The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

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*Environmental enrichment for maned wolves (*Chrysocyon brachyurus*): group and individual effects*

AS Vasconcellos[*†], *MABV Guimarães*[‡], *CA Oliveira*[‡], *CS Pizzutto*[‡] *and C Ades*[†]

[†] Av Prof Mello Moraes 1721, Institute of Psychology, Cidade Universitária, São Paulo, CEP 05508-030, Brazil
[‡] Av Prof Dr Orlando Marques de Paiva 87, VRA Department of Animal Reproduction, Cidade Universitária, São Paulo, CEP 05508-270, Brazil
* Contact for correspondence and requests for reprints: angelv@usp.br

Abstract

*Procedures that increase foraging and exploratory behaviours are generally accepted as effective at improving welfare and reducing stereotypies in captive animals. To determine the effect of food and toy enrichment on the behaviour and hormonal levels of maned wolves (*Chrysocyon brachyurus*), 11 wolves were submitted to a baseline-enrichment-baseline schedule with four enrichment conditions: food scattering, toy presentation, edible toy presentation, and a combination of food and toy conditions. Behaviour and glucocorticoid metabolite concentrations (GCM) were assessed throughout the experiment. Our hypotheses were that enrichment procedures would lead to an increase in activity in the short and long term, a decrease in pacing, and a reduction in GCM concentrations. We also hypothesised that the concentrations of GCM would show a correlation with behaviour. Enrichment conditions increased immediate locomotion but did not produce long-term changes of activity nor reduced pacing. The procedures had a clear effect on individual behaviour and induced, under food enrichment conditions, an increased interest in food. Enrichment did not lead to decreased GCM concentration in the group as a whole, but there was a tendency for male and female animals to differ in hormonal reactions to environmental change. A positive correlation between GCM concentrations and pacing behaviour and a negative correlation between GCM concentrations and social behaviour were found. Results suggest that food scattering as an enrichment procedure may be used with maned wolves to enhance welfare, whilst also revealing the importance of taking individual and gender differences into account when planning enrichment techniques.*

Keywords: *animal welfare,* Chrysocyon brachyurus, *environmental enrichment, faecal corticoid metabolites, maned wolf, stress*

Introduction

Maned wolves (*Chrysocyon brachyurus*) are the largest, South American canids. They have a wide distribution, which includes regions in north and north-east Argentina, west Bolivia (Cabrera 1957; Nowak & Paradiso 1985), Peru (Dietz 1984; Beccaceci 1991) and Uruguay (Mones & Olazarri 1990). In Brazil, they are found in the central, south-eastern and southern regions (Cabrera 1957; Dietz 1984; Nowak & Paradiso 1991), mainly in the cerrado (dry forest and savannah; Rodden *et al* 2004). Despite this widespread distribution, maned wolves are at risk in the wild (Dietz 1984; Beccaceci 1991), mainly due to large-scale losses of habitat, in particular the conversion of forest to agricultural land (Fonseca *et al* 1994). They are classified as near threatened by the International Union for the Conservation of Nature (IUCN; [2007]). As wild maned wolf populations decrease, the establishment of a sustainable *ex-situ* population is essential.

Maned wolves are predominantly nocturnal but display some daytime activity (35% of the hours during the day, 91% during the night [Dietz 1984]). Their diet consists of fruit, small mammals (mainly rodents) and birds, according to seasonal availability, and considerable time is spent foraging (Santos *et al* 2003; Queirolo & Motta-Junior 2007; Rodrigues 2007). Maned wolves travel relatively long distances — up to 16 km per day (Bandeira de Melo *et al* 2007). The length of nocturnal foraging trips may depend upon the extent of illumination and tend to be shorter on full-moon nights (Sábato *et al* 2006). Although maned wolves are primarily solitary, pairs have been observed together in the daytime (Bandeira de Melo *et al* 2007). Maned wolves appear to be facultatively monogamous; while mated pairs may share between 50 to 90% of their observed ranges, adjacent individuals of the same gender presented smaller overlapping territories: 10–20% for females and 1% for males (Trolle *et al* 2007).

Maned wolves are common in Brazilian zoos. Although adequate to ensure the survival of adults, handling techniques still preclude appropriate reproductive success (Rodden & Rosenthal 1995; Maia & Gouveia 2002). Gestation lasts for approximately 63 days, and parental care is provided mainly by the female (Dietz 1984, however, see Bandeira de Melo *et al* 2007 for a report of parental care supplied by a male maned wolf). Births in captivity are rela-

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tively common, but infant mortality rate prior to the end of the first year of life is high (71%), due mostly to parental incompetence; a probable result of the constraints of captivity (Maia & Gouveia 2002).

Severe and chronic stress may reduce individual fitness through immunosuppression and tissue atrophy (Munck *et al* 1984), decrease the animals' reproductive success (Liptrap 1993; Dobson & Smith 1995), and may lead to the development of stereotypies (McBride & Cuddeford 2001). Stimulating captive environments, which promote higher levels of activity and exploration as well as increase the performance of typical behaviours, improve the animals' welfare (Shepherdson 1998). For bears and felids, enrichment was found to increase activity levels and normal exploratory behaviours, while reducing abnormal pacing and hiding (Forthman *et al* 1992; Carlstead *et al* 1993a,b; Shepherdson *et al* 1993; Wielebnowski *et al* 2002a,b; Bashaw *et al* 2003). Maned wolves forage for a large variety of items (Queirolo & Motta-Junior 2007; Rodrigues 2007) and those in captivity are highly investigative (Vasconcellos personal observation 2008). Therefore, they may suffer from being kept in sterile environments with little opportunity to explore or display foraging activities analogous to those exhibited in the wild. The lack of novelty and complexity of many captive environments may indeed generate atypical behaviour in maned wolves. Bestelmeyer (1998) recorded lower activity levels in captive maned wolves relative to activity estimates for their wild conspecifics. The study showed pacing to make up about 1.25% of time spent (including inactive time), which may be regarded as an indication of poor welfare.

There have only been a few studies on environmental enrichment in wild canids, eg on red foxes (*Vulpes vulpes*; Nimon & Broom 2001), blue foxes (*Alopex lagopus*; Ahola *et al* 2000; Korhonen & Niemela 2000; Nimon & Broom 2001), bush dogs (*Speothos venaticus*; Ings *et al* 1997) and maned wolves (*Chrysocyon brachyurus*; Daley & Lyndaker-Lindsey 2000; Cummings *et al* 2007). Daley and Lyndaker-Lindsey (2000) reviewed the types of enrichment and the enrichment items used for 87 maned wolves in 27 North American zoos. They gathered information on the type and frequency of enrichment items but not on their effect on the animals' behaviour. Cummings *et al* (2007) conducted an experiment in which the physiological and immediate behavioural responses to enrichment were assessed in four, captive maned wolves. The activity and exploratory behaviour of the wolves was monitored during a 30-min period, immediately following hidden-food or novel-object presentation. Three-out-of-four displayed an increase in activity scores relative to baseline levels; all four animals increased their exploratory rates during this post-introduction period. Faecal glucocorticoid metabolite (GCM) concentrations were increased in the males but not the females.

In order to examine the hypothesis that novelty and environmental stimulation can improve the well-being of captive maned wolves, we presented maned wolves in three Brazilian zoos with: (i) toys for exploration (a one-meter piece of rope, a wooden halter and a plastic bone for every animal) (Boinski *et al* 1999; Korhonen & Niemela 2000; Wells 2004); (ii) objects which could be used as food or toys (pumpkins filled with blood) and (iii) hidden food (amongst vegetation, inside trunk holes or buried in the ground), thereby increasing the difficulty of gaining access to it. In an attempt to assess the wolves' welfare, we collected and analysed both behavioural data and faecal GCM concentrations (Carlstead *et al* 1993b; Schapiro *et al* 1993; Boinski *et al* 1999; Ahola *et al* 2000; Wasser *et al* 2000).

Based on the existing literature on environmental enrichment (Cummings *et al* 2007; Hansen *et al* 2007; Powell & Svoke 2008), the biology of maned wolves (Dietz 1984; Bandeira de Melo *et al* 2007; Queirolo & Motta-Junior 2007), hormonal reactions to challenges (Boinski *et al* 1999; Liu *et al* 2006; Moreira *et al* 2007), and personal observations of the study species, we developed five hypotheses. These are that the provision of objects to manipulate and the dispersion of food would lead to the following: (i) short-term increases in activity (higher frequency of locomotion, foraging or exploratory behaviours following food presentation); (ii) long-term increases in activity (ie, increased activity throughout the day); (iii) a reduction in pacing and (iv) a reduction in GCM concentrations, all of which are generally taken as signs of enhanced levels of welfare. It was also predicted that (v) the GCM concentrations would correlate with behaviour.

Materials and methods

Animals

The experimental animals consisted of 11 maned wolves from three zoos in the State of São Paulo, Brazil: São Bernardo do Campo Zoo (SBCZ), Fundação Parque Zoológico de São Paulo (FPZSP) and Parque Zoológico Municipal Quinzinho de Barros (PZMQB) (Table 1). Behavioural and hormonal data were collected from August to December 2002 (São Bernardo do Campo Zoo), from March to July 2003 (Fundação Parque Zoológico de São Paulo), and from August to December 2003 (Parque Zoológico Municipal Quinzinho de Barros).

All animals with the exception of one individual (Joana) were pair-housed. All enclosures (the size of which varied from 177 to 615 m^2) were planted with grass and furnished with live trees and a pond, and most parts of the enclosures contained only natural substrates. All animals had free access to both indoor quarters and outdoor exhibit for 24 h per day. The wolves were fed once a day, in the morning; the meal consisted of seasonal fruits (apple, banana, papaya) and meat (beef heart, chicken necks, moist dog food or slaughtered rats). Prior to the study, and during the baseline conditions, food was placed on trays, one for each individual, in the indoor quarters.

Qualitative observations of wolves' interactions with humans indicated that they fell into one of three cate-

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Table 1 Characteristics of the study animals.

Animal	Gender	Mate	Zoo born (B) or wild caught (C)	Institution	Enclosure size (m^2)
D	Female	P	1994 B	SBCZ	490
P	Male	D	1999 B	SBCZ	490
Um	Female	Dois	1993 C	FPZSP	615
Dois	Male	Um	1995 B	FPZSP	615
Três	Male	Quatro	2000 C	FPZSP	490
Quatro	Female	Três	1997 C	FPZSP	490
Joana	Female	–	1999 C	PZMQB	177
Preta	Female	Lobão	1991 C	PZMQB	202
Lobão	Male	Preta	1991 C	PZMQB	202
Filhote	Male	Sorocaba	1998 B	PZMQB	397
Sorocaba	Female	Filhote	2002 C	PZMQB	397

SBCZ: São Bernardo do Campo Zoo;
FPZSP: Fundação Parque Zoológico de São Paulo;
PZMQB: Parque Zoológico Municipal Quinzinho de Barros.

gories: 'strong avoidance' (Sorocaba, Filhote and P ran into the indoor quarters whenever the keeper entered the enclosure, and remained there until he left); 'wariness' (Preta, Lobão, D, 1, 2, 3 and 4, although displaying the solitary and shy profile of maned wolves, remained outdoors in the keeper's presence, waiting for food to be offered) and 'approach and affiliation' (Joana, an individual raised in close contact with humans, approached the keeper whenever he came into the enclosure, wagging her tail and rubbing the side of her body against his legs).

Experimental design

All procedures reported in this paper are carried out in accordance with all relevant and applicable state and federal laws in Brazil.

The experimental procedure followed an ABA experimental design (baseline 1/enrichment/baseline 2). In baseline 1 and baseline 2, normal zoo handling and feeding conditions were maintained. The order of the four enrichment conditions — Food, Toy, Food + toy and Edible toy — each lasting two weeks, was different at each zoo in order to control for sequential effects. The temporal order was: SBCZ (Toy, Food + toy, Edible toy, Food); FPZSP (Food, Edible toy, Food + toy, Toy); PZMQB (Food, Toy, Food + toy, Edible toy). Animals were observed for 20 h during every baseline phase and 40 h in the enrichment period. During the enrichment period (Food, Toy, Food + toy and Edible toy), the enrichment techniques were used on all weekdays, even when there were no observations.

Enrichment conditions

Food

The usual food items were divided into two equal portions, one presented as usual in a tray, indoors (where the animals were retained during the preparation of enrichment conditions in the enclosure) and the other, divided amongst four of the 12 pre-defined hiding sites in the enclosure. The 12 sites were located throughout the enclosure and consisted of holes in the ground (with the food covered by leaves), holes in the tree trunks, and locations amongst the vegetation.

Toy

A rope (one meter long and four centimetres in diameter, with knots at the ends), a plastic bone and a wooden halter for each animal were placed in the enclosure.

Food and toy

Food (as in the Food condition) was distributed in the enclosure in the presence of the toys (as in the Toy condition).

Edible toy

Perforated seedless pumpkins, filled with blood (one per animal) were placed in the enclosure.

Behavioural observations

A focal animal sampling observation procedure was used, three times a week, on weekdays, according to the categories listed in Table 2. Animals were observed during the daytime to avoid disturbing the zoos' husbandry protocol. Although mainly nocturnal or crepuscular, maned wolves do forage and travel in the

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Table 2 Description of the behavioural categories recorded in the study.

Observed behaviours	Description
Inactivity	
Resting	Lies laterally or ventrally.
Standing	Upright, supported on all four legs.
Out of view	Stays in an area which is visually inaccessible to observer.
Activity	
Observing	Interrupts current behaviour to stare at a specific stimulus (directing head and ears).
Aggression	Display threat (opens the mouth, growls, barks or bites).
Social	Sniffs another wolf, raises tail without growling, lowers front part of the body, gets closer to a human, wagging the tail, rubs neck or genital region on the vegetation.
Foraging/Exploration	Engages in searching, handling, ingestion and burying of food; hunts insects, birds etc.
Locomotion	Moves forward in an apparently intentional manner.
Pacing	Moves to-and-fro or in circles in a repititive manner, completing at least three repetitions.
Other behaviours	Behaviours as yet to be described.

daylight (Dietz 1984), thus making it feasible to present enrichment and observe its effects during the day. In fact, preliminary observations revealed that the maned wolves spent about 21% of the morning and 14% of the afternoon active, which is less than the estimated diurnal activity of wild animals (35% of daylight hours) and corroborates previous findings (Bestelmeyer 1998).

Each subject was observed for 20 min, five times a day, beginning: (i) between 0730 and 0800h; (ii) between 0930 and 1000h; (iii) between 1130 and 1200h; (iv) between 1330 and 1400h and (v) between 1530 and 1600h. Enrichment was introduced immediately preceding the third observation period. Due to a change in schedule, animals D and P were not observed between 1130 and 1200h.

Observations were made every 30 s (interval sampling) during the allotted period through the use of a handheld computer (Psion Workabout®, Psion Teklogix, Ontario, CA, USA). The behavioural recording programme was The Observer (Version 3.0, Noldus Information Technology, BV, Wageningen, The Netherlands), and the order of individuals observed was rotated randomly each day.

Hormone radioimmunoassay (RIA)

Faecal samples, collected in the morning of observation days (three times a week), were immediately homogenised and frozen for storage at –20°C. The steroid extraction method of Brown *et al* (1994) was used with minor modifications as already described for maned wolves (Cummings *et al* 2007). In a pilot test, a high correlation between GCM concentrations in wet and lyophilised samples was found (Pearson's correlation coefficient, $r = 0.8915$; n = 11; $P < 0.01$); therefore wet faeces were used. The hormonal data of a pair of animals who bred during the experiment (Três and Quatro) were excluded from analyses.

GCM were quantified using a commercially-available double-antibody corticosterone radioimmunoassay kit (MP Biomedicals, Irvine, CA, USA), following the manufacturer's protocol. This assay was shown to be able to detect changes in adrenocortical activity in a wide range of species (Wasser *et al* 2000). The assay was validated for maned wolves (Vasconcellos *et al*, in preparation): there was a correlation between corticosterone metabolic dilution curves and those of the commercial kit and an ACTH challenge was run. Before analysis, faecal extracts were diluted in RIA diluent (1:100 and 1:20, where necessary). Faecal concentrations of GCM are expressed as micrograms per gram of wet faeces.

There are still no studies on the time necessary for the glucocorticoid metabolism to take place in maned wolves, but Velloso *et al* (1998) quantified the excretion of marked testosterone, demonstrating that 97% of testosterone is excreted through the faeces, reaching a peak five hours after injection of the marked hormone. There was 75.8% excretion of testosterone up to 16 h afterwards. To take the delay of hormone secretion into account, GCM concentrations were paired with behavioural data recorded on the previous day.

Due to problems with black buzzards (*Coragyps atratus*) which entered the enclosures and consumed the faeces, some faecal samples could not be collected in FPZSP. For this reason, in analyses of the whole sample, the number of individual faecal samples (n) varied from two-to-twelve in baseline 1, from one-to-six in the Food condition, from two-to-six in the Toy condition, from one-to-six in the Food + toy condition, from three-to-six in the Edible toy condition, and from seven-to-twelve in baseline 2.

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Statistical analyses

Behavioural and hormonal data were tested for normality (Kolmogorov-Smirnov test) and non-parametric data were normalised using rank-transformation (SPSS Inc 2003). The Bonferroni correction was conducted for multiple comparisons. Tests were performed through GraphPad InStat (version 3.00, GraphPad Software Inc 2000), SPSS (version 12.0, SPSS Inc 2003) and SAS System for Windows (SAS Institute Inc 2000) programmes.

For behavioural analyses, the number of scores for each behavioural category for each subject was averaged, generating a subject mean for each of the five 20-min periods, in each condition. A subject mean for observation day was also calculated, and both means (period and day) for each experimental condition were used in repeated measures ANOVAs. The analysis was also applied to individual data; however, due to the possibility of a pseudoreplication bias, these results should be considered carefully.

For hormonal analyses, we used the individual mean of GCM concentrations per experimental condition. To analyse these data, a repeated measures ANOVA with two factors, experimental condition and sex, was applied. This method allows a comparison of groups under each condition, conditions within each group, and interaction between the variables (experimental conditions × gender) (Winer 1971; Timm 1975). When the ANOVA overall analysis was significant, planned *post-hoc* contrast analyses, between baseline 1 and each of the enrichment conditions, were performed.

Covariance between each of the behavioural categories and GCM concentrations and between individual behavioural scores under different experimental conditions was assessed through Pearson's correlation.

Results

Description of responses to enrichment

All animals demonstrated an immediate interest in scattered food (Food and Food + toy conditions). They explored the enclosure, approaching and sniffing vegetation and tree trunks, and attempted to draw out the food items placed in burrows or inside tree trunks. They searched for hidden food at all possible sites, probably locating the food via smell, and extracted it with their muzzle and/or paws. Searching persisted even after the last food morsel had been obtained. Searching behaviour was never observed during baseline 1 or 2, when food was presented in trays.

Wolves Um, Dois, Três and Quatro, which were slow to approach food when it was offered in a tray (baseline 1 and 2), quickly visited all food sites as soon as released in the Food and Food + toy conditions. When male and female animals approached food sources together, priority was generally given to the animal that arrived first. Animals D and P, when released from indoor quarters, abandoned the food they were eating in the trays and ran outdoors to search for and consume the scattered morsels. They returned to the trays after approximately 40 min.

Toys were not removed at night, therefore the wolves could have interacted with them during this period when their interactions could not be observed directly. At the beginning of each observation day, broken pumpkins (Edible toy) and frayed ropes (Toy) were found scattered around the enclosure. During the day, Joana, who had been raised in close interaction with humans, was the only wolf seen to engage in play bouts, either with the offered toys or with other objects.

Effects of enrichment on behaviour

'Locomotion' was significantly higher in the Food condition than in baseline 1 during Observation period 3 (when enrichment had just been introduced, n = 9; $F = 3.79$; $P < 0.05$), considering the total 11-animal sample. However, no behavioural categories differed significantly between conditions when the five daily observation periods were taken together for analysis (n = 11; ns). Analysis by subject revealed significant differences between enrichment conditions and baseline 1 (Table 3) in most animals, but not in the same direction. Um, for instance, exhibited decreased 'Out of view' and increased 'Resting' and 'Activity' in enrichment conditions involving food. Quatro exhibited increased 'Out of view', 'Activity' and 'Foraging/Exploration' but reduced 'Resting'. Lobão displayed a reduction in 'Aggression'. Filhote and Sorocaba exhibited increased 'Out of view' and decreased 'Resting'.

The constancy of individual characteristics was assessed by calculating the overall correlation between the behaviours performed during each pair of experimental conditions (Food × Toy, Food × Food + toy, etc). The number of significant correlations (out of 15 possible correlations) was relatively high for 'Pacing' (15), 'Activity' (13), 'Standing' (12) and low in other categories such as 'Foraging/Exploration' (5), 'Observing' (5), 'Social' (4), and 'Locomotion' (3); (Table 4).

Effects of enrichment on GCM concentrations

There were no significant differences in mean GCM concentrations among experimental conditions in the group (n = 9; $F = 1.688$; ns) and no significant differences were found between male and female wolves' scores during the whole experimental procedure ($n_{male} = 4$, $n_{female} = 5$; $F = 1.632$; ns) or in any of the experimental conditions (ns in all conditions).

The condition × gender interaction approached significance (n = 9; $F = 2.06$; $P = 0.09$). Female scores decreased in baseline 2 (n = 5; $F = 4.05$; $P < 0.05$) and male scores showed a tendency to decrease in the Edible toy condition (n = 4; $F = 3.50$; $P = 0.06$).

Individual variation in hormonal profiles was marked (Figures 1 and 2). In four animals (D, Preta, Sorocaba, and Filhote), GCM concentrations increased as a reaction to enrichment (especially in the Food + toy condition). In contrast, P, Lobão, Um and Dois showed decreases in

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Table 3 Significant results in the comparison of individual scores in every condition with baseline 1 scores.

Individual	Behaviour	Condition	*P*-value	*F*-value	Change
P	Out of view	F	$P = 0.0005$	$F = 4.779$	Increase
	Resting	F	$P = 0.0008$	$F = 4.541$	Decrease
Um	Out of view	T, F + T, ET	$P < 0.0001$	$F = 15.081$	Decrease
	Resting	T, F + T, ET	$P < 0.0001$	$F = 13.108$	Increase
	Activity	F + T, ET	$P = 0.0018$	$F = 4.051$	Increase
Três	Observing	BL2	$P = 0.0499$	$F = 2.277$	Increase
	Activity	BL2	$P = 0.0267$	$F = 2.620$	Increase
Quatro	Out of view	F + T, ET	$P < 0.0001$	$F = 10.213$	Increase
	Resting	F + T, ET	$P < 0.0001$	$F = 7.084$	Decrease
	Foraging/Exploration	F	$P = 0.0006$	$F = 4.634$	Increase
	Activity	F	$P = 0.0003$	$F = 4.924$	Increase
Joana	Out of view	ET, BL2	$P = 0.0112$	$F = 3.086$	Increase
Preta	Observing	ET	$P = 0.0417$	$F = 2.376$	Decrease
Lobão	Aggression	F, T, F + T, BL2	$P = 0.0087$	$F = 3.222$	Decrease
Filhote	Out of view	T, F + T, ET	$P = 0.0013$	$F = 4.213$	Increase
	Resting	F, T, F + T, ET, BL2	$P = 0.0002$	$F = 5.176$	Decrease
Sorocaba	Out of view	T, F+T, ET, BL2	$P < 0.0001$	$F = 9.522$	Increase
	Resting	T, F+T, ET, BL2	$P < 0.0001$	$F = 9.023$	Decrease

Repeated Measures ANOVA followed by Dunnet *post-hoc* test, df = 5.
F: Food; T: Toy; F + T: Food + toy; ET: Edible toy; BL1: baseline 1; BL2: baseline 2.

Table 4 Significant correlations of behavioural scores between conditions.

Behavioural categories	BL1 × F	BL1 × T	BL1 × FT	BL1 × ET	BL1 × BL2	F × T	F × FT	F × ET	F × BL2	T × FT	T × ET	T × BL2	FT × ET	FT × BL2	ET × BL2
Pacing	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Activity	*		*	*	*	*	*	*	*	*	*		*	*	*
Standing	*	*	*	*	*	*	*	*		*	*		*		*
Other	*	*	*	*	*	*	*			*	*	*	*	*	
Resting	*				*					*	*	*	*	*	*
Out of view	*				*					*	*	*	*	*	*
Aggression			*	*	*								*	*	*
Foraging/Exploration			*	*				*	*						*
Observing	*			*	*			*	*						
Social	*	*				*							*		
Locomotion			*	*										*	

* $P < 0.05$.

hormonal levels in the enrichment conditions (with the lowest values in the Food + toy and Edible toy conditions).

There was a positive correlation between GCM concentrations and the frequency of 'Pacing' (n = 9; $r = 0.621$; $P = 0.01$) and a negative correlation between GCM concentrations and 'Social' (n = 9; $r = 0.6366$; $P < 0.01$).

Discussion

Direct qualitative behavioural observations indicated that the food enrichment procedures used in the present study were effective in generating interest in the animals. Wolves reacted promptly to enrichment conditions and, in some cases, as observed in animals D and P, foraging for hidden

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food took precedence over eating food available in a tray. Unpublished results from our department confirm the hypothesis of a preference of maned wolves for scattered and hidden 'costly' sources of food. When both free food (in trays) and concealed food parcels were available, wolves spent relatively more time searching than eating at the tray.

This allocation of time to a costly way of acquiring food is analogous to the contra-freeloading effect (Jensen 1963; Neuringer 1969; Krebs *et al* 1978; Inglis *et al* 1997; Bean *et al* 1999; Lindqvist & Jensen 2008, but see Young & Lawrence 2003) and may be taken as a corroboration of the Ethological Necessities model (Hughes & Duncan 1988), according to which animals, regardless of their goal or consummatory activities, value the instrumental or appetitive behaviour, *per se*. It may also be taken as supporting Barnard's adaptive self-expenditure view, which equates welfare "with the animal's ability to spend itself in the manner dictated by its adaptive life history strategy" (Barnard 2007). Maned wolves travel long distances to forage on plants or small mammals (Bandeira de Melo *et al* 2007), and this may predispose them to preferring a relatively active way of acquiring food under captive conditions.

As for the toys, we found indications of interaction, but no effect on the measurable variables. It is possible that if the toys had been changed throughout the Toy condition, we would have found a measurable effect, as in some other studies (Hubrecht 1993; Korhonen & Niemela 2000; Wells 2004).

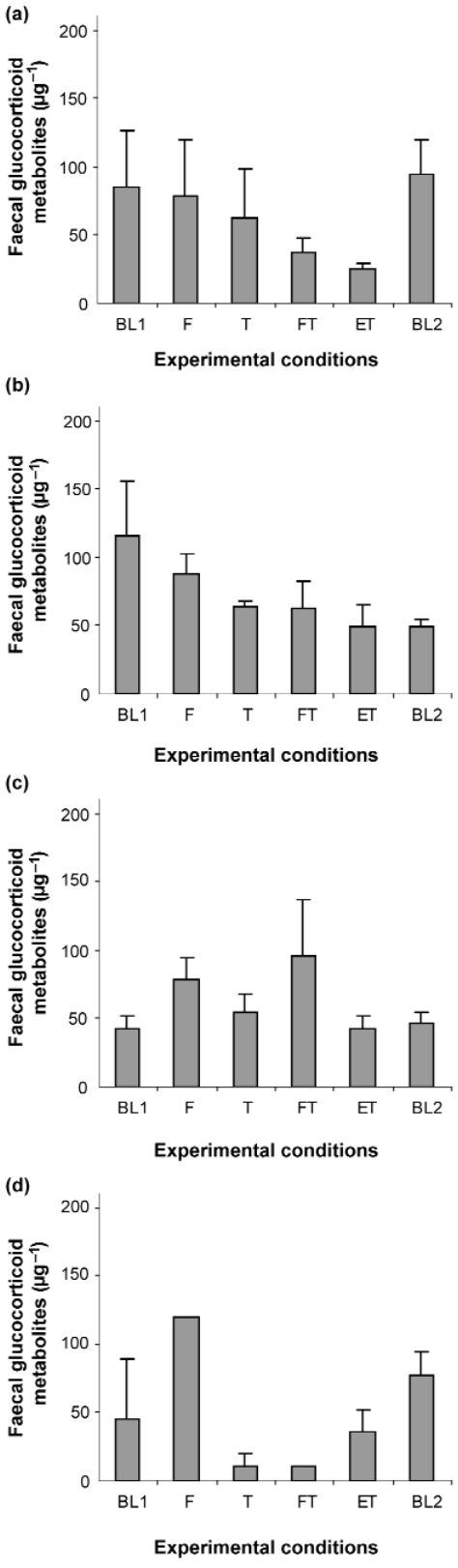
Quantitative analysis revealed that the effects of enrichment procedures were complex and were the probable outcome of an interplay of factors. Our first and last hypotheses (short-term activity increases and correlation between GCM concentrations and behaviour) were verified but the other three (long-term increases in activity, reductions in pacing and in GCM concentrations) were not.

Short-term effects are behavioural (or hormonal) responses to treatment, which depend on the actual or recent presence of enrichment stimuli. Long-term effects, generally more relevant to husbandry and welfare concerns, are those that may remain after treatment is discontinued and signal a long-term change in stress conditions.

Short-term effects of food enrichment were obtained in our study. Increased levels of 'Locomotion' were recorded during observation period 3, immediately after food was made available. Exploratory and activity rate increases found by Cummings *et al* (2007) in captive maned wolves were also a short-term effect since, in that study, records were taken only during a 30-min period after the enrichment presentation. Results such as these do not support a guarantee that a specific treatment is stress-lowering or welfare-promoting on a long-term basis.

When combining the five daily observation periods for analysis, that is, when looking for long-term, durable effects of enrichment, we did not find statistically-significant results for the whole 11-animal sample in any of the behavioural categories. Aspects of our procedure (duration of enrichment treatment, challenge and complexity value of

Figure 1



Mean (± SEM) GCM profiles of male maned wolves during the experimental conditions for (a) P, (b) Lobão, (c) Filhote and (d) Dois. BL1 = baseline 1, F = Food, T = Toy, FT = Food and toy, ET = Edible toy and BL2 = baseline 2).

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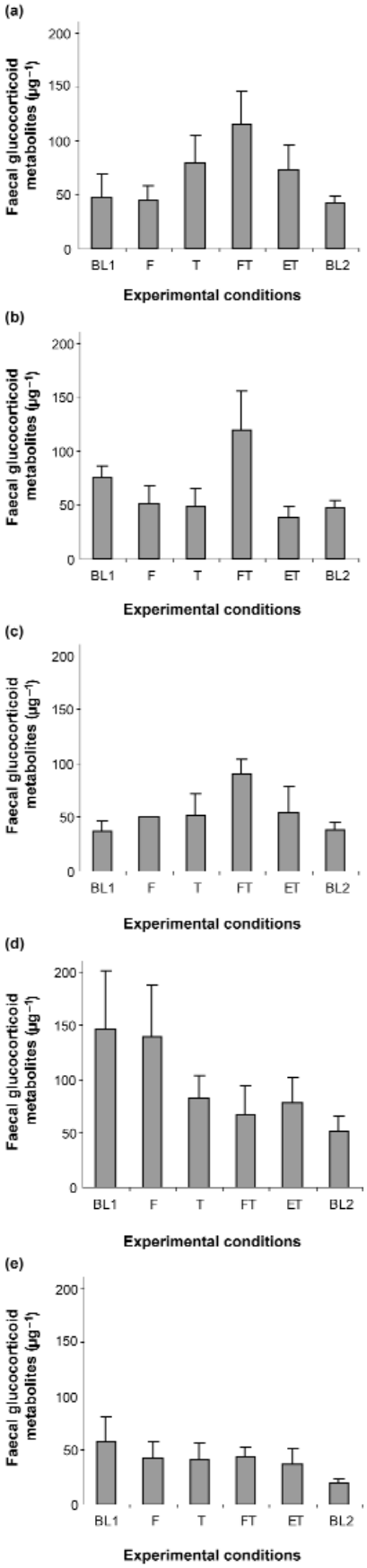
Figure 2



Mean (± SEM) GCM profiles of female maned wolves during the experimental conditions for (a) D, (b) Preta, (c) Sorocaba, (d) Um and (e) Joana.

the food searching task, nature of the toys offered, period of the day in which interventions were made, etc) may eventually explain this lack of homogeneous, significant, long-term effects. But one aspect that seems particularly relevant and that deserves attention is our subjects' variability in performance and reaction to enrichment techniques.

When analysed at the individual level, our results reveal significant long-term effects of enrichment: there were significant differences in performance between experimental conditions and baseline 1 (Table 3) in nine of the eleven animals, mostly in food conditions (Food, Food + toy, and Edible toy). The wolves in our study, as is the case with many zoo animals, had different origins; some were born in the zoo and others were wild-captured (Table 1). They differ in age and past social experience and it is not improbable that their ontogenetic trajectories might have produced differences in behavioural propensities and sensibility to enrichment procedures.

Differences in temperament and individual traits were found to influence the response to stress or environmental challenges in several species (Mülleder *et al* 2003; De Palma *et al* 2005; Kralj-Fiser *et al* 2007; Slotta-Bachmayr & Schwarzenberger 2007; Albert *et al* 2008). Inter-individual variability has been found by Carlstead and Kleiman (1998) and Cummings *et al* (2007) in studies on maned wolves. Tests have been devised to assess animal temperament and behavioural differences and to evaluate their influence on the effectiveness of environmental enrichment techniques (Walsh & Cummins 1976; Cooper & Mason 2000; Harri *et al* 2000; Mason *et al* 2001; Raffa *et al* 2002; Bremner-Harrison *et al* 2004; Kirkden & Pajor 2006; Powell & Svoke 2008).

The three profiles the wolves presented in the presence of humans ('strong avoidance', 'wariness', and 'approach and affiliation') may explain some of the behavioural differences observed during the enrichment conditions. For example, Joana, the least shy of the wolves, was the only one to interact with toys during daylight. Contrasting performances were observed in shy animals ('strong avoidance'), which showed increased 'Out of view' and decreased 'Resting' relative to baseline 1, and hid more when exposed to enrichment procedures.

In order to assess the existence of lasting individual characteristics, we calculated the correlation between scores of specific behaviours in different experimental conditions, assuming that a correlation throughout conditions would reveal stable inter-individual differences. Significant correlation coefficients were indeed obtained in a relatively high number of comparisons. For certain categories ('Activity', 'Out of view', 'Standing', 'Resting'), the animals maintained inter-individual rankings under different external conditions. The number of correlations between conditions was lower for other categories ('Aggression', 'Social', 'Locomotion', 'Foraging/Exploration' and 'Observing'). Further studies are needed to specify the degree of stability (in terms of inter-individual differences) of traits or behavioural predispositions and to understand their influence on animals' responses to enrichment.

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We expected, in accordance with the results of previous studies (Carlstead *et al* 1993b; Schapiro *et al* 1993; Pedersen 1996; McGreevy & Nicol 1998; Boinski *et al* 1999; Ahola *et al* 2000; Beattie *et al* 2000; De Groot *et al* 2000; De Jong *et al* 2000; Roy *et al* 2001; Belz *et al* 2003; Hansen *et al* 2007; Moreira *et al* 2007), that the concentrations of GCM in maned wolves would decrease in response to environmental enrichment procedures. GCM concentrations did not decrease with enrichment when looking at the entire sample of wolves; however, there were noticeable individual differences in GCM concentrations between baseline and enrichment conditions (Figures 1 and 2). Unexpected variability in hormone production has also been recorded in several species (Weiss *et al* 2004; Haubenhofer *et al* 2005; Liu *et al* 2006; Cummings *et al* 2007; Gorgasser *et al* 2007; Svedsen *et al* 2007) and is consistent, in the present experiment, with inter-individual variability in behaviour.

GCM changes in response to enrichment may depend, as behaviour does, on ontogenetic factors. Joana, the wolf raised in intense contact with humans, displayed stable GCM concentrations throughout the experimental conditions (Figure 2). Since instability in hormone levels may be a sign of poor welfare conditions and may be associated with indicators of chronic stress (such as proneness to agonistic interactions and stereotypic pacing [Carlstead & Brown 2005]), Joana probably experienced the best welfare conditions of all the wolves in the study. It is possible that Joana's deviation from the rest of the sampled wolves, both in behaviour and hormonal levels, were due to her history of close contact with humans.

The reduction in the females' GCM concentrations in baseline 2, at the end of the experiment, and the tendency for hormone concentrations to decrease in males during the Edible toy condition suggest that male and female maned wolves may react differently to enrichment stimuli. Differences in GCM concentrations related to gender have been found in different species and may be attributed to differences in basal corticoid levels (Wielebnowski *et al* 2002a), metabolite metabolism and excretion routes (Touma *et al* 2003), reaction to stressors (Belz *et al* 2003), and levels of inter-individual variability (Lepschy *et al* 2007). Differences in GCM concentrations between male and female responses to enrichment, which have not been described previously in maned wolves, may result from differences in their reproductive strategies. These differences are worth further investigation, due to their implications for welfare.

The occurrence of both an increase and decrease in GCM concentration, as a consequence of enrichment, indicates that the causal relationship between hormonal indicators and welfare is not a simple one. These results should not lead to the conclusion that enrichment produced beneficial effects in some animals and harmful ones in others. Positive enrichment effects are not necessarily those obtained through the lowering of GCM concentrations (Natelson *et al* 1988; Haemisch *et al* 1994; Smith & Dobson 2002). Stress is indeed activated under conditions of a perceived threat to homeostasis and is the reaction to a challenge to adaptation and survival. Nevertheless, not all stress agents are harmful (causing distress), and their (positive or negative) effects may depend on contextual factors. There is a minimum level of stress needed for adaptations to occur and enrichment, in order to establish an optimum activation level, may require increased hormone production. Pigs (*Sus domesticus*) in enriched environments may show increases in cortisol concentrations relative to baseline levels, where such increases may not be attributed to reduced welfare (Beattie *et al* 2000). Marashi *et al* (2003) also obtained higher levels of plasma corticosterone in mice (*Mus domesticus*) housed in enriched environments as compared to mice in barren environments, an increase thought to be possibly associated with behaviours indicative of welfare.

The positive correlation between GCM concentrations and 'Pacing' may be taken as an indication that higher GCM concentrations can be associated with increases in excitability. There was, however, no reduction in 'Pacing' due to enrichment procedures. Cummings *et al* (2007), similarly, did not find reduction in GCM concentrations after the application of environmental enrichment. The walking habit of wild maned wolves may explain why, under the constraints of captivity, these animals continue to pace, both during and after enrichment conditions. These results are consistent with the correlation between a carnivore's home-range size in the wild and pacing in captivity described by Clubb and Mason (2003). Negative correlation between GCM concentrations and 'Social' may also be interpreted in terms of a heightened activation under hormonal influence, which competes with affiliative interactions (Carlstead & Brown 2005).

Animal welfare implications

According to our study, enrichment techniques, in particular those that stimulate interest in foraging activities, may influence behaviour and hormonal concentrations in captive maned wolves. Most relevant, however, is the clear indication that individual and gender aspects may influence the effectiveness of these enrichment techniques. Further evaluation of individual differences and the interaction of such differences with handling treatments seem essential for designing effective maned wolf enrichment techniques.

Conclusions

In addition to demonstrating the welfare relevance of stimulus variability and elicitation of foraging-like searching behaviour in maned wolves, our results suggest the need for a conceptual change in the planning of environmental enrichment protocols. Contrary to our expectations, the procedures did not promote, at the group level, long-term increased activity, reduced pacing, or reduced GCM concentrations. Long-term performance changes in our animals were individualised and indicated the importance of individual history and temperament as factors in the outcome of husbandry manipulations. Prior assessment of individual temperament and behavioural tendencies should enhance the success of an enrichment

programme. Hormonal analysis revealed that the relationship between glucocorticoid levels, behaviour, and welfare may be more complex than commonly thought and requires further examination.

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