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Original article

Treatment of ligno-cellulosic residues with urea. Influence of dosage, moisture, temperature and addition of ureases

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Summary — Comparative studies were made at a laboratory level to assess the effectiveness of the treatment of straw with anhydrous ammonia (anhydrous NH_3) and urea. The influence of moisture, dosage, and temperature were also assessed. The influence of the addition of an urease source was studied in treatments with urea. Two experiments were performed: 1), Anhydrous-NH_3 and urea were used separately to treat a total of 13 samples of barley straw. Anhydrous NH_3 was applied to 3 samples at a dosage of 4% and at moisture levels of 9.2, 22.2 and 32.7%. Urea was applied to 9 batches at doses at 4.3, 6.5 and 8.0% and at 3 moisture levels: 22.2, 31.1 and 42.7%. One sample was left untreated. 2), Urea at a dosage of 3% was applied to 24 samples of wheat straw and 24 samples of maize stover at 4 moisture levels, 10, 20, 30 and 40% and 3 temperatures: 15, 25 and 35 °C for wheat straw and 5, 15 and 25 °C for maize stover. All these parameters were studied both with and without the addition of crude soya bean (CSB).

In experiment 1 the increase in moisture content when NH_3 was applied reduced the neutral detergent fibre (NDF) content, but did not modify the *in vitro* dry matter digestibility (IVDMD) values, which were similar to those obtained on the application of urea at moisture levels of 31.1 and 42.7%. In experiment 2, the increase in moisture content to 30% produced a positive response in most of the chemical parameters analyzed. However, with an increase in humidity to 40% the results were less homogeneous. The temperatures used were not limiting, and only had a significant effect ($P < 0.05$) on the NDF and total nitrogen (N) contents. For maize stover the temperature did not influence ($P > 0.05$) either the degree of ureolysis or the IVDMD. The addition of CSB had no significant effect ($P > 0.05$) when applied to the maize stover, and when applied to wheat straw its only significant effects ($P < 0.05$) were on the NDF and IVDMD values. At low moisture levels the addition of CSB increased the degree of ureolysis in wheat straw.

straw / chemical treatment / anhydrous ammonia / urea / moisture / temperature / urease

Résumé — Traitement de résidus ligno-cellulosiques à l'urée. Influence de la dose, de l'humidité, de la température et de l'addition d'uréase. *Des essais comparatifs ont été effectuées à l'échelle d'un laboratoire, pour évaluer l'efficacité du traitement de la paille à l'ammoniac anhydre ou à l'urée, ainsi que l'influence de l'humidité, de la dose d'urée, de la température et de l'addition d'une source d'uréase dans le traitement à l'urée. On a réalisé 2 essais :*

– sur 13 échantillons de 2 kg de paille d'orge, 3 traitements à l'ammoniac anhydre ont été effectués à une dose de 4% et à des niveaux d'humidité de 9,2, 22,2, et 32,7%. On a effectué en outre 9 traitements à l'urée à 3 niveaux d'humidité (22,2, 21,1 et 42,7%) et 3 doses d'urée (4,3, 6,5 et 8,1%);

– 24 échantillons de paille de blé et 24 échantillons de cannes de maïs ont été traités à l'urée à 3%, à 4 niveaux d'humidité 10, 20, 30 et 40%, 3 températures (15, 25 et 35 °C pour la paille de blé et 5, 15 et 24 °C pour les cannes de maïs) avec addition ou non de farine cru de soja (CSB), source d'uréase.

Dans l'expérience 1, on a observé que l'augmentation de l'humidité dans le traitement à l'ammoniac réduisait la teneur en NDF (fibre neutre détergente), mais qu'elle ne modifiait pas les valeurs de la

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IVDMD (dégradabilité de la matière sèche in vitro*) qui étaient similaires à celles obtenues dans les traitements à l'urée à des niveaux d'humidité de 31,1 et 42,7%.*
Dans l'expérience 2, l'augmentation du taux d'humidité jusqu'à 30% a provoqué une réponse positive de la plupart des paramètres analysés mais moins uniforme de 30 à 40%. Les températures utilisées n'ont pas été limitantes, et ont eu un effet significatif (P < 0,05) sur les teneurs en NDF et en azote total (N). La température n'a eu d'influence ni sur le degré d'uréolyse, ni sur la IVDMD des cannes de maïs. L'addition de CSB n'a pas affecté (P > 0,05) l'efficacité du traitement des cannes de maïs et n'a eu un effet significatif (P < 0,05) que sur la teneur en NDF et la IVDMD de la paille de blé.

paille / traitement chimique / ammoniac-anydre / urée / humidité / température / urease

INTRODUCTION

Ligno-cellulosic residues are an important source of feed for ruminants in many parts of the world. They are produced in large quantities, but have a low nutritional value. Ways of improving their nutritional value which are compatible with the practical needs and practices of farmers are being investigated. The application of alkalis (Sundstøl and Owen, 1984) has being developed unevenly over the last few decades. The most widely used treatment among farmers is the application of anhydrous NH_3.

Hadjipanoyiotou (1982) used the addition of urea as an alternative to anhydrous NH_3. The method was based on the fact that urea would turn into ammonia in the presence of adequate quantities of ureases under specific moisture and temperature conditions. To a large extent, these conditions determine the effectiveness of the method.

Existing information on the influence of moisture content and temperature on the application of anhydrous NH_3 or urea is often contradictory (Borhami and Sundstøl, 1982; Cloete *et al,* 1983; Ørskov *et al,* 1983; Mandell *et al,* 1988). The addition of a source of urease to facilitate the process also produces results that are sometimes contradictory (Kiangi *et al,* 1981; Jayasuriya and Pearce, 1983; Williams *et al,* 1984b; Wanapat *et al,* 1985).

This paper attempts to determine the effects of treatments with urea on different materials at different levels of moisture content, temperature and dose, and the presence or not of a source of urease.

MATERIALS AND METHODS

Experiment 1: Treatment of barley straw with anhydrous NH_3 and urea

Thirteen samples of 2 kg shredded (2 cm) barley straw (cv Georgia) were treated and kept in closed plastic bags (60 x 100 cm). Three treatments with anhydrous NH_3 (40 g/kg DM) at moisture levels of 10, 20 and 30% and 9 treatments with urea (40, 65, and 80 g/kg DM) at 3 moisture levels: 20, 30 and 40%.

The anhydrous NH_3 treatments were injected directly into the bags containing the previously moistened straw. The urea treatments were applied manually as evenly as possible. The bags were closed and kept at laboratory temperature for 2 months.

Experiment 2: Influence of temperature, moisture and addition of crude soya bean (CSB) on the effectiveness of the treatment of different ligno-cellulosic materials with urea

Forty-eight 1.5-kg samples of wheat straw (cv Anza) (WS) and maize stover (cv Pionner-3283) (CS) were treated with commercial urea (46%

Table I. Experimental design for urea solution treatments on wheat straw and maize stover, applied in plastic containers (Exp 2).

Wheat straw (WS)			*Maize stover (CS)*		
Temp (°C)	*CSB*	*Moisture (%)*	*Temp (°C)*	*CSB*	*Moisture (%)*
	*	11.6		*	15.2
	–	10.9		–	15.5
	*	24.2		*	26.0
	–	25.2		–	26.0
15	*	36.3	5	*	39.2
	–	38.0		–	36.0
	*	47.9		*	51.8
	–	44.7		–	54.2
	*	13.3		*	15.2
	–	13.3		–	16.1
	*	25.2		*	29.8
	–	25.8		–	30.0
25	*	38.6	15	*	39.9
	–	39.2		–	41.9
	*	51.0		*	50.8
	–	50.7		–	49.9
	*	12.7		*	14.5
	–	12.9		–	15.6
	*	23.9		*	28.2
	–	23.6		–	27.9
35	*	36.5	25	*	39.5
	–	36.9		–	41.4
	*	49.0		*	50.5
	–	51.7		–	48.4

* Crude soya bean added (CSB).

nitrogen). Table I shows the different temperature and moisture conditions, and whether or not CSB was added. Temperatures were chosen for their similarity to ambient temperatures at different harvest times. The urea doses applied were 30 g/kg DM (WS) and 32.5 g/kg DM (CS), and the CSB doses were 33 g/kg DM (WS) and 35 g/kg DM (CS).

All the samples were shredded in a hammer mill through a 2-cm diameter sieve. The treatments were performed manually. The treated material was kept in double-capped plastic containers for 2 months in 4 climatic chambers at a constant temperature.

Chemical and biological analysis

After drying in a ventilated oven at 60 °C the samples were ground in a bladed mill through a 0.8-mm sieve. The following parameters were analyzed: ash, crude fibre (CF) and total nitrogen (N) (AOAC, 1984); neutral detergent fibre (NDF) (Goering and Van Soest, 1970); unhydrolysed urea (Watt and Chrisp, 1954); *in vitro* dry matter digestibility (IVDMD) (Tilley and Terry, 1963); organic matter or dry matter cellulase digestibility * (OMD or DMD-cellulase) (Aufrere, 1982); *in sacco* dry matter degradability (48 h) (DMD, *in sacco*) using 4 rumen fistulated wethers (Mehrez and Ørskov, 1977).

Statistical analysis

The results obtained were subjected to variance analysis (Steel and Torrie, 1980) using the statistical analysis system (SAS) (1987). Since repetitions were lacking, triple interaction was taken as error. To determine the main effects, nonsignificant double interactions were added to the error. When the double interaction was significant, analysis was not continued (Baucroft, cited by Sokal and Rohlf, 1979).

RESULTS

Experiment 1

Table II shows the results of the chemical parameters analyzed. In treatment with anhydrous NH_3 the increased moisture caused a reduction in NDF content of 4.3, 10.5 and 13.5 percentage units for moisture levels of 9.2, 22.2 and 32.7% respectively.

In the treatments with urea (table II) the presence of variable amounts of residual urea permitted an estimation of the degree of ureolysis achieved. A positive response was obtained in line with moisture. At a moisture level of around 40%, ureolysis was practically total, while at around 20% moisture the degree of ureolysis was between 30–50%. On the other hand, increasing the dose of urea brought about a negative response in ureolysis.

Crude fibre and ash contents were apparently unaffected by the urea treatments, and NDF content decreased. This tendency was greater at the intermediate moisture level (30%). The results obtained by means of IVDMD and OMD-cellulase also showed some degree of moisture influence, and coincided with the largest falls in NDF content.

Experiment 2

Table III shows the effects of temperature, moisture and CSB application on the chemical values carried out on the urea treatment of wheat straw and corn stover. The analytic values of these treatments are shown in figures 1 and 2.

Wheat-straw treatments

The increase in temperature (15, 25 and 35 °C) caused a reduction in OM content (92.4, 90.8, 90.7%) ($P < 0.001$), an increase in total N (0.95, 1.01, 1.32%) ($P < 0.001$) and a reduction in NDF content (78.6, 76.1, 75.8%) ($P < 0.001$).

The effect of moisture gave a clear response ($P < 0.001$) in total N content, where it was 1.42, 1.01, 0.99 and 0.97% respectively for moisture levels of 10, 20, 30 and 40%. The degree of ureolysis is influenced by moisture content; however, there is an interaction ($P < 0.01$) with the temperature and the presence of ureases. The DMD-cellulase and DMD *in sacco* parameters were affected ($P < 0.01$, $P < 0.05$) by the moisture content, with temperature–urease interactions ($P < 0.05$).

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Table II. Chemical composition, unhydrolysed urea, *in vitro* DM digestibility (IVDMD) and cellulase organic matter degradability (OMD-cellulases) for barley straw treated with anhydrous NH3 or urea under differents conditions (Exp 1).

Treatment	*Urea (%) DM basis*	*Moisture (%)*	*Ash (%)*	*Nitrogen (%) NT (%)*	*Crude fibre (%)*	*NDF (%)*	*ADF (%)*	*ADL (%)(*	*N-urea unhydrol (%)*	*IVDMD (%)*	*OMD cellulases*
1 Untreated	–	9.2	4.0	0.53	44.6	84.6	49.8	7.6	–	–	–
2 NH_3 Treatment	(4.0)	9.2	4.3	1.34	44.5	80.3	49.6	8.9	–	50.8	–
3 NH_3 Treatment	(4.5)	22.2	4.2	1.75	45.9	74.1	51.9	9.8	–	50.6	–
4 NH_3 Treatment	(4.1)	32.7	4.9	1.72	46.2	71.1	52.6	13.6	–	52.9	–
5 U-Treatment	(4.3)	22.2	3.8	1.98	44.9	83.2	51.8	12.4	1.0	42.8	30.8
6 U-Treatment	(4.3)	31.1	4.0	1.55	45.0	80.3	–	–	0.3	51.5	34.6
7 U-Treatment	(4.3)	42.7	3.8	0.98	46.2	82.8	51.9	8.3	0	47.6	31.2
8 U-Treatment	(6.5)	21.2	3.9	3.59	44.1	81.7	49.6	8.1	1.7	43.2	31.1
9 U-Treatment	(6.5)	31.6	4.6	1.86	44.1	79.4	53.7	8.3	0.6	50.8	35.0
10 U-Treatment	(6.5)	43.1	4.3	1.39	46.5	80.3	53.3	8.4	0.2	51.4	32.5
11 U-Treatment	(8.0)	21.8	4.7	3.50	43.6	82.3	50.3	8.0	2.6	40.5	31.3
12 U-Treatment	(8.0)	32.5	4.4	2.61	44.7	78.7	51.3	8.7	1.4	53.3	36.6
13 U-Treatment	(8.0)	43.1	4.9	1.98	44.7	77.6	51.6	8.7	0.5	51.4	36.5

Table III. Significance of the effects of temperature, moisture and urease application on different parameters (Exp 2).

Neutral	*Organic matter*	*Deterg fibre*	*N-urea resid*	*Total N*	*IVDMD*	*DMD cellulases*	*DMD in sacco*
Wheat straw treatments							
Temperature (T)	***	***	–	***	–	–	–
Moisture (M)	NS	NS	–	***	–	**	*
Urease (U)	NS	***	–	NS	*	–	–
Interactions :							
(T x M)	NS	NS	**	NS	*	NS	NS
(T x U)	NS	NS	NS	NS	NS	*	*
(M x U)	NS	NS	**	NS	NS	NS	NS
X	91.30	76.83	0.30	1.10	29.26	27.15	44.19
Root MSE	0.430	1.040	0.065	0.117	1.660	0.983	3.347
Corn stover treatments							
Temperature (T)	*	**	NS	*	NS	–	–
Moisture (M)	NS	NS	***	**	NS	**	–
Urease (U)	NS	NS	NS	NS	NS	–	NS
Interactions :							
(T x M)	NS	NS	NS	NS	NS	NS	***
(T x U)	NS	NS	NS	NS	NS	**	NS
(M x U)	NS	NS	NS	NS	NS	NS	NS
X 90.65	72.75	0.28	1.48	46.32	34.93	53.43	
Root MSE	1.047	1.865	0.183	0.207	4.527	1.163	2.204

Significance : * $P < 0.05$; ** $P < 0.001$ and NS = $P > 0.05$; MSE : mean square error.

The application of CSB caused a reduction in NDF content ($P < 0.001$), with a reduction from 77.7 to 75.9%, and a slight increase ($P < 0.05$) in the IVDMD value. It had no effect on the total N content ($P > 0.05$).

Maize stover treatments

With the increase in temperature (table III) there were changes in total N ($P < 0.05$) and NDF ($P < 0.01$). There were no changes in the degree of ureolysis or in the IVDMD ($P < 0.05$). On average, at temperatures of 5, 15 and 25 °C, NDF was 71.8, 74.8 and 71.7%, and total N content was 1.39, 1.47 and 1.63% respectively.

The increases in moisture level led to a considerable drop in the residual urea content ($P < 0.001$) (from 0.84 to 0.08% of N, on average), and the total N content decreased ($P < 0.01$) (from 1.81 to 1.40% of N). There was a significant variation in DMD-cellulase ($P < 0.01$) with increases in moisture content (33.2, 35.9, 36.1 and 34.6% respectively for the 4 moisture levels). The application of ureases caused no significant response ($P < 0.05$) in any of the parameters studied.

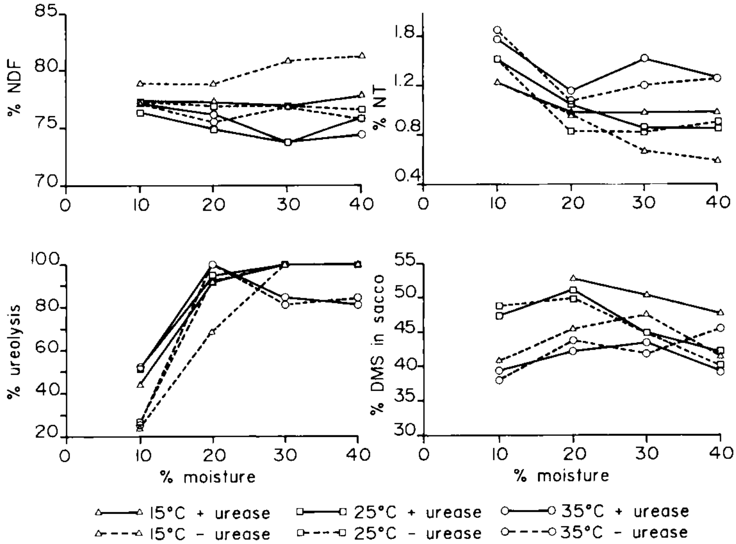

Fig 1. Neutral detergent fibre (NDF) and total N contents, urea hydrolysation rate, dry matter degradability *in sacco* (48 h) (DMD- *in sacco*) from wheat straw treated with 3% urea at 3 different temperatures and four levels of moisture (Exp 2).

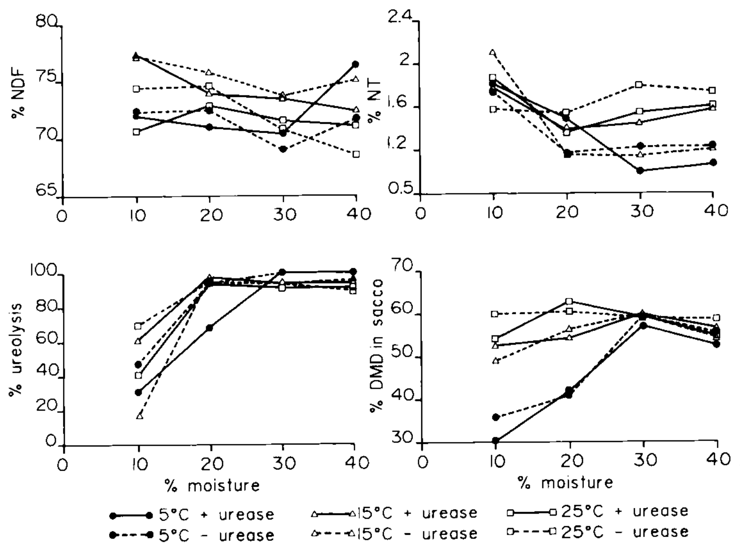

Fig 2. Neutral detergent fibre (NDF) and total N contents, urea hydrolysation rate, dry matter degradability *in sacco* (48 h) (DMD- "*in sacco*") from maize stover treated with 3% urea at 3 different temperatures and 4 levels of moisture (Exp 2).

DISCUSSION

Anhydrous NH_3 treatment vs urea treatment

In anhydrous NH_3 treatment, the variation in moisture content did not affect the IVDMD, although there was less N retained in the driest treatment. This coincides with the findings of Waiss *et al* (1972) and Solaiman *et al* (1979) who used ammonia in an aqueous solution, and with Kiangi *et al* (1981) and Mandell *et al* (1988) who used higher moisture levels. According to Borhami and Sundstøl (1982) increases in moisture of up to 10% would give positive responses, and the addition of more water would only be necessary if very high doses of ammonia were employed, which would lead to the retention of a quantity of N which would be unsuitable for animal nutrition (Dryden and Leng, 1986). In practical conditions of treatment with anhydrous NH_3, Ibbotson *et al* (1983, 1984) found no influence due to moisture content.

The increase in the residual urea and total N content with the increase of the urea dose coincides with the findings of Jayasuriya and Pearce (1983) who estimated the maximum dose of urea at 6%, at moisture levels higher than those studied here. There was no influence of the 3 doses of urea on the IVDMD and OMD-cellulase. This coincides with the observations of Williams *et al* (1984a) in *in sacco* results and of Abdouli and Khorchani (1987) for *in vitro* results with treatments at 37 °C. There could be a tendency for the NDF content to decrease when the urea dose is increased. This coincides with the results of Abdouli and Khorchani (1987). The values obtained in IVDMD for anhydrous NH_3 treatments are comparable to those obtained with urea at 40–60 g/kg DM and 30% moisture content.

Influence of moisture on urea treatment

The increase in moisture content caused an increase in the degree of ureolysis and greater nitrogen retention. In WS the application of ureases at low moisture levels had a positive effect on the degree of ureolysis. This coincides with the findings of Besle *et al* (1990a,b). However in CS the application of ureases did not have any effect. Williams *et al* (1984a), working at moisture levels of between 25–35%, also observed a greater degree of ureolysis with increased moisture.

In general, both experiments (*ie*, 1 and 2) were more effective when the moisture content was raised from 20 to 30%. However, increasing moisture to 40% did not lead to better results in all cases. Ibrahim *et al* (1986) also estimated optimum moisture content to be ≈ 30%.

Influence of temperature

In the anhydrous NH_3 treatments carried out in stack, Alibes *et al* (1984) refer to the necessity of high ambient temperatures to produce optimum results and, in particular, good nitrogen retention. Thus, the limited effectiveness of some treatments (Mandell *et al,* 1988; Mann *et al,* 1988) has been attributed to low temperatures. It is reasonable to consider, therefore, that in treatments with urea in which the temperature does not rise due to the treatment, ambient temperature is a factor which determines successful treatment.

Temperature increase (between 5–35 °C) was not decisive in determining the specific degree of ureolysis, but did seen to determine the accompanying greater nitrogen retention. Cloete and Kritzinger (1984) did not observe sufficient ureolysis at 4 °C; Bennahmed and Dulphy (1985) and Besle *et al* (1990b), working in low winter temperatures, observed ureolysis of

19 and 17%. However, Williams *et al* (1984b) indicated that there could be sufficient ureolysis at 5.5 °C, although the process would be slower.

In our experiments, none of the temperatures used had an important effect on the process. However, at extreme temperatures (5 and 35 °C) there are occasional interactions which should be taken into account. On the other hand, Cottyn and De Boever (1988) did not achieve effective treatments at an average daily temperature of 5 °C.

Influence of adding ureases

In general, the effects on digestibility and other chemical/biological parameters of adding a urease source was little or none (Kiangi *et al,* 1981; Jayasuriya and Pearce, 1983; Wanapat *et al,* 1985; Williams *et al,* 1984b). This could be due to the great diversity of added CSB sources (Ibrahim *et al,* 1986) to the wide variety of dosages applied: 6 g/kg DM (Dias Da Silva *et al,* 1988); 50 g/kg DM (Williams *et al,* 1984b) and of moisture content. The presence of ureases considerably reduces the treatment time (Jayasuriya and Pearce, 1983; Williams *et al,* 1984b). On the other hand, the best responses have been shown by Besle *et al* (1990a), where moisture content was reduced to 20–25%; these responses not only affected ureolysis, OMD and N retention but also intake (for reasons still to be identified).

In maize stover treatments there was no response likely to the addition of 3% CSB, whereas in wheat straws treatments there were some changes. Cloete and Kritzinger (1984) found worse results at 35 °C and according to them this could be due to the reduction in ureasic activity at this temperature.This coincides with Du Preez 1983 (cited by Cloete and Kritzinger, 1984), who concluded that ureases showed the greatest activity at 30 °C.

CONCLUSION

Treatment with urea in an aqueous solution has always improved the nutritional quality of the original by-product. The factors studied (dosage, moisture content, temperature and presence of ureases) play a fundamental role. These treatments could be applied specifically in tropical or arid climates, and would adapt particularly well to Mediterranean climates. In this group of climatic regions, it could prove of interest to reduce as far as possible the amount of water applied. This seems to be possible (optimum moisture levels between 25 and 30%), even at lower moisture levels, where additional ureases would have to be used.

A 4% urea dose seems to be optimum, and the range of temperatures studied (5–35 °C) is compatible with the treatment. The ureolytic activities of the straws used is probably sufficient, making the addition of ureases unnecessary.

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