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Plasma biochemical values in the guanaco (*Lama guanicoe*) and a comparison with the sheep

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Abstract

An initial experiment was conducted to investigate the variability of selected metabolites in the plasma from guanacos. A second experiment directly compared plasma biochemical values for guanacos with those for sheep. During the first experiment, jugular blood samples were collected from five mature castrated guanacos using an embedded experimental design. Weekly blood samples were collected at the same time (09.00 h) on the same day each week for 7 weeks. Daily blood samples were collected at the same time each day (09.00 h) during week 5. On day 2 of week 5, blood samples were collected every 3 h from 09.00 h for a 24-h period. No evidence of a cyclical pattern of plasma parameters was observed on a weekly, daily or 3-h basis. During the second experiment, the metabolic profiles of 11 mature castrated guanacos and 11 mature barren ewes (Merino × Welsh Mountain) were compared. Significant differences in plasma concentrations of all metabolites except urea-nitrogen (guanacos – 15·42 mmol/l, sheep – 15·60 (s.e.d. 1·506) mmol/l) were found with values for guanacos v. *sheep as follows: glucose (7·63* v. *3·63 (s.e.d. 0·268) mmol/l); acetate (0·26* v. *0·48 (s.e.d. 0·035) mmol/l); β-hydroxybutyrate (0·06* v. *0·50 (s.e.d. 0·019) mmol/l); albumin (33·4* v. *29·5 (s.e.d. 0·93) g/l); and total protein (53·8* v. *65·6 (s.e.d. 2·12) g/l); (P < 0·001 for all previous variables); non-esterified fatty acids (0·48* v. *0·29 (s.e.d. 0·048) meq per l; P < 0·01) and α-amino N (2·44* v. *2·66 (s.e.d. 0·088) mmol/l; P < 0·05). This study indicates that the reference plasma metabolite concentrations of sheep are not suitable alternatives for use for nutritional or veterinary purposes with guanacos, but those of llamas or alpacas are. The results also suggest that energy capture and transport in camelids may be markedly different from that in conventional ruminants.*

Keywords: *camelidae, glucose, 3-hydroxybutyric acid,* lama guanicoe, *metabolites.*

Introduction

Like the alpaca, the guanaco *(Lama guanicoe) is* a potential source of fine fibre for commercial production and has an undercoat comparable in fibre diameter to cashmere (Russel, 1993). Despite their economic potential, comparatively little is known about this species of South American camelid and this dearth of knowledge extends to plasma reference ranges for biochemical values (Hastings and Gasgoyne, 1992).

The use of metabolic profiles in dairy cattle and other conventional livestock species is a well established technique for monitoring physiological changes and determining the health status of animals (Payne *et al.*, 1970 and 1974; Russel and Wright, 1983). While reference ranges for serum biochemical values in the llama (*Lama glama)* (Lassen *et al.*, 1986; Fowler, 1989; Fowler and Zinkl, 1989) and alpaca *(Lama pacos)* (Fowler, 1989; Simons *et al.*, 1993) have been published, most of the information currently available relates to proteins and/or minerals and little consideration has been given to energy metabolites other than glucose. Few or no similar reference values for guanacos have been published.

Previously published reference ranges for plasma biochemical values in South American camelids have for the most part been obtained through herd-sampling programmes (Lassen *et al.*, 1986; Fowler and Zinkl, 1989; Simons *et al.*, 1993). This type of sampling programme offers the advantage that values from healthy animals covering a wide variety of nutritional and physiological states or

environmental conditions can be obtained. However, it suffers from the disadvantage that no measure of the within-animal variation can be determined. Likewise, in these studies no consideration has been given to temporal variation in levels of metabolites. The design of the initial experiment reported in this paper was therefore chosen to allow changes in metabolite levels over time to be monitored within individual animals and to provide an estimate of the amount of variation that may be expected both between and within animals. Frequently reported metabolites such as glucose, total protein and urea were measured to allow comparisons with data for llamas and alpacas to be made. The levels of metabolites such as β-hydroxybutyrate (BHB) and acetate, which had not previously been reported for any species of South American camelid, were also recorded. The results obtained from this initial experiment led to a second experiment which directly compared standard indicators of nitrogen and energy status for the guanaco with those of sheep.

Material and methods

Two separate experiments were conducted. During the first experiment a series of repeated measurements were made on housed guanacos offered a standard diet, allowing changes in plasma metabolite concentrations over time (hours, days, weeks) to be monitored. The second experiment consisted of a direct comparison of plasma metabolites from guanacos and sheep grazing upland pasture.

Sample collection

Experiment 1. This experiment was carried out between the beginning of November and the middle of December, and was based on five clinically healthy, captive-bred castrated males aged 4 years old and weighing approximately 110 kg. All were familiar with the restraining equipment (a drop-floor crush) used during sample collection and had undergone regular handling. The animals were housed in individual loose pens (3 m × 5 m) and given about 0·6 kg high-quality chopped grass hay and 0·6 kg concentrate (Coarse 16 Mixture, Dalgety Agriculture Ltd, Bristol, UK) twice daily at 09.00 h and 16.00 h. Subsamples from each bale of hay and bag of concentrate given were collected and bulked prior to subsequent analysis for dry matter (DM), ash, gross energy (GE), acid-detergent fibre (ADF), neutral-detergent fibre (NDF), starch, water-soluble carbohydrate (WSC) and total nitrogen (TN) contents. Trace-mineral salt mix and water were available *ad libitum.*

Blood samples were taken over a number of weeks, days and hours using an 'embedded' sampling programme. Weekly blood samples were taken via jugular venipuncture at 09.00 h for 7 weeks. During week 5, daily samples were taken at 09.00 h for 8 days via temporary jugular catheters. From day 2 to day 3 of week 5, samples were taken via the temporary jugular catheters at 3-h intervals from 09.00 h for 24 h. Polyvinyl chloride jugular catheter lines were inserted percutaneously at the base of the neck under local anaesthetic using an introducer set the day before the first sample was to be taken. After insertion and after each sampling, the catheters were flushed and left filled with a solution of sterile heparinized saline (approx. 25000 i.u. heparin per l saline). Care was taken to ensure that waste heparinized saline was completely removed from the catheter lines before the blood samples were collected.

At each sampling, approximately 10 ml of blood was collected per animal into a heparinized Vacutainer (Becton, Dickinson and Co., Rutherford, New Jersey) or a syringe prepared with one drop of heparinized saline (approx. 1500 i.u. heparin per ml). Samples were collected onto ice before being immediately spun down at approximately 1500 × **g** for 15 min at 4°C. Plasma was then decanted into separate 1·5 ml microtubes and frozen at –18°C until analysed.

Experiment 2. The metabolic profiles of 11 guanacos and 11 sheep were compared. The guanacos were captive-bred castrated males aged between 4 and 6 years old, and weighed about 105 kg. The sheep were 3-year-old barren ewes (Merino × Welsh Mountain) weighing about 70 kg. All were clinically healthy and underwent regular anthelmintic dosing. The animals grazed the same area of *Agrostis* spp./*Festuca* spp.-dominated upland pasture maintained at a target sward height of 8 cm, had *ad libitum* access to a trace mineral block, and had been grazing the same area for a minimum of 6 weeks prior to the collection of samples. One sample per animal was collected and to reduce the level of restraint required the samples were taken from a tail vessel.

Sampling was conducted at the end of May. All the samples were taken within a 1-h period (13.00 h to 14.00 h). Approximately 10 ml blood was collected per animal into vacutainers containing oxalate/fluorate (for glucose and non-esterified fatty acid (NEFA) analysis) or lithium heparin (all other analyses). Samples from both species were analysed as a single batch, with replicate analyses carried out on each sample. After collection, the samples were stored in an insulated box containing ice packs, and were spun down within 3 h of collection. Subsequent sample handling and storage was as described for experiment 1.

Sample analysis

Analysis of food samples was done using standard techniques. DM content was determined at 100°C and ash content was measured after sample ignition at 550°C for 16 h. NDF and ADF analyses were carried out on freeze-dried samples according to the methods of Van Soest and Wine (1967) and Van Soest (1963) respectively. Starch determinations were based on the method of MacRae and Armstrong (1968) and WSC determinations on the method of Thomas (1977). GE was determined by adiabatic bomb calorimetry, and TN by the Kjeldahl method (Bradstreet, 1969).

Plasma samples were analysed by discrete analysis methods for glucose (Sigma kit no. 16-UV, Sigma-Aldrich Company Ltd, Poole, UK), BHB (Sigma kit no. 310A), acetate (Clarke and Payton, 1983), total protein (Sigma kit no. 541-2), albumin (Sigma kit no. 621-3P), urea-N (Sigma kit no. 66) and α-amino N (Oddy, 1974). Plasma globulin concentration was calculated as the difference between the total protein and albumin concentrations. In addition, plasma samples from experiment 2 were analysed by discrete analysis for NEFA (Wako kit no. 994-75409, Wako GMBH, Neuss, Germany).

Statistical analysis

Simple descriptive statistics (means, standard deviations, ranges and coefficients of variation) were used to summarize the data from across each time series (weekly, daily, 3-h). Autoregression analyses were carried out to establish whether there were defined time-dependent fluctuations in the levels of metabolites, while means for each animal within each time series were used in pair-wise correlation analyses to investigate the relationships between concentrations of the various plasma metabolites. Between-species differences in plasma metabolite concentrations were analysed using one-way analysis of variance (Genstat 5; Lawes Agricultural Trust).

Table 1 *Plasma biochemical values in the guanaco as determined by weekly, daily and 3-h sampling regimes*

		Weekly	Daily	3-hourly
Glucose (mmol/l)	mean	7·65	7·59	7·70
	s.d.	0·837	0·691	1·150
	CV	0·11	0·09	0·15
	range	6·26-11·29	6·29-10·23	5·74-11·15
Acetate (mmol/l)	mean	0·246	0·252	0·318
	s.d.	0·1341	0·1448	0·1295
	CV	0·55	0·58	0·41
	range	0·062-0·633	0·062-0·657	0·110-0·657
β-hydroxybutyrate		trace	trace	trace
Total protein (g/l)	mean	70·5	68·2	66·0
	s.d.	5·37	4·21	3·19
	CV	0·08	0·06	0·05
	range	62·8-82·4	61·7-79·3	60·3-73·3
Albumin (g/l)	mean	45·8	42·6	39·7
	s.d.	3·10	3·41	2·39
	CV	0·07	0·08	0·06
	range	39·3-53·9	38·3-53·9	34·3-47·7
Globulins (g/l)	mean	24·7	25·7	26·4
	s.d.	5·03	3·68	3·30
	CV	0·20	0·14	0·13
	range	14·5-36·5	14·5-32·4	17·7-34·3
Urea-N (mmol/l)	mean	9·31	8·61	8·44
	s.d.	1·321	1·268	1·572
	CV	0·14	0·15	0·19
	range	6·71-12·15	5·90-11·20	5·81-11·12
α-amino N (mmol/l)	mean	2·51	2·47	2·19
	s.d.	0·263	0·318	0·352
	CV	0·10	0·13	0·16
	range	1·98-2·98	1·54-3·10	1·19-2·66

Results

Experiment 1: weekly, daily and 3-h plasma profiles in guanacos

The quantities of food offered to the animals were kept constant throughout the period of the experiment. The hay was of relatively good quality (912 g DM per kg; and per kg DM: 65 g ash, 125 g crude protein, 261 g ADF, 539 g NDF, 31 g WSC, 61 g starch, and 19·0 MJ GE) and the concentrate was of a standard composition (870 g DM per kg; and per kg DM: 110 g ash, 181 g crude protein, 122 g ADF, 236 g NDF, 167 g WSC, 334 g starch, and 17·7 MJ GE).

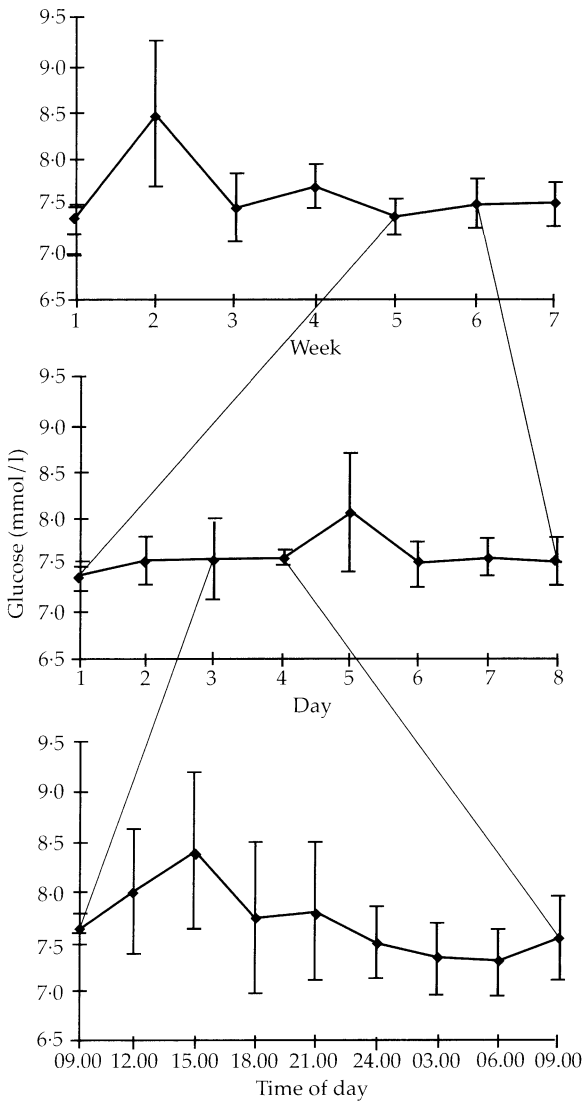

Figure 1 Mean plasma glucose concentrations (mmol/l) in the guanaco as determined by weekly, daily and 3-h sampling regimes.

Table 1 summarizes the plasma biochemical values for all the metabolites measured, giving figures for the mean, standard deviation, and range of values recorded across each of the three time series. The concentrations of BHB in the guanaco plasma from this experiment was undetectable by the standard analytical methods used. Autoregression of the data from each of the time series yielded no significant serial correlations, indicating that the mean data are representative of whole data sets, and that no significant cycling of metabolite concentrations was apparent over the course of the weeks, days and hours observed. As an example of this, and to illustrate the experimental design, mean plasma glucose data are presented in Figure 1.

Pair-wise comparisons, used to investigate relationships between metabolites, identified few correlations. There were strong correlations between acetate and α-amino N (r^2 = 0·91, 0·88 and 0·75 for weekly, daily and 3-h means respectively), although these data must be viewed with caution due to the small number of animals sampled (no. = 5). In comparison, the correlations between glucose and urea were more variable (r^2 = 0·39, 0·65 and 0·85 respectively). Interestingly, there was a relatively strong correlation between plasma total protein and globulin concentrations (r^2 = 0·92, 0·87 and 0·694 for weekly, daily and 3-h means respectively), whereas the relationship between total protein and albumin was very weak (r^2 = 0·001, 0·290 and 0·061 respectively).

Coefficients of variation for each plasma metabolite associated with the three time series are also given in Table 1. Plasma acetate concentration data were found to have the greatest variation, whilst the lowest variation was observed with plasma total protein or albumin.

Experiment 2: comparison of plasma biochemical values in the guanaco and the sheep

Plasma metabolite concentrations for guanacos and sheep are presented in Table 2. With the exception of urea, the concentrations of all metabolites were significantly different between species ($P < 0·05$ or less).

The concentration of glucose in the guanaco plasma was almost twice that in the sheep plasma. The level of NEFA was also substantially higher in the guanaco plasma. In contrast, the acetate concentration of the guanaco plasma was approximately half that of the sheep plasma, whilst the BHB concentration was an order of magnitude lower.

Table 2 *Comparison of plasma biochemical values in the guanaco with equivalent data for sheep*

	Guanaco	Sheep	s.e.d.
Glucose (mmol/l)	7·63	3·63***	0·268
Acetate (mmol/l)	0·263	0·479***	0·0352
β-hydroxybutyrate (mmol/l)	0·060	0·502***	0·0191
Non-esterified fatty acids (meq/l)	0·480	0·293**	0·0478
Total protein (g/l)	53·8	65·6***	2·12
Albumin (g/l)	33·4	29·5***	0·93
Globulins (g/l)	20·4	36·1***	1·59
Urea-N (mmol/l)	15·42	15·60	1·506
α-amino N (mmol/l)	2·44	2·66*	0·088

The plasma concentrations of total protein, globulins and α-amino N were significantly lower in the plasma from the guanacos than in the plasma from the sheep. However, the concentration of albumin in the guanaco plasma was higher than that in the sheep plasma.

Discussion

The current study was carried out to collect baseline metabolic profile data from guanacos for veterinary and nutritional comparisons. The study then compared basic plasma biochemistry of guanacos with that of a true ruminant, the sheep.

Weekly, daily and 3-h profiles in guanacos

The plasma glucose values for guanacos recorded during experiment 1 were similar to those reported for llamas (Lassen *et al.*, 1986; Fowler and Zinkl, 1989; Kaneko, 1989), and higher than those reported for true ruminants (Kaneko, 1989). Lassen *et al.* (1986) suggested that higher glucose concentrations for camelids could be a reflexion of transient increases due to excitement and subsequent adrenaline release associated with restraint and sampling. The sampling protocol adopted during the current experiment was chosen to minimize stress. All the experimental animals were familiar with regular restraint in the specialized handling equipment used. Distress as a result of frequent needle puncture during the housed study was eliminated by the use of temporary indwelling catheters. Therefore, it is unlikely that the glucose concentrations recorded in the present study were artificially high.

The urea N values recorded for guanacos were similar to those reported for llamas (Lassen *et al.*, 1986; Kaneko, 1989) and alpacas (Simons *et al.*, 1993). During the present experiment with animals offered high-quality hay and concentrates the plasma urea concentrations did not vary significantly over the course of a day. Previous studies with sheep given hay-based diets found similar results (Lewis, 1957), when, despite marked fluctuations in rumen ammonia concentrations, plasma urea N concentrations were relatively constant over a 12-h period. In contrast, on a restricted diet, plasma urea concentrations in llamas have been found to vary markedly depending on the time of feeding and the amount of food consumed (Hinderer and Engelhardt, 1975), which concurs with studies in cattle (e.g. Gustafsson and Palmquist, 1993).

The mean plasma total protein values recorded during experiment 1 were generally slightly higher than those quoted for llamas (Lassen *et al.*, 1986; Fowler and Zinkl, 1989; Kaneko, 1989) and alpacas (Simons *et al.*, 1993), whereas the albumin values were similar to those reported for llamas (Kaneko, 1989) and alpacas (Simons *et al.*, 1993). This difference emphasizes the need to define specific reference ranges if biochemical data are to be a useful diagnostic tool for camelid management. If the albumin values obtained from this experiment were compared with equivalent information for cattle or sheep (Kaneko, 1989) they may have been assumed to have come from an animal suffering from dehydration.

The plasma globulin concentrations for guanacos were also similar to those expected for llamas (Lassen *et al.*, 1986; Kaneko, 1989) and alpacas (Simons *et al.*, 1993) and again are higher than those of domesticated ruminants. It is interesting to note that the correlation between globulins and total protein was greater than the correlation between plasma albumin and total protein, and that the coefficients of variation were greatest for globulins, thus indicating that changes in plasma total protein were influenced more by globulin concentration than by albumin concentration.

There do not appear to be any reports in the literature which include plasma concentrations of α-amino N, acetate and BHB in the guanaco, or any other species of South American camelid. The concentrations of α-amino N found during this study were of the order reported in the literature for true ruminants (Reynolds and Huntington, 1988; Kunz *et al.*, 1985) and are presumably similarly dependent to a certain extent on the animal's diet. The concentrations of plasma α-amino N were highly correlated to the observed concentrations of acetate, which are also dependent on diet. The values for acetate recorded were lower than those reported for cattle (Reynolds *et al.*, 1992). Plasma concentrations of BHB were found to be extremely low, undetectable by the standard method used for the

determination of BHB in cattle plasma. NEFA were not measured during experiment 1 since the heparin used as an anticoagulant agent could potentially have influenced *in vivo* the lipoprotein lipase activity, thus affecting the NEFA values. Any heparin remaining in the catheter and mixed with the sampled blood could also have had an effect on subsequent NEFA determinations.

The coefficients of variation calculated indicate a large amount of individual animal variation. Serial correlations within each of the time series were not found to be significant, indicating that there was no diurnal, daily or weekly cycling of metabolites and suggesting that the mean values calculated describe the data well. Despite this, the coefficients of variation for the 3-h samples were greatest for those metabolites that would be expected to have a short half life in the blood, e.g. urea N, glucose and α-amino N, and lowest for those with a longer half life, e.g. total protein.

In general the plasma biochemical values obtained for guanacos were similar to those previously reported for the domesticated species of South American camelid. This implies other reference values obtained from sampling llamas or alpacas are likely to be applicable to guanacos. The low levels of BHB in the guanaco blood, together with the high concentrations of glucose, indicate that reference values for sheep or other ruminants are unlikely to be immediately applicable to guanacos due to intrinsic differences in basic energy metabolism.

Comparison of plasma biochemical values in the guanaco and the sheep

There are no previous accounts in the literature of a direct comparison between the metabolic profiles of a species of camelid and a conventional ruminant. Experiment 2 directly compared plasma biochemical values for guanacos with those for sheep when consuming the same diet and in the same environment to eliminate these as contributing factors to the interpretation of species differences.

The total protein and albumin values recorded for the guanacos during experiment 2 were lower, and the urea values higher, than those recorded during experiment 1. Accounting for these differences is difficult. Although the diets were different, good quality hay plus concentrates *v.* early season permanent pasture, the condition score of the animals on both trials was constant and similar (condition score 3). Seasonality may have influenced the results, since experiment 1 was conducted November/December and experiment 2 at the end of May. Whatever the factor or factors affecting the level of these nitrogenous metabolites, the health of the animals was maintained.

With the exception of plasma urea N, the levels of all the plasma metabolites measured were significantly different in the guanaco when compared with the sheep. BHB was quantifiable in the plasma from experiment 2, indicating that the concentrations of this metabolite in the plasma from experiment 2, although very low, were higher than those in the plasma collected during experiment 1. The concentrations of glucose in the guanaco plasma were approximately twice those of the sheep. Due to the nature of food digestion by the true ruminant animal, most, if not all of the glucose used by ruminant tissues is produced through gluconeogenesis by the liver and kidney, and ruminant tissues are also able to use products of rumen fermentation such as acetate and butyrate as energy sources. Molar proportions of volatile fatty acids (VFAs) in rumen liquor have been found to be similar for guanacos and sheep offered a standard diet (M. D. Fraser, unpublished data). Despite this, there were between-species differences in the plasma derivatives of VFA absorption in the present study, with guanaco plasma acetate concentrations being half those of the sheep and the BHB concentrations being 10 times lower.

In the true ruminant, acetate is absorbed from the gut and that which is not oxidized is passed unchanged into portal blood. Thus, the lower plasma concentrations of acetate in the guanacos may reflect a greater utilization of acetate by gut tissues than occurs in the sheep. In the ruminant BHB is largely derived from two sources. The first is by conversion from butyrate produced in the rumen (Stangassinger and Giesecke, 1986; Britton and Krehbiel, 1993). The second is through ketone body production as fatty acids are used for gluconeogenesis, leading to its use as an indicator of energy intake at high plasma concentrations (Russel *et al.*, 1967). In this study, plasma concentrations of BHB in the guanacos were negligible compared with the true ruminant species and were more like those of a monogastric animal (e.g. the horse; Kaneko, 1989), suggesting that butyrate conversion to BHB may not occur in the guanaco. The value for BHB recorded, together with the glucose results, suggest that energy capture and transport in camelids may be markedly different from that in conventional ruminants.

Despite similar nutritional histories for both animal species, the concentration of NEFA in the guanaco plasma was significantly higher than that in the sheep plasma. Increased plasma concentrations of

NEFA in cattle (Russel and Wright, 1983) and sheep (Russel *et al.*, 1967) are associated with energy deficiency, since they are released from fat stores. However, there had been no detectable change in the condition score of the experimental animals of the present study over the 4 weeks preceding the sampling date, with the guanacos and sheep maintaining a condition score of 3 and 3·5 respectively. Thus, these data are another illustration of the need for species-specific biochemical reference ranges to be available. Similarly, plasma albumin concentrations are related to long-term dietary protein intake, and can be regarded as an indicator of whole-body protein status in sheep (Sykes, 1976). The between-species difference in plasma albumin cannot, however, be attributed to differences in dietary protein intake in this study, and again highlights the need for baseline values for comparative purposes.

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