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Use of In-Vitro Gas Production to Evaluate Rumen Fermentation of Untreated and Urea Treated Finger Millet Straw (*Eleusine coracana*) Supplemented with Different Levels of Concentrate

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Abstract: Measurement of gas produced during in-vitro fermentation was used to assess the fermentability of urea treated and untreated finger millet straw to which various levels of concentrate (maize/wheat bran/groundnut cake 35 : 32 : 30) had been added. Data obtained from this technique were compared with in-vitro digestibility data obtained earlier on the same feeds. Similar trends for the effect of supplementation on digestibility were observed in both in-vitro and in-vivo measurements. Linear correlation equations were obtained between gas produced and the proportion of dry matter disappearing, enabling in-vitro gas production to be expressed in in-vitro digestibilities. A single pool exponential equation was fitted to the gas production data enabling estimates to be made of the time when in-vitro digestibilities best matched in-vivo digestibility data. These times were 45·8 and 47·9 h of untreated straw, 43·5 and 61·0 h for treated straw for trials 1 and 2, respectively. Statistically significant ($P < 0{\cdot}05$) interactive effects between supplement and both treated and untreated straws were observed. The digestibility of untreated straw was particularly stimulated by small quantities of supplement. The digestibility of treated straw was higher than that of untreated straw and less stimulated by supplementation. These findings are consistent with the hypothesis that fibre digestibility can be increased by providing a supplement which provides sufficient nutrients to stimulate the activity of rumen micro organisms.

Key words: straw, supplementation, urea treatment, gas production, rumen fermentation, digestibility.

INTRODUCTION

In-vivo measurement of the intake and digestibility of ruminant feeds can be a good guide to the potential performance of animals on particular diets, but they are time consuming, involve a high labour input and require a large number of animals of the same age, breed and sex to minimise experimental variations. In-vitro techniques are relatively simple and large numbers of samples can be assessed at one time but have some limitations.

Enzymic methods (eg Jones and Hayward 1975) may be insensitive to factors such as associative effects and toxins which can affect microbial degradation. Both enzymic and the standard Tilley and Terry (1963) methods measure end point digestion and do not provide information on the kinetics of digestion. As digestibility is affected by rate of passage (Orskov and McDonald 1979), methods which give data for a fixed incubation period could be misleading. Incubations

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could be ended at different times to obtain kinetic data but this greatly increases the effort involved.

Measurement of gas production, based on head-space pressure as developed by Theodorou and co-workers (Merry *et al* 1991; Theodorou *et al* 1991) is a simple in-vitro procedure, not requiring expensive glass syringes like the comparable Menke gas production method (Menke *et al* 1979). The Menke method has been shown to be a useful indicator of in-vivo dry matter intake, digestible dry matter intake and growth rate for barley and wheat straws (Blummel and Orskov 1993). Not much information is available, however, about how data obtained using the Theodorou method compare with in-vivo data, nor on whether this method is useful for investigating associative effects between different feeds. The present study was undertaken to look at associative effects in-vitro using the Theodorou gas production technique and compare them to in-vivo dry matter digestibilities of urea treated and untreated finger millet straw at different levels of concentrate supplementation. The concentrate was formulated to provide a balanced supply of protein and energy.

EXPERIMENTAL

Feed samples

The feed samples used in the gas production technique were from the same batch of feeds which had been used in in-vivo digestibility trials. The composition of these feeds is presented in Table 1. Finger millet (*Eleusine coracana*) straw, which is referred to subsequently as treated straw, was sprayed with an aqueous solution of urea (50 g urea litre^{-1}) at the rate of 1 litre urea solution per kg straw and stored in a pit for 14 days (Prasad *et al* 1991). Untreated finger millet straw was from the same batch of straw which had been used to prepare the treated straw. The concentrate mixture used as a supplement had the following ingredients (on a fresh weigl basis):

maize: 35%
wheat bran: 32%
ground nut cake: 30%
mineral mixture: 2%
salt: 1%

For the in-vivo experiment 16 crossbred heifers (18–2‹ months of age) were used, eight receiving untreatec straw and the other eight the treated straw *ad libitum* From each group two animals received the supplement at levels of 0, 1·0, 2·0 and 3·0 kg per day. The feeding trial was carried out for a period of 45 days, after which total faeces was collected for 7 days to measure digestibility (Prasad *et al* 1991). The samples were stored for about 2 years at ambient temperatures in airtight containers after completing in-vivo trials until the in-vitro studies were conducted.

Experimental design

Two trials using the gas production technique were conducted with a total substrate weight (fresh basis) of 1 (±0·0020) g per treatment replicate and different proportions of supplement/straw. In the first, the weights of concentrate were selected to be similar to the range of proportions of these feeds actually consumed by heifers in the in-vivo trials (see Table 2 for the in-vivo data and the weights of concentrate required *in vitro* to give the same supplement/straw proportions). Untreated and treated straw were fermented alone and with the following weights of concentrate (g fresh basis): 0·20, 0·35, 0·50 plus untreated straw to give a total of 1 g substrate; 0·15, 0·27, 0·35 plus treated straw to give a total of 1 g substrate. The concentrate was also fermented alone.

In the second trial 10 different proportions of concentrate/straw plus straw and supplement alone

TABLE 1
Composition of feeds offered for in-vivo digestibility and used in in-vitro gas production experiments[a]

	Organic matter (g kg^{-1} dry matter	*Nitrogen (g kg^{-1} dry matter)*	*Neutral detergent fibre (g kg^{-1} dry matter*	*Ether extract (g kg^{-1} dry matter*
Untreated finger millet straw	930	4·8	780	11·7
Urea treated finger millet straw	927	15	765	9·8
Concentrate mixture[b]	883	32	272	57

[a] Data taken from Prasad *et al* (1991) except at note[b] which is taken from Prasad *et al* (1994).

TABLE 2
In-vivo dry matter digestibility and time during in-vitro gas production at which corresponding gas production was achieved for untreated and treated finger millet straw at different levels of concentrate supplement

Proportion concentratrate/straw consumed in vivo	*Weight of concentrate (g) in 1 g substrate required for in-vitro gas production to give the concentrate/ straw proportions consumed in vivo*	*In-vivo digestibility*[a] (%)	*Length of incubation to achieve digestibility (h)*	
			Trial 1	*Trial 2*
Untreated straw				
Straw only	0	52·1	46·3	55·6
Straw only	0	51·6	46·3	54·7
0·34	0·25	59·2	46·8	43·7
0·32	0·24	62·8	52·0	49·0
0·67	0·40	59·5	39·7	39·3
0·65	0·39	63·1	44·0	43·7
0·67	0·40	64·9	46·0	46·5
1·00	0·50	66·5	45·0	50·4
Average		60·0	45·8[b]	47·9[b]
Treated Straw				
0	0	65·2	44·2	57·0
0	0	72·8	55·2	81·8
0·20	0·17	74·0	48·6	68·5
0·25	0·20	72·1	46·0	62·1
0·33	0·25	70·7	39·1	57·8
0·37	0·27	73·3	42·1	65·5
0·54	0·35	65·5	35·0	45·6
0·66	0·40	68·4	37·6	49·6
Average		70·2	43·5[c]	61·0[c]

[a] Data taken from Prasad *et al* (1991) for individual animals.
[b] Pooled standard deviation of average = 4·67. Averages not significantly different ($P > 0·05$).
[c] Pooled standard deviation of average = 9·26. Averages significantly different ($P < 0·05$).

were used to simulate an extended range of supplementation in greater detail. The weights of concentrate (g fresh basis) were: 0·13, 0·20, 0·25, 0·29, 0·35, 0·40, 0·45, 0·50, 0·55, 0·59 plus untreated straw to give a total of 1 g substrate; 0·05, 0·10, 0·15, 0·20, 0·23, 0·25, 0·32, 0·35, 0·40, 0·45 plus treated straw to give a total of 1 g substrate.

Gas production method

The method described by Merry *et al* (1991) was used. This consists of the anaerobic fermentation at 39°C of dried substrate (ground to pass through a 1 mm dry mesh screen) in stoppered 125 ml serum bottles. The buffer used was similar to that described by Tilley and Terry (1963) while the inoculum was prepared from strained fresh rumen fluid. Following suggestions by Theodorou (Theodorou M K pers comm), Trypticase peptone was not included in the buffer and 5 ml of inoculum was used for each bottle. Gas production was monitored using a pressure transducer attached via a three way valve to a syringe and needle. At intervals the needle was put through the serum bottle stopper, the pressure adjusted to atmospheric using the syringe and the volume of gas removed noted. The gas removed was then discarded.

Each fermentation was performed in triplicate and gas production monitored at intervals up to a total duration of 166 h. The samples were autoclaved at the end of the experiment and the residual dry matter was estimated by filtering each sample into preweighed filter crucibles. The particulate material was washed with distilled water and oven dried overnight at 100°C.

Computation of data and statistical analysis

Cumulative gas production data were corrected to 1 g dry matter (DM). In-vitro digestibility (%) was calculated assuming that all of the residual dry matter after 166 h fermentation was unfermented substrate. In-vitro digestibility was correlated against cumulative gas produced after 166 h using a linear equation. Only data

from samples which contained straw and control (no substrate) treatments were used. The resultant correlation equation was used to convert the gas produced during shorter fermentation intervals into in-vitro digestibility to compare with in-vivo digestibilities.

To give a more precise estimate of the duration of in-vitro fermentation which gave the closest agreement with the digestibilities observed *in vivo* the following calculations were made. In-vitro gas production obtained at the same or similar concentrate to straw ratios as those of the in-vivo data were fitted to the single pool exponential equation described by Merry *et al* (1991):

$$\text{Volume} = a + b(1 - \exp(-k \times \text{time})$$

where constants a, b and k are a scale factor, the gas pool size and the rate constant, respectively. Data from 12 h fermentation onwards only were used. The in-vivo digestibilities for each ratio of concentrate to straw were converted to gas volumes using the linear regression equation described above. These were substituted into the single pool exponential equation using the constants a, b and k derived as above, enabling the corresponding time (t) to be calculated.

The data from both trials were analysed to see if there were interactive effects between the straws and concentrate supplement, ie if the gas produced by mixed substrates was greater than predicted by summing the gas produced by the two substrates when fermented individually. A pooled standard error was calculated and used to determine 95% confidence intervals for supplemented substrates. Gas production, assuming no interactions, was predicted by a straight line joining the mean gas production values obtained when the straws and supplement were fermented by themselves. If the predicted levels of gas production fell outside the 95% confidence levels of measured gas production by supplemented straws this was taken as the statistically significan ($P < 0{\cdot}05$) interactive effect.

RESULTS

Gas production versus time

The cumulative in-vitro gas production at different times for unsupplemented treated and untreated straws, and for the concentrate mixture by itself is given in Fig 1. The initial rate of gas production was highest for the concentrate mixture, followed by the treated straw and untreated straw. The total gas produced when fermentation had almost been completed (after 166 h) was similar for all three substrates.

Comparison of in-vitro gas production data and in-vivo digestibility

The correlation equations between gas produced after 166 h (in ml per g DM) and the proportion of dry matter disappearing during fermentation (in-vitro digestibility) were

- trial 1: in-vitro digestibility = (gas produced × 0·320) −0·458 (correlation coefficient 0·968, R^2 93·62%)
- trial 2: in-vitro digestibility = (gas produced × 0·293) −5·970 (correlation coefficient 0·978, R^2 95·68%)

Table 2 shows the proportions of concentrate/straw obtained *in vivo* (in-vivo digestibility data obtained by Prasad *et al* (1991)) and the calculated duration of in-vitro fermentation which corresponded to the observed in-vivo digestibilities. For untreated straw these lay in the range 39·7–52·0 h for trial 1, 39·3 and 55·6 for trial

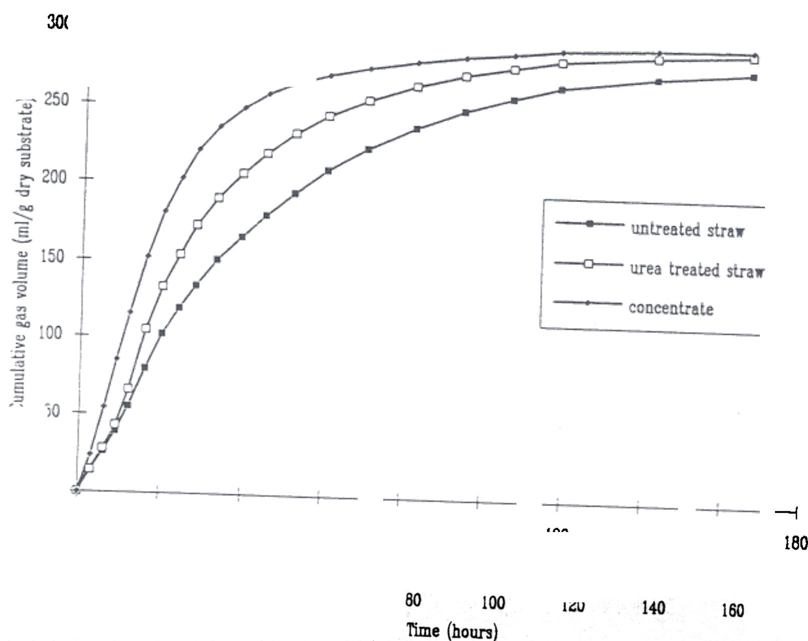

Fig 1. Cumulative gas produced for untreated and urea treated finger millet straw and concentrate

2, means 45·8 and 47·9 h, respectively (means not significantly different, $P > 0{\cdot}05$). For treated straw the ranges were 35·0–55·2 h and 45·6–81·8 h, means 43·5 and 61·0 h, respectively (means significantly different, $P < 0{\cdot}05$).

Figure 2 shows the in-vitro digestibility predicted from gas production in trial 2 after 45 and 52 h for untreated straw with different levels of supplementation together with the digestibility data obtained *in vivo*. Figure 3 shows the corresponding data for treated straw at the most closely fitting sampling times of 52 and 60 h together with the in-vivo data. For the untreated straw the trends between supplement level and digestibility for the in-vitro and in-vivo data are strikingly similar; a relatively steep increase in digestibility from unsupplemented straw to the lowest level of supplementation examined *in vitro* (0·13 g supplement), then a more gradual increase in digestibility as the level of supplement increased. The unsupplemented untreated straw appeared to be more digestible *in vivo* than was indicated by the in-vitro data (trial 2), reflected in the relatively high fermentation time for these samples in Table 2. This was not, however, evident in trial 1.

Comparison of supplemented untreated and treated straw

Figure 4 compares the effect of supplementation (as expressed by the quantity of concentrate in the 1 g substrate used) on the in-vitro digestibility of untreated and treated straw after fermentation for 52 h. Statistical analysis of the data indicated that there were significant ($P < 0{\cdot}05$) interactive (ie positive associative) effects between both untreated and treated straw with the concentrate supplement. Significant interactions were found

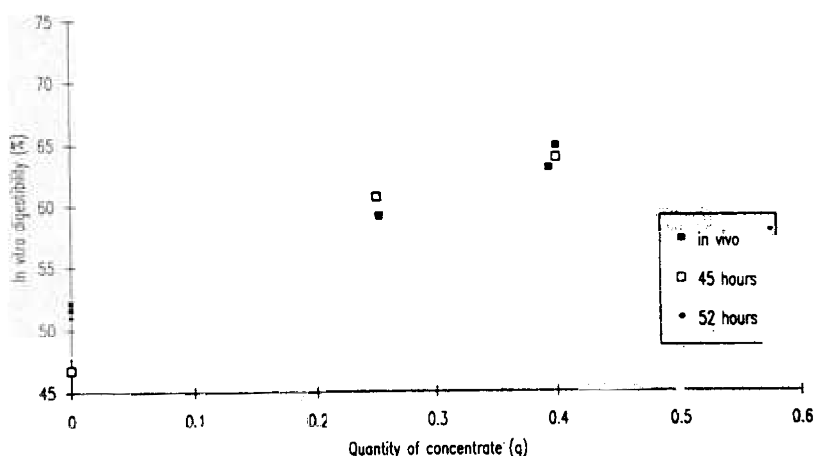

Fig 2. In-vitro digestibility predicted from in-vitro gas production using untreated finger millet straw after 45 and 52 h incubation, with in-vivo digestibilities at different levels of supplementation with concentrate. Quantities of concentrate are given in g fresh basis of 1 g total substrate (straw plus concentrate).

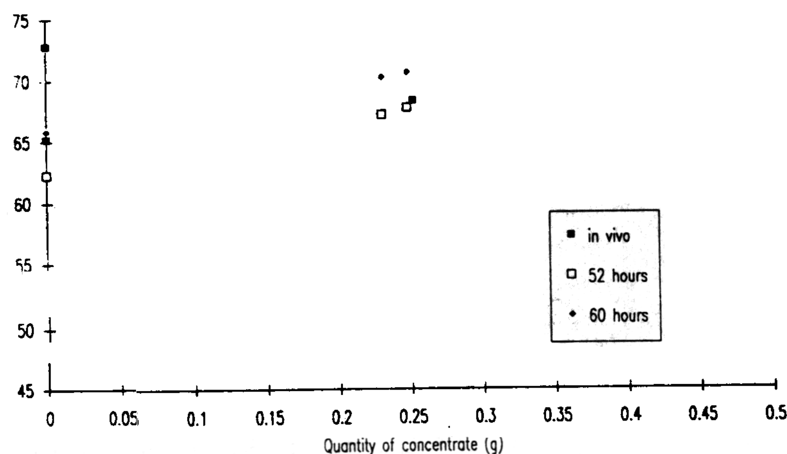

Fig 3. In-vitro digestibilities predicted from in-vitro gas production using urea treated finger millet straw after 52 and 60 h incubation, with in-vivo digestibilities at different levels of supplementation with concentrate. Quantities of concentrate are given in g fresh basis in 1 g total substrate (straw plus concentrate). For ease of comparison the in-vitro digestibility is presented on the same scale as Fig 2.

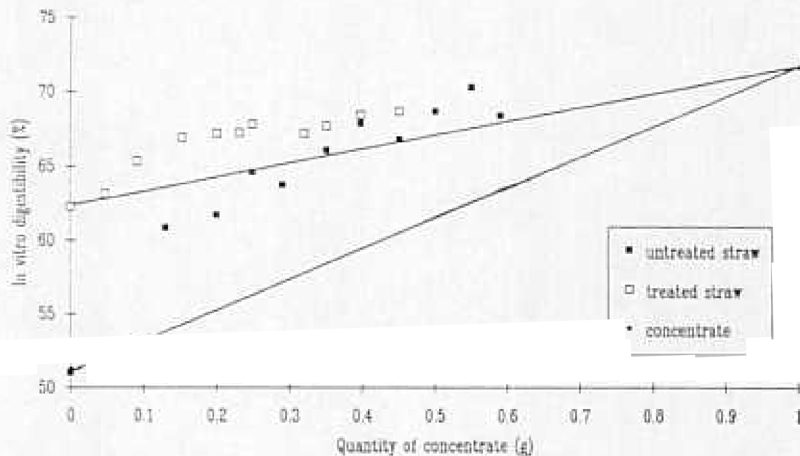

Fig 4. Comparison of the in-vitro digestibility predicted from in-vitro gas production after 52 h incubation between urea treated and untreated finger millet straw supplemented with different levels of concentrate. The straight lines drawn between data for the straws alone (on the vertical axis, 0 concentrate) and the concentrate alone (1 g concentrate) indicate the predicted in-vitro digestibilities of supplemented straws if there were no interactive effects. Quantities of concentrate are given in g fresh basis in 1 g total substrate (straw plus concentrate).

for all supplement levels in trial 1, and for all supplement levels for untreated straw in trial 2. Significant interactions were observed for treated straw for concentrate levels of 0·15–0·25, higher levels of supplement giving consistently higher gas productions than predicted by the no interaction model (although differences generally do not reach statistical significance, $P > 0{\cdot}05$).

DISCUSSION

Gas production versus time

The initial rate of gas production was highest for the concentrate mixture presumably due to the presence of readily fermentable carbohydrates. The treated straw had a higher initial rate of gas production than untreated straw indicating that it was more readily digested by the rumen microbes. Urea ammoniation of straw loosens the lignocellulolytic complexes in the cells and enhances fibre digestion by rumen microbes (Rai *et al* 1990). Improved fibre digestibility of urea treated straw *in vivo* has also been reported (Joshi *et al* 1990; Prasad *et al* 1991). The rates of initial gas production of the three individual substrates were, therefore, ranked in the order expected.

Comparison of in-vitro gas production data and in-vivo digestibility

The differences observed between the two trials in the correlation equations relating in-vitro digestibility and gas production were presumably due to the differences between the different batches of fresh inoculum used for each trial.

The data in Table 2 indicated that 45 and 52 h were the gas production monitoring times which most closely fitted in-vivo digestibility data. This is similar to the incubation time of 48 h used by Tilley and Terry (1963) for the degradation of fibre by rumen microorganisms. These times are long compared to the in-vivo mean rumen retention times of 30–36 h for mature steers quoted by Mir *et al* (1991) for animals fed *ad libitum* on alfalfa hay, orchardgrass hay, alfalfa silage, maize silage, cracked maize plus orchardgrass hay (70 : 30) and barley straw plus alfalfa hay (70 : 30). Uden and Van Soest (1984) noted that *in vivo* there was a balance between digestion and passage in the rumen, so that in-vitro batch systems may be expected to give higher digestibilities. There are also several factors which could slow in-vitro systems compared with *in vivo*. The fermenting mixture is much more dilute *in vitro* to prevent the rapid build up of excessive levels of end-products. The rumen inoculum used was not adapted to the feeds so some adaptation presumably took place during the gas production run itself, possibly extending the initial lag phase.

In-vivo digestibility is a combination of rumen digestion plus digestion in the lower digestive tract. The gas production method simulates only rumen conditions so material which is not readily digested in the rumen but readily digested elsewhere, such as herbage protein (Tilley and Terry 1963), may be digested to a greater extent *in vivo* than by the gas production method. Another factor may be the contribution of bacterial mass to the residual dry matter reducing the in-vitro digestibility. Both factors could help account for the best fitting incubation times in the gas production method being longer than mean rumen retention times for the same digestibility.

In Fig 2 the higher in-vivo digestibility of unsupplemented untreated straw compared to in-vitro digestibility could be due to an increased retention time of

unsupplemented untreated straw *in vivo*, but this effect was not observed in trial 1 where the times of best fit with the in-vivo data were similar to the average for the data set as a whole (Table 2). It may be related to the differences in the inocula for the two trials, the inoculum for trial 2 being apparently less active.

For treated straw the in-vivo data were scattered making it difficult to discern a clear trend. The in-vitro data indicated that supplementation up to about 0·2 g of supplement increased the digestibility of the feed mixture but higher levels of supplementation produced only minor additional increases.

Comparison of supplemented untreated and treated straw

Figure 4 indicates that untreated straw required higher levels of supplementation to achieve the same level of digestibility as supplemented treated straw. This was also evident from the in-vivo studies (Prasad *et al* 1991) where the value of treating straw became less important as the level of supplementation increased. Creek *et al* (1984) similarly found that supplementation with concentrate had a greater stimulatory effect on liveweight gains of steers fed on untreated straw than those fed ammonia treated straw.

The digestibility of untreated straw is particularly stimulated by low levels of supplementation presumably because the additional nutrients allow faster microbial growth enabling a faster rate of degradation of the fibrous straw. As the basic requirements of the rumen microorganisms are met, further supplementation stimulates a decreasing response. The finding of interactive effects between straws and supplements is consistent with the hypothesis that supplementation increases the degradation of straw by stimulation of the rumen bacteria, hence supplemented straw is more digestible than predicted from the digestibilities of the supplement and straw when fermented separately.

CONCLUSIONS

Similar trends were observed between in-vitro gas production and in-vivo digestibility data on the effect of supplementing untreated and urea treated straw with a concentrate mixture, although scatter in the in-vivo data on the supplementation of urea treated straw makes detailed comparisons difficult. The best fit with in-vivo digestibility was obtained after 45–52 h of in-vitro gas production. The in-vitro technique gave results comparable to those obtainable from in-vivo studies and hence could be a useful tool for evaluating feeds for ruminants. However, there is a need to make more extensive comparisons between gas production and in-vivo data with different classes of feeds.

The digestibility of untreated straw was particularly stimulated by small quantities of supplement. The digestibility of treated straw was higher than that of untreated straw and less stimulated by supplementation. These findings are consistent with the hypothesis that fibre digestibility can be increased by providing a diet which contains sufficient nutrients to stimulate the activity of rumen microorganisms.

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