effect of resting time or resting conditions on bruising in cows.

With the aim of contribute to the determination of an adequate resting time for cattle after long transportation, the study was carried out to evaluate the effects of different lairage time on some haematic parameters and meat quality of bulls subjected to long commercial journeys.

Material and methods

Animals, pre-slaughter handling, and behavioural evaluation

The study was carried out on 39 Limousine bulls of the same age (14±1 months, mean ±S.D.) and body weight (600±50 kg, mean ±S.D.) supplied by one farm located near Saragoza (Spain). The animals were examined after 5 consignments carried out using a semi-trailer vehicle in accordance with Reg. CE 411/98 , after a journey of 2,550km with a final destination of the "San Giorgio" abattoir in Gangi (Palermo, Italy). At each consignment, 32 animals were loaded onto the vehicle, which was divided in pens where the available surface per head was more than 1.7 m^2. A part of these animals were unloaded during the journey, thus, those examined at the abattoir were 8 in the early two consignments, 10 in the third, 9 in the fourth and 4 in the fifth. Transport time was of 53.6±10.9h (mean ±S.D.); the high variability was due to the time spent for the intermediate unloading as well as to road traffic during the journeys. Instead, lairage duration was conditioned by the day of arrival of the lorry at the abattoir, considering that all the animals were slaughtered on Mondays. For bulls of the 1st, 3rd and 5th consignments, which arrived between Saturday and Sunday, lairage duration was from 24 to 36h, 31 h on average, whereas for those of the 2nd and 4th consignments, which arrived on Friday, lairage was of 59 and 57h, respectively. Therefore, in relation to the wide variability of lairage duration, subsequently, the subjects were collected into two groups, called "Short lairage" and "Long lairage".

During lairage the environmental temperature from the 1st to the 5th consignment, was of 28 °C, 18.4 °C, 5.3 °C, 20.2 °C, 17.4 °C, respectively. The available surface per head in the resting box was over 2.1 m^2. Moreover, during the lairage period animals received water and straw *ad libitum* and were not mixed with

unfamiliar subjects. During the unloading and driving to the resting pens, and during handling through the race leading to the trap, number of falls, reversals, heads, mounts, balks, jumps, slips, evacuations and vocalizations were recorded for each subject. A description of behavioural events and loading and unloading terms were reported by Maria *et al.* (2004). At the abattoir, all animals were stunned by captive bolt.

Blood analysis

Immediately after unloading and at the exsanguination, individual blood samples (10mL) from each subject were collected from the jugular vein in a vacutainer® containing K-EDTA; then, they were split into two aliquots: on the first one (2mL), Leukocyte count (WBC), Erythrocyte count (RBC), Haemoglobin (Hgb), Hematocrit (Hct), Platelet count (Plt) were determined by using an automatic analyser (GENIUS - VET, SEAC®); the second aliquot (8mL) was centrifuged (ALC 4237R) at 3500xg for 15min within two hours of drawing and plasma was frozen at -20 °C until analysis (ASPA, 1999). On individual plasma sample Glucose, Non Esterified Fatty Acids (NEFA), Creatine Kinase (CK) contents were determined by using a biochemical automatic analyser (BM HITACHI 911 - Roche). Plasma cortisol was determined by enzyme-immunoassay using an automatic analyser DPC Immulite (Medical System) and a COR LKCO1 kit (Medical System).

Carcass and meat quality

At 30 min *post mortem*, carcass bruising was assessed using a 3 point scale (1 = none; 2 = slight; 3 = severe) on the basis of a photograph standard (Honkavaara *et al.*, 2003). Bruises were scored separately in different parts of carcass, i.e. shoulder, side, back, round and tail. The composite whole carcass score was the highest value assigned to each carcass part. At 45 min *post mortem*, pH measurement (pH_1) was made on *longissimus thoracis* muscle of each left half carcass, at the level of the 8th thoracic vertebra. The pH_1 was determined with WTW 330/SET (Weilheim, Germany) pH-meter equipped with glass electrode (Hamilton Double Pore™, Reno (NV) USA). After 24h *post mortem*, the pH (pH_u) measurement was repeated on the same muscle at the same position. Moreover, a sample of *longissimus thoracis* muscle between the 8th thoracic and 1st lumbar vertebra was collected from each left half carcass and transported (0°C - +2°C) to the laboratory for further analyses (ASPA, 1996). Colour (Illuminant D 65; Photometer SPECTRAL Scanner, DV), cooking loss (Honikel, 1998) and tenderness (WBS-INSTRON 5542) were determined. Colorimetric co-ordinates L* (Lightness), a* (redness index) and b* (yellowness index), were measured after 30 minutes of blooming, at 24h and 7 d *post mortem*. Hue angle (H) was also calculated. At 7d post mortem, water holding capacity of muscle samples kept at 4 °C was measured as cooking losses. Samples were held in plastic bags and immersed in a water-bath set to 95 °C until the internal temperature reached 65 °C as monitored by a thermocouple. Bags were then cooled under running water for 30 min at 15 °C (ASPA, 1996), dried with paper towels and reweighed; cooking losses were measured by dividing the difference between weights of uncooked and cooked samples by weight of uncooked samples and expressed as percentage. On the same cooked muscle samples tenderness was measured as a Warner Bratzler Shear Force (WBS); six "cores", of diameter of 10 mm, were removed from each cooked sample parallel to muscle fibre and sheared perpendicularly to fibres' direction by INSTRON 5542 equipped with Warner Bratzler shearing device, with a crosshead speed of 200 mm/min (ASPA, 1996).

Statistical analysis

The values of haematic parameters and

those of meat quality were analysed for normal distribution. The functions used to normalise data were the following: Log_{10} for Hgb, Hct, NEFA and CK, 1/n for RBC, $1/n^2$ for glucose and $1/\sqrt{n}$ for cortisol. Untransformed mean values are given in the tables. Blood analysis data were processed using a repeated measure analysis of variance by GLM procedure of SAS (2001). This model included transport time and environmental temperature during resting as covariates. Meat quality data were subjected to analysis of variance with the same procedure of SAS using the following model: $J_{ij} = \mu + Lairage_i + b_1 * Transport\ time_{ij} + b_2 * Resting\ environmental\ temperature + \varepsilon_{ij}$. including, as the previous model, transport time and the environmental temperature during resting as covariates. Exact Fisher test was used to evaluate differences in bruising frequencies between carcasses from the two lairage groups.

Results and discussion

Behavioural evaluation

The occurrence of behavioural events was extremely low in both lairage groups (data not shown). Due to adequate races and good handling before stunning, only slip and reversal events occurred on two subjects during unloading and driving.

Blood analysis

As regards the blood cell counts, lairage duration affected (P<0.001) leukocyte and platelet counts (Table1). However, values (Table 2) are within the physiological ranges observed for this species and age (Jain, 1986). Leukocyte count decreased during the lairage period (Table 2) and the decrease was higher in "Long lairage" group than "Short lairage," 18% and 10%, respectively. An increase in WBC during a long transport followed by a decrease at the end of the journey was observed by Earley *et al.* (2003) on heifers.

Lairage determined a decrease in platelet count which was so much higher as shorter was the period spent in the resting pens; this reduction was of 36% for animals of "Short Lairage" group and of 10% for those of "Long Lairage" (Table 2). Irrespective of lairage duration, higher platelet counts (Table 2) observed at the unloading could be due to vascular microtraumas caused by the cardio-circulatory effects of catecholamine (adrenalin and noradrenalin), released during handling operations, and by action of cortisol, (Zavy *et al.*, 1992).

In this study, lairage time showed no significant effect on erythrocyte count, haemoglobin and hematocrit (Table 1). Repeated measure analysis of variance showed that, irrespective of lairage duration, Hct increased significantly from unloading to slaughter (Table 2). Similar results were achieved by Tadich *et al.* (2005) who observed an increase in PCV in

Table 1. F value for repeated measure of analysis of variance and their significance

		Between subjects	Within subjects		
		Lairage	Time	Time x Lairage	Time x Transport time
WBC		17.68 ***	0.01 ns	5.04 *	1.04 ns
RBC	1/n	0.32 ns	0.33 ns	1.24 ns	0.51 ns
Hgb	Log_{10}	2.68 ns	2.55 ns	1.03 ns	0.13 ns
Hct	"	1.11 ns	12.21 ***	0.60 ns	5.32 **
Plt		22.15 ***	4.02 *	6.78 *	0.98 ns

*ns: not significant; * P<0.05; ** P<0.01; *** P<0.001.*

relation to the increase in lairage time from 3 to 24h in steers subjected to journey of 3 and 16h. Knowles *et al.* (1999) observed an increase in PCV only until 12h of recovery period and a decrease after 72h on steers and heifers subjected to 31h transport. Instead, Earley *et al.* (2003), in steers subjected to long transportation, observed lower hematocrit values during recovery time than during the journey. As is well known, dehydration or splenic contraction caused by sympathetic nerve activity or circulating catecholamine might increase HCT and PCV values (Mitchell *et al.*, 1988). In the present study HCT variation after lairage, that was not affected by its duration, could be due to splenic contraction at stunning rather than to dehydration of animals, because all subjects, in particular those of the "Long Lairage" group, had the opportunity to drink enough to recover from dehydration that probably occurred during long journeys.

Table 2. Changes in blood constituents as result of Short and Long Lairage (means).

		Short Lairage		Long Lairage	
		Unloading	Slaughter	Unloading	Slaughter
WBC	$10^3/\mu l$	6.40	5.76	7.96	6.53
RBC	$10^6/\mu l$	10.46	10.31	9.70	10.51
Hgb	g/dl	14.16	15.29	14.79	15.63
Hct	%	41.04	43.97	42.22	44.53
Plt	$10^3/\mu l$	505.09	322.19	622.25	559.01

Statistical analysis of the haematological parameters used as physiological indices of stress showed a significant effect of lairage time only on CK and cortisol (Table 3). CK enzyme, as reported in Table 4, showed plasmatic levels increase from unloading to exsanguination, particularly in "Short Lairage" group (33.2% *vs.* 14.3%).

High CK levels observed after unloading are due to the conditions and duration of transport whereas, the increase observed during lairage time could be due to the muscular activity of the animals during lairage as well as in the stunning trap. Warriss *et al.*

Table 3. F value for repeated measure of analysis of variance and their significance.

		Between subjects	Within subjects		
		Lairage	Time	Time x Lairage	Time x Transport time
Glucose	$1/n^2$	0.00 ns	0.07 ns	0.62 ns	0.08 ns
NEFA	Log_{10}	1.64 ns	1.88 ns	0.00 ns	1.21 ns
CK	Log_{10}	5.11 *	0.08 ns	0.31 ns	0.56 ns
Cortisol	$1/\sqrt{n}$	5.85 *	0.63 ns	1.42 ns	1.28 ns

*ns: not significant; * P<0.05.*

Table 4. Changes in plasma constituents as result of Short and Long Lairage (means).

		Short Lairage		Long Lairage	
		Unloading	Slaughter	Unloading	Slaughter
Glucose	mmol/l	4.49	4.79	4.49	4.79
NEFA	meq/l	0.86	0.63	0.86	0.63
CK	U/l	1422.59	1895.34	1908.53	2180.92
Cortisol	nmol/l	27.58	26.54	32.37	20.62

(1995) and Knowles *et al.* (1999) observed on steers and heifer, after a long journey of 15 and 31 h respectively, an increase in CK from arrival to 36h, reaching the control group level after 72h. Tadish et al. (2005) after a transport of 16h observed a high CK value upon arrival at the abattoir without further increase during 24h lairage time.

Cortisol plasmatic level was significantly influenced by lairage time ($P<0.05$) and the decrease was higher in the "Long lairage" group (36.3%) than the "Short lairage" group (3.8%) (Table 4). This result agrees with Mitchell *et al.* (1988) who showed a decline in plasmatic cortisol level after transport due to a decrease in stress inducing the stimulation of hypothalamic-adrenal cortex axis. Knowles *et al.* (1999), after a transportation of steers and heifers for up to 31h, found that the level of cortisol continued to increase after the journey, reached a peak after 12h of resting and then decreased steadily. Tadich *et al.* (2005) found an increase in cortisol during resting periods ranging from 3 to 24h, concluding that the handling prior to stunning could be responsible for this variation. In the present study, cortisol reduction observed after lairage could be due to a combined effect of the absence of psychological stress during handling and stunning and the physiological recovery, more evident after longer resting.

No significant difference was observed for glucose and NEFA (Table 3). The slight increase observed for glucose during short and long lairage time (Table 4) could be due to glycolysis caused by catecholamine according to Warriss *et al.* (1995). As regards NEFA levels, a slight decrease was observed probably related to the feeding availability during lairage.

Carcass and meat quality

Different lairage duration did not significantly affect incidence of slight and severe bruises ($P>0.05$) (data not shown). The 35.9% of the examined carcasses showed bruises that were mainly located (64.9%) on the rump and tail. This result is related to the frequent use of twist doors to push the animal on the trap. Thus, in this study the incidence of bruising was due, to a very

Table 5. F values of analysis of covariance and their significance for meat quality traits.

		F (Lairage)	F (Transport time)	F (T°)	SEM
pH_1		0.70 ns	0.10 ns	0.12 ns	0.196
pH_u		20.55 ***	16.34 ***	4.68 *	0.117
Colour 24 h post mortem:					
L*		4.61 *	1.34 ns	0.08 ns	4.912
a*		2.00 ns	4.02 *	0.02 ns	2.402
b*		4.19 *	8.29 **	13.92 ***	2.667
H		1.93 ns	8.90 **	11.00 **	0.104
Colour 7d post mortem:					
L*		0.96 ns	0.04 ns	0.16 ns	12.850
a*		12.28 **	4.35 *	10.98 **	2.959
b*		8.64 **	6.85 *	6.94 *	2.735
H		0.99 ns	1.32 ns	0.90 ns	0.113
Cooking losses	%	10.90 ***	24.65 ***	2.13 ns	4.598
Shear force	kgf/cm^2	12.25 **	10.02 **	4.21 *	1.593

*ns: not significant; * P<0.05; ** P<0.01; *** P<0.001.*

Table 6. Effect of lairage time on meat quality traits (least square means ± SE).

		Short Lairage	Long Lairage
pH:			
pH1		6.64 ± 0.05	6.56 ± 0.06
pHu		5.30 ± 0.03 ^{A}	5.56 ± 0.03 ^{B}
Colour 24 h post mortem:			
L*		38.74 ± 1.34 ^{a}	33.40 ± 1.60 ^{b}
a*		22.33 ± 0.65	24.06 ± 0.78
b*		6.95 ± 0.73 ^{a}	9.71 ± 0.87 ^{b}
Hue		0.31 ± 0.02	0.38 ± 0.03
Colour 7 d post mortem:			
L*		39.69 ± 1.35	37.23 ± 1.64
a*		22.14 ± 0.81 ^{A}	27.39 ± 0.97 ^{B}
b*		6.31 ± 0.74 ^{A}	10.38 ± 0.89 ^{B}
Hue		0.29 ± 0.03	0.35 ± 0.04
Cooking losses	%	28.94 ± 1.25 ^{A}	36.62 ± 1.50 ^{B}
Shear force	kgf/cm2	5.14±0.46 ^{A}	8.85 ±0.74 ^{B}

a, b: P<0.05; A, B: P<0.01.

large extent, to a questionable practice applied just before stunning.

The lairage duration has significantly influenced the pH_u (Table 5), which was higher (P<0.01) in the muscle of "Long Lairage" animals than that of the muscle of "Short Lairage" subjects, although this value (Table 6) was under DFD meat threshold levels (Shackelford et al., 1994). The high ultimate pH of meat was probably a consequence of depleted muscular glycogen reserves prior to slaughter, in relation to the longer diets restriction of the "Long Lairage" group (Silva *et al.*, 1999).

Regarding colorimetric parameters, luminosity at 24h post mortem was significantly higher (P<0.05) in the "Short Lairage" group than the "Long Lairage", whereas, in the latter, yellow index was higher (Table 6), probably due to lower meat acidification. In accordance with the variation in pH_u, the meat of animals that rested for a longer time showed grater redness at 7 days post mortem. These results are in accordance with the observations of Gallo et al. (2003) about the negative effect of long lairage on meat colorimetric parameters.

The "Short Lairage" group showed significantly (P<0.01) lower values of ultimate pH, cooking loss and tenderness than those observed for the "Long Lairage" group. These results are in accordance with Guignot et al. (1994) who found a linear relationship between ultimate pH and tenderness. The hypothesis to explain this linear relationship suggested that low pH_u value causes injury to the lysosome membranes and protease leak, and therefore, autolysis, with a consequent increase of the tenderness.

Conclusions

Data show that pre-slaughter lairage duration after a long transport may influence, although slightly, the blood parameters as well as the meat quality. On the whole, the increase in the lairage duration over 36h does not determine any benefit for

the animal's well-being whereas it can cause a reduction in the beef quality. For such long transports, it would be better to have an adequate organization of the facilities in order to diminish the pre-slaughter lairage duration

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