





**Features of psittacine birds in captivity:  
focus on diet selection and digestive characteristics**

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2011**

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# **Features of psittacine birds in captivity: focus on diet selection and digestive characteristics**

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*Thesis submitted in fulfilment  
of the requirements for the degree of  
doctor in Veterinary Science (PhD)  
Faculty of Veterinary Medicine  
Ghent University  
2011*

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**Kalmar, I.D.** (2011) Features of psittacine birds in captivity: focus on diet selection and digestive characteristics

PhD thesis, Ghent University, Belgium.

With references, with summaries in English and Dutch.

**ISBN 978-90-5864-250-9**

...

Soon you will not see us  
in the forest if you look,  
the library is where we'll be  
inside a picture book.

...

(Plight of the Parrot - Terri L. Doe)





# TABLE OF CONTENTS

<b>LIST OF ABBREVIATIONS</b>	<b>1</b>
<b>CHAPTER 1</b> General introduction	<b>3</b>
1.1. Background information on psittaciform birds	<b>5</b>
1.2. Housing and environmental enrichment	<b>15</b>
1.3. The use of parrots as laboratory animals	<b>21</b>
1.4. Digestive characteristics and nutrition	<b>34</b>
<b>CHAPTER 2</b> Scientific aims	<b>61</b>
<b>CHAPTER 3</b> Effect of dilution degree of commercial nectar and provision of fruit on food, energy and nutrient intake in two rainbow lorikeet subspecies	<b>65</b>
<b>CHAPTER 4</b> Effects of segregation and impact of specific feeding behaviour and additional fruit on voluntary nutrient and energy intake in yellow-shouldered amazons ( <i>Amazona barbadensis</i> ) when fed a multi-component seed diet <i>ad libitum</i> .	<b>83</b>
<b>CHAPTER 5</b> Apparent nutrient digestibility and excreta quality in African grey parrots fed two pelleted diets based on coarsely or finely ground ingredients.	<b>105</b>
<b>CHAPTER 6</b> General discussion	<b>123</b>
<b>SUMMARY</b>	<b>135</b>
<b>SAMENVATTING</b>	<b>141</b>
<b>ACKNOWLEDGEMENTS</b>	<b>147</b>
<b>SCIENTIFIC PUBLICATIONS</b>	<b>151</b>
<b>CURRICULUM VITAE</b>	<b>165</b>



<b>aD</b>	apparent digestibility	<b>H</b>	<i>Trichoglossus h haematodus</i> (green-naped lorikeet)
<b>AF</b>	as fed (on fresh matter basis)	<b>M</b>	<i>Trichoglossus h mitchelli</i> (red-breasted lorikeet)
<b>AWA</b>	Animal Welfare Act	<b>MBW</b>	metabolic bodyweight
<b>BW</b>	bodyweight	<b>ME</b>	metabolizable energy <sup>1</sup>
<b>Ca</b>	calcium	<b>N</b>	nectar
<b>CF</b>	crude fibre	<b>NfE</b>	nitrogen free extract
<b>CITES</b>	Convention on International Trade of Endangered Species	<b>NSP</b>	non-starch polysaccharides
<b>CP</b>	crude protein	<b>OM</b>	organic matter
<b>C-pellets</b>	coarse particle pellets	<b>P</b>	phosphorous
<b>D</b>	dilution degree	<b>S</b>	subspecies
<b>DM</b>	dry matter	<b>St</b>	starch
<b>ECI</b>	excreta consistency index	<b>Su</b>	sugar
<b>EE</b>	ether extract (crude fat)	<b>VAM</b>	extruded pellets enriched with vitamins, amino acids and minerals
<b>F-pellets</b>	fine particle pellets		
<b>F</b>	fruit		

<sup>1</sup> Due to the lack of established equations to calculate ME for psittacine species, distinct equations are applied in the species-specific literature. In order to facilitate comparison of data to the available literature, corresponding equations were used in the different chapters:

**Chapter 3:** equation developed for poultry [Larbiere and Leclercq (1992)]

$$ME_{\text{lorikeets}} \text{ (MJ ME kg}^{-1}\text{)} = 0.1551 \text{ CP (\%)} + 0.3431 \text{ EE (\%)} + 0.1301 \text{ Su (\%)} + 0.1669 \text{ St (\%)}$$

**Chapter 4 and 5:** modified equation originally developed for poultry [Schoemaker and Beynen (2001)]

$$ME_{\text{granivorous parrots}} \text{ (MJ ME kg}^{-1}\text{)} = 0.18 \text{ CP (\%)} + 0.39 \text{ EE (\%)} + 0.17 \text{ NfE (\%)}$$



# CHAPTER 1

## GENERAL INTRODUCTION

In part after:

**Kalmar ID**, Janssens GPJ and CPH Moons. 2010. Guidelines and ethical considerations for housing and management of psittacine birds used for research purposes. *Institute for Laboratory Animal Research (ILAR) Journal* **51**, 409-423.

**Kalmar ID**, Moons CPH, Meers L and GPJ Janssens. 2007. Psittacine birds as laboratory animals: reflections on welfare. *Journal of the American Association for Laboratory Animal Science* **46**, 8-15.



## 1.1. BACKGROUND INFORMATION ON PSITTACIFORM BIRDS

The Psittaciformes or parrots form a large order of land birds comprising over 350 species in about 83 genera (Collar, 1997). In Belgium and the rest of Europe, the total number of parrots kept in captivity is estimated to be 3 and 45 million, respectively (Ghysels, 2005). In the United States they are the third most popular companion animal, estimated at 10.1 million individuals in 2002 (AVMA, 2002).

### SYSTEMATICS

Psittaciform birds have distinct morphological traits, such as a stout curved beak topped by a cere (the bump where the nostrils are located), zygodactyl feet (two toes pointing forward and two backward), and colourful plumage. Traditionally, psittacines are phylogenetically placed between the Columbiformes (pigeons) and Cuculiformes (cuckoos). Using molecular techniques, Sibley and Ahlquist (1990) suggested a closer relationship with Apodiformes (swifts), but this would be inconsistent with the plesiomorphic or primitive distal centriole in swift sperm (Jamieson and Tripepi, 2005).

Within the order of Psittaciformes, two distinct groups are generally recognized as separate families: Cacatuidae (cockatoos) and Psittacidae (parrots) (Collar, 1997; Forshaw and Cooper, 2002). External morphological features of cockatoos that distinguish them from other parrots include an erectile crest and lack of Dyck texture<sup>2</sup> in feathers. As a result of the latter, cockatoos never display blue or green colours in their plumage. An internal difference includes the presence in cockatoos of a gallbladder, which is absent in other parrots (Rowley, 1997). Recent phylogenetic data (Tokita *et al.*, 2007) suggest a third family in the order of Psittaciformes, the Nestoridae, which includes the New Zealand parrots: kakapo (*Strigops habroptilus*), kea (*Nestor notabilis*), and kaka (*Nestor meridionalis*). Lories and lorikeets form another separate group of parrot species. These birds are distinguished by certain anatomical features and feeding preferences, but their classification into a subfamily within the Psittacidae (Loriinae) or into a separate family (Loriidae) remains controversial (Russell, 1987; Forshaw and Cooper, 2002). The most prominent anatomical characteristic of lories is their long, brush-tipped tongue and slender beak, which reflect a high degree of diet specialization towards nectar and pollen (Cornejo and Clubb, 2005).

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<sup>2</sup> Blue feather colour is formed by nanostructures in the feather called Dyck texture (Dyck 1971a,b).

## GEOGRAPHIC DISTRIBUTION

Most psittacines are native to the southern hemisphere and are predominantly, but not exclusively, found in tropical regions. Lories, lorikeets, and cockatoos occur naturally only in Australia and surrounding Indonesian islands. Genera in the family of Psittacidae, on the other hand, are indigenous to Asia, Central and South America, Africa, and the Australian continent. There are only two genera of Asiatic parrots: hanging parrots (*Loriculus* spp.) and ring-necked parakeets (*Psittacula* spp.). Pionus parrots (*Pionus* spp.), macaws (*Ara*, *Cyanopsitta*, and *Anodorhynchus* spp.) and conures (e.g., *Aratinga*, *Cyanoliseus*, and *Pyrrhura* spp.) are native to South America, and the amazons (*Amazona* spp.) originate from Central and South America. African greys (*Psittacus* spp.), poicephalus parrots (*Poicephalus* spp.), and lovebirds (*Agapornis* spp.) originate from Africa; whereas grass parakeets (*Neophema* spp.), budgerigars (*Melopsittacus* spp.), and rosellas (*Platycercus* spp.) are indigenous to Australia (Russell, 1987).

Irrespective of geographical origin, descendents of escaped cage birds have been establishing self-sustaining populations in regions other than their native habitat. For instance, monk parakeets (*Myopsitta monachus*), which are indigenous to South America, have formed feral populations on four other continents (Russello *et al.*, 2008), and in the United States the American Ornithologists' Union has identified seven parrot species as self-sustaining exotics, with another seven likely to be recognized as such in the near future. The two native US parrot species, the Carolina parakeet (*Conuropsis carolinensis*) and the thick-billed parrot (*Rhynchopsitta pachyrhyncha*), on the other hand, disappeared during the 20<sup>th</sup> century (Butler, 2005). Efforts to re-establish the latter, however, have not resulted in self-sustaining wild populations. Still, release experiments with wild-caught birds from Mexico, where the species is still extant, have resulted in a limited number of surviving, trans-located birds and some reproductive success in Arizona (Snyder *et al.*, 1994).

## CONSERVATION STATUS

Unlike most taxa, parrots are vulnerable to multiple sources of extinction risk, which include a.o. habitat loss, introduction of predators and competitors, and human persecution such as trade (Juniper and Parr, 1998; Owens and Bennett, 2000). As a result, a higher proportion of species within the order of psittacine birds is threatened with extinction compared to most other bird orders (Bennett and Owens, 1997; Owens and Bennett, 2000).



Almost all parrot species are listed by the Convention on International Trade in Endangered Species (CITES). This is an international treaty that regulates the import and export of endangered wild flora and fauna, based on their classification into one of three Appendices. These regulations comprise not only live or dead animals, but also their products and tissues.

The classification of species in CITES Appendices occurs as follows:

(CITES, 1973 and 2009)

- Appendix I : species at risk of global extinction
- Appendix II : (a) species that may become threatened with extinction if international trade restrictions would be absent
  - (b) non-endangered species that may be in danger because of resemblance to specimens listed in the appendices
  - (c) second-generation captive-born offspring of Appendix I species
- Appendix III : species for which trade is regulated in request of certain countries

Appendix I lists 51 psittacine species, which account for about one-third of all avian species in this CITES appendix; nearly all remaining psittacine species appearing in Appendix II. Only four species of Psittaciformes are not considered at risk: peach-faced lovebirds (*Agapornis roseicollis*), budgerigars (*Melopsittacus undulatus*), cockatiels (*Nymphicus hollandicus*), and rose-ringed parakeets (*Psittacula krameri*) (CITES, 2009).

## MAIN AREAS OF WELFARE CONCERN

### *Domestication*

Parrots, commonly admired for their vocal abilities and exotic appearance, have been kept in captivity for at least 2500 years. The first parrots introduced into Europe were probably Alexander parakeets (*Psittacula krameri*), which were brought from the Far East by Alexander the Great during the 4<sup>th</sup> century BC. Later on, North African and Indian species were spread by the Romans. In the 15<sup>th</sup> and 16<sup>th</sup> centuries, European explorers brought back American and Asian parrot species as living proof of their voyages (Forshaw and Cooper, 1989; Silva, 1991; Collar, 1997), and in the 18<sup>th</sup> century colonists brought small Australian parakeets to Europe (Silva, 1991). Budgerigars were successfully bred in captivity by the end of the 19<sup>th</sup> century, largely because of their early reproductive maturity and sexual dimorphism (Earle and Clarke, 1991; Silva, 1991; Alderton, 2001).

Captive breeding in other parrot species, however, has become truly successful only in the past 40 years. Aside from their late maturity and sexual monomorphism to the human eye, other contributing factors to this delay are the animals' strict monogamy and need for prolonged periods of parental care. A major improvement in captive breeding was the development of endoscopic and genetic sexing, as well as histological appraisal of breeding potential from testis biopsies. Thanks to these techniques, besides improved husbandry practices and advances in veterinary care, about 85% of parrot species have bred in captivity to date (Silva, 1991; Crosta *et al.*, 2002; Gartrell, 2002; Harcourt-Brown, 2000).

Yet, the vast majority of captive parrots is still either wild-caught or belonging to one of the first few generations born in captivity. Domestication, which is the process of adaptation to captivity through genetic changes, in contrast, requires several generations of selective breeding. Only a couple of dozen animal species have been domesticated yet, but domestication of parrots is still in its infancy (Meehan *et al.*, 2003b). As captive parrots belong at most to the first few captive-bred generations, they have, unlike for example dogs and cats, not yet undergone selection for behavioural traits that facilitate adaptation to life in captivity (Davis, 1999). In consequence, although parrots can become very docile and adapted to captive environments within their own lifespan, they still share the natural behaviour and response thresholds with their wild counterparts. Hence, these bird species should be considered wild animals, with the possible exceptions of budgerigars and cockatiels (Bergman and Reinisch, 2006). Therefore, parrots are likely to experience frustration when confined in conditions that limit the expression of species-specific behaviours, especially those associated with high levels of motivation.

### *Cognition*

The remarkable cognitive abilities in psittacine birds have been intensively studied and documented (e.g., Pepperberg, 2006; Giret *et al.*, 2009; Shuck-Paim and Borsari, 2009). Even in the oldest known writings about parrots, dating from 500 BC, Ctesius described the ability of vocal mimicking of Indian and Greek words in a plum-headed parakeet (*Psittacula cyanocephala*) (Collar, 1977). More eminent with regards to the cognitive abilities of certain parrot species is recent data that confirm levels of intelligence, for instance in symbolic learning, similar to those of great apes and some marine mammals (Pepperberg, 2002). This high level of cognition in parrots is consistent with several characteristics generally associated with advanced cognitive processing, such as a relatively large brain, slow development,

longevity, and a long period of parental association (Shuck- Paim and Borsari 2009). Parrots' vocalizations, and especially their ability to mimic the human voice, are a distinctive manifestation of their cognition<sup>3</sup>. Herein, in contrast to most songbirds, in which vocal learning is limited to a sensitive period in early development, parrots exhibit lifelong vocal learning (Marler, 1970; Scarl and Bradbury, 2009). Scarl and Bradbury (2009) suggest that the ability in some parrot species to mimic vocal calls in a single interaction is an efficient vocal technique for mediating fission fusion flocks (constantly changing social groups).

Numerous scientifically sound laboratory studies on parrot cognition have demonstrated that several parrot species display a much higher intellectual capacity than the ability merely to mimic the human voice. The African grey parrot 'Alex' is probably the most well-known individual in this field of research. Pepperberg (2006) worked extensively with Alex and reviewed his trained cognitive abilities with regard to meaningful use of the English spoken language. This included the labelling of over 50 objects and the functional use of short queries that demonstrate accurate comprehension of concepts such as colour, shape, material, relative size, and quantity. Studies with other African greys have demonstrated mirror-mediated discrimination, spatial location, and spontaneous categorization into unlabeled edible or non-edible items (Pepperberg, 1995; Giret *et al.*, 2009). In addition, Schachner and colleagues (2009) demonstrated the ability to align synchronized motor actions (head movements and foot lifting) in response to music in an African grey parrot (*Psittacus erithacus erithacus*) and a sulfur-crested cockatoo (*Cacatua galerita*). Although most cognitive research in parrots involved African grey parrots, advanced cognitive processing has been evaluated in other parrot species as well. Werdnich and Huber (2006), for instance, showed successful performance of complex string-pulling tasks in keas, and Shuck-Paim and Borsari (2009) demonstrated this ability in neotropical parrot species (two macaw and one amazon species).

Altogether, these cognitive abilities suggest a level of intelligence that leads to questions regarding the psychological welfare of parrots that are confined for lengthy periods in barren enclosures without behavioural diversions (Dilger, 1982b; Pepperberg, 2004). Hence, besides being one of the grounds for their popularity as pet animals, the high level of cognition in parrots also comprises a source of welfare concerns when these birds are kept in captivity,

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<sup>3</sup> This ability in psittacines is distinctive but not unique: songbirds (Passeriformes) and hummingbirds (Trochilidae) also display vocalization through imitation (Matsunaga and Okanoya 2009), in contrast to the vocalizations of most avian groups, which depend on the production of innate sounds.

which necessitates careful consideration of the choice of species as well as their housing conditions.

### *Longevity*

The most reliable records regarding the lifespan of free-ranging parrots mainly originate from a large-scale study in Australia, in which native birds were banded starting at the beginning of the 20<sup>th</sup> century. Among these tagged birds was a little corella (*Cacatua sanguinea*) that was at least 71 year old when it died. In captivity, larger species have a potential lifespan of 30 to 50 year, with individual birds living as long as 80 years (Collar, 1997). Given this considerable attainable lifespan, one should bear in mind that parrots might well outlive their owners, which necessitates careful planning of the long-term fate of pet parrots.

## **INDICATIONS OF DISTRESS AND COMPROMISED WELFARE**

### *Excessive Vocalization*

Almost all psittacine species are highly social and flockdwelling animals. Their flock cohesion is promoted by the display of a wide variety of vocalizations, such as calm contact calls, loud alarm calls, food-begging calls, and interspecific agonistic calls (Bergman and Reinisch, 2006). Vocalization is thus an inherent component of the normal repertoire of parrot behaviour. Still, it may become excessive in both loudness and frequency, although, humans often consider any loud vocalization in parrots undesirable, excessive, or abnormal. While a certain expression of vocal behaviour is normal in parrots, vocalizations may also indicate disturbed well-being. Alternatively, it may also simply reflect unintentionally learned attention-seeking behaviour.

It is important to try to distinguish between normal, distress-signalling, and learned vocalization in parrots. The underlying motivation should be considered by taking into account both the animals and elements in their environment. Normal vocalization is displayed according to a daily pattern that includes extensive morning and evening vocalization, as well as several 15- to 20-minute loud vocalizations throughout the day (Wilson, 2005a). In contrast, prolonged and repetitive screaming, especially if it occurs throughout the day, may either indicate boredom, fear, distress or the desire for increased attention from caretakers (Davis, 1991; Alderton, 2001; Bergman and Reinisch, 2006).

### *Fear Behaviour*

Fear is generally considered an unpleasant emotional state (Jones, 1997), which necessitates the ability of human caretakers to readily recognize species-specific signs of it. Parrots' fear of humans is often directed at the hands, while human faces rarely elicit fear reactions. Therefore, if the sight or proximity of a friendly face does provoke fear, it could indicate extreme fright (Wilson, 2007).

Although parrots may respond to fear by biting, it is in their nature, as prey-animals, to instinctively attempt to avoid a threat by flying away (Welle and Luescher, 2006). Other fear-induced behaviours include reluctance to approach novel objects, humans or conspecifics, withdrawal, hiding, or alarm calls. If such signals are ignored, the animal may express stronger responses, such as fearful vocalization or a passive-aggressive posture, indicated by erection of the contour feathers (Welle and Luescher, 2006; Wilson, 2007). As a last resort, the animal may bite, which usually leads to the desired outcome: cessation of interaction with the caretaker. Herein, repetitive exposure to fearful situations in which humans ignore a bird's warning signals increases the risk of apparently impulsive fear biting (Welle and Luescher, 2006; Wilson, 2006). In general, all sources of fear should be kept to a minimum and causal factors of seemingly unfounded dread actively be sought, followed by an attempt to overcome or minimize them. Diverse environmental enrichment strategies can also reduce the likelihood of fear responses (Meehan *et al.*, 2003a).

Several standardized methods have been developed to objectively evaluate the level of fear in parrots. These usually assess either fear towards novel objects (neophobia) or fear towards humans. For instance, fear towards novel objects can be measured by the latency to feed on a highly favoured food item in the presence of the object (Mettke-Hofmann *et al.*, 2002). Alternatively, the latency of first contact and the duration of interaction within a fixed time interval can be measured (Meehan and Mench, 2002; Mettke-Hofmann *et al.*, 2002). Yet, a longer latency for approach does not necessarily reflect greater fear, it might also indicate a low motivation for exploration, which is likely if the duration of interaction is also low (Meehan and Mench, 2002). A third method of testing fear towards novel objects is to introduce it to the parrot in a familiar environment and evaluate the animal's behaviour, proximity to the object, and bite marks resulting from manipulation (Fox and Millam, 2004). Still, because exploratory behaviour and neophobia are influenced by species, results of such standardized tests always should be interpreted with caution (Mettke-Hofmann *et al.*, 2002).

Assessments of fear towards familiar or unfamiliar people can be evaluated using a handler response test, as described by Meehan *et al.* (2003a). This 10-point handler-response test comprises 5 components that are scored from 0 to 2 depending on the bird's reactions to a series of stimuli, such as the willingness of the bird to approach a familiar or unfamiliar handler and the acceptance of food or touch to different parts of the body (**Table 1**; Meehan *et al.*, 2003a).

**Table 1.** 10-point handler-response test to assess fear towards humans in parrots (after Meehan *et al.*, 2003a).

Test Component	Score 0	Score 1	Score 3
Extend finger	retreat / aggression	no response	approach
touch back	retreat / aggression	accept with flinch / vocalization	accept
touch head	retreat / aggression	accept with flinch / vocalization	accept
offer food	retreat / aggression	accept with flinch / vocalization	accept
position after 15 s	> 90 cm	30 - 60 cm	0 - 30 cm

### *Abnormal Repetitive Behaviour*

According to Garner (2007), abnormal repetitive behaviours are those that “are inappropriately repetitive in goal or motor pattern and functionless, maladaptive, or self-injurious.” They comprise stereotypies and impulsive/compulsive behaviours such as feather picking parrots (for review, Van Zeeland *et al.*, 2009). In rare cases, parrots have been described to develop vocal stereotypies (screaming) (Bergman and Reinisch, 2006), but Meehan and Mench (2006) classify parrot stereotypies into three main categories:

1. locomotor : route tracing, pacing, corner flipping, ...
2. oral : spot pecking, sham chewing, bar biting, tongue rolling, ...
3. object-directed : repetitive invariant manipulation of objects, ...

The development of a stereotypy is often linked to frustration caused by constraint of a highly motivated behaviour (Mason, 1991; Meehan and Mench, 2006). Afterwards, this stereotypic behaviour can become disengaged from its original cause and continue to be performed even after a.o. environmental conditions are improved. Display of stereotypic behaviour may thus not necessarily indicate currently compromised welfare, but rather indicates the animal's psychological history.

Conditions known to elicit stereotypic behaviour in parrots include impaired foraging opportunities, insufficient opportunity for locomotion, and lack of (physical) social contact with conspecifics (Meehan *et al.*, 2003a and 2004; Meehan and Mench, 2006). Meehan and colleagues (2004) demonstrated that environmental enrichment in the form of foraging opportunities and increased level of cage complexity significantly reduced - but did not completely eliminate - the performance of stereotypies in single-housed amazons. In pair-housed amazons, however, the same environmental enrichment protocol completely inhibited development of stereotypies (Meehan *et al.*, 2003a).

A typical example of stereotypic behaviour in parrots includes psychogenic feather destructive behaviour. Herein, a strong genetic predisposition exists for a.o. African greys, cockatoos and lovebirds (Gill, 2005). Frustration, resulting from inappropriate husbandry or a barren environment that conflicts with the species-specific behavioural repertoire, is often the underlying cause (Jenkins, 2005). However, evaluation of signs of feather or skin mutilation should consider medical conditions as well. Moreover, self-inflicted damage must be differentiated from aggression from conspecifics. Meehan *et al.* (2003b) developed a scoring system to quantify the plumage condition of parrots, allowing reliable evaluation of change over time (**Table 2**) (Meehan *et al.*, 2003b). This system uses a questionnaire to score feather, down, and skin condition on 5 body areas.

**Table 2.** Scoring system to determine feather condition in parrots  
(adapted from Meehan *et al.*, 2003b)

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<b>Chest, back, and legs</b>			
<u>Score</u>	<u>Feathers</u>	<u>Down</u>	<u>Skin</u>
0	all or most removed	all or most removed	injured
1	all or most removed	all or most removed	exposed
2	all or most removed	some removed	patches exposed
3	all or most removed more than half removed	exposed some removed	not exposed patches exposed
4	less than half removed	some removed	patches exposed
5	more than half removed	exposed	not exposed
6	less than half removed	exposed	not exposed
7	marked fraying or breakage	not exposed	not exposed
8	little or no fraying or breakage	not exposed	not exposed

<b>Wings</b>			
<u>Score</u>	<u>Coverts, primaries (I), and secondaries (II)</u>	<u>Down</u>	<u>Skin</u>
0	all or most coverts, I, and II removed	removed	injured
2	all or most coverts, I, and II removed	removed	exposed
4	more than half of coverts removed more than half of I and II removed	exposed exposed	not exposed not exposed
6	less than half of coverts removed less than half of I and II removed	exposed exposed	not exposed not exposed
	I and II markedly frayed or broken	not exposed	not exposed
8	little or no fraying or breakage	not exposed	not exposed

<b>Tail</b>	
<u>Score</u>	<u>Feathers</u>
0	all or most removed or broken
4	some markedly frayed, broken or removed
8	little or no fraying or breakage

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## 1.2. HOUSING AND ENVIRONMENTAL ENRICHMENT

Despite their widespread geographic distribution, most parrot species have similar behavioural repertoires and thus similar housing and management requirements in captivity (Russello *et al.*, 2008). With a few exceptions, they are highly social, diurnal prey animals that establish strong, monogamous pair bonds and often form gregarious flocks (Evans, 2001; Seibert, 2006).

### ENVIRONMENTAL CONDITIONS

Parrots, like most warm-blooded animals, tolerate a relatively wide range of ambient temperatures and relative humidities. Still, extremes should be avoided (Dilger, 1982a). The diurnal cycle, in contrast, is more likely to be inadequate when parrots are artificially kept in regions of the northern hemisphere, and such a disturbed diurnal cycle may induce behavioural problems. Most psittacine species originate from equatorial areas where day-length is 12 h year-round (Evans, 2001). Consequently, when considering the lighting schedule in captive confinement, psittacines should be allowed 10 to 12 h of darkness daily to promote normal sleep, regardless of whether light is supplied naturally, artificially, or both (Dilger, 1982a). Furthermore, in their natural habitat, transition between darkness and light occurs gradually at twilight, and ideally, this transition should be imitated in captive environments (Wilson, 2005a). Parrots thus require a year-round circadian light-dark schedule in which the dark period is without disruptions and of an adequate duration of about 12 hours, as insufficient or disturbed sleep may adversely affect their physical and emotional health (Bergman and Reinisch, 2006; Lightfoot and Nacewicz, 2006). In addition, during daytime, parrots are noisy, gregarious animals themselves, and are surrounded by noise in their natural habitat. In consequence, complete silence indicates the presence of a predator. Therefore, provision of soft background music is advised to help avoid continuous apprehensive states in parrots (Evans, 2001). Yet, an excessively noisy environment can render the birds nervous or even noisier (Davis, 1999).

With regards to artificial lighting, species-specific studies that address the perception of the flicker frequency associated with discontinuous illumination in parrots are lacking. However, it has been demonstrated that poultry perceive light emitted from fluorescent lamps driven by high-frequency (100 Hz) alternating current as continuous light, whereas they experience light emitted by low-frequency (50 Hz) lamps as stroboscopic light (Nuboer *et al.*, 1992;

Prescott *et al.*, 2003). Therefore, low-frequency illumination might also be disturbing to parrots. In albino animals, light intensity requires additional consideration in order to prevent light-induced retina damage. Albino rats, for example, develop retinal damage within 13 weeks of exposure to light intensities as low as 50 lux (Williams *et al.*, 1985). Then again, a study of Wilken (1998) could not reveal microscopic evidence of light-induced retinal damage in either normally pigmented or hypo-pigmented lutino or albino budgerigars. However, Wilken (1998) noted the presence of melanin granules in all investigated eyes; therefore, none of these birds could strictly be considered albinotic. In accordance with these findings, cones dominate the avian retina, whereas the retinal underdevelopment in hypo-pigmented mammals is confined to rods, which may result in only a relatively minor deficit in the retina of albino bird strains (Jeffery and Williams, 1994).

### **TYPE OF ENCLOSURE**

There are several options for bird housing: cage or aviary, solitary or non-solitary, and indoor or outdoor confinement. Each type has its advantages and limitations. A cage is a relatively small enclosure for solitary or pair housing, whereas an aviary is a large, most often outdoor enclosure that can accommodate a flock of birds. Solitary housing is inadvisable because of the social nature of most parrot species, but group housing can give rise to intra-species aggression and thus requires careful monitoring (Romagnano, 2006). Moreover, it is prudent to take species-specific characteristics into account when considering group housing, as certain parrot species are considerably more prone to aggressive behaviour toward conspecifics than others. Male cockatoos in particular, but also macaws and amazons, often inflict serious mate trauma when housed in pairs, although this apparently does not occur in free-ranging specimens (Romagnano, 2006). Indoor housing is usually more constraining in dimension, but outdoor housing increases the risk of escape, theft and infectious diseases contracted from wild birds (Lightfoot and Nacewicz, 2006). As regards the latter, Wilson (2006) advises not to house species that originated on different continents in the same airspace. Then again, outdoor confinement offers the advantage of full-spectrum sunlight, through which ultraviolet B radiation enables endogenous vitamin D<sub>3</sub> synthesis and thus promotes calcium metabolism (Stanford, 2006; Wilson, 2006).

### **CAGE POSITION AND ACCESSIBILITY**

The preferred position of a parrot enclosure inside a room is next to an opaque wall and away from doors or windows, as the sudden entrance of humans, and passage of vehicles, cats or birds could startle the animal (Dilger, 1982a; Evans, 2001; Wilson, 2005a). For outdoor enclosures, it is important to allow easy passage along at least two free ends of the cages. The passage at the front side of the cages should be kept clear and be used for daily husbandry procedures; whereas the rear end ideally offers a protective barrier (e.g., through dense plant covering), its usage being limited to facilitate non-routine procedures such as capture of the birds.

Wide door-like openings should permit easy access to all corners of the cage. Hence both the birds and all enclosure items can be easily reached (Luescher and Wilson, 2006). Water and feeding bowls should be easily accessible without the need to enter or open the enclosure. The same is true for solid cage bottoms, for which drawer-like designs facilitate removal for cleaning and disinfection. Likewise, in hanging cages or aviaries, a mesh bottom enables removal of excrement and food spillage with minimal disturbance to the birds. These features also minimize the risk of animal caretakers being bitten by the parrots, which can inflict serious flesh wounds.

### **CONSTRUCTION MATERIALS, CAGE DIMENSIONS AND INTERNAL DESIGN**

Materials used to construct cages must be non-corrosive and non-toxic. Galvanized wire, which is steel wire coated with zinc to prevent corrosion, is a common construction material of animal enclosures. However, this material encompasses a toxic hazard to parrots unless all white rust (lumps of oxidised zinc) is removed prior to first use. Washing in dilute acetic acid (vinegar) will facilitate the removal of these oxidized deposits, as this yields soluble salts that are easily brushed off. Otherwise, toxic concentrations of zinc could be ingested, resulting in 'new-wire disease', which is an important differential diagnosis in birds showing neurologic symptoms (Howard, 1992; Harcourt-Brown, 2000). Howard (1992) investigated the effects of ingestion of different zinc sources in adult cockatiels, as the toxic hazard associated with new galvanized cages has been questioned to originate from the zinc itself or other metal contaminants. Ingestion of pure zinc, stripped zinc coating containing 1% lead, or white rust all resulted in dose-dependent illness and mortality. The main clinical signs were dullness, lethargy, periodic dysphagia, ataxia, muscle wasting and greenish diarrhoea (Howard, 1992). Scientifically based recommendations for species-specific cage dimensions are scarce.

Nevertheless, enclosures should at least enable the birds to spread their wings and turn around while perched without touching the cage floor or walls with their tail or wings; larger or long-tailed birds, or a greater number of birds requiring bigger enclosures (Dilger, 1982a; Turner, 1992; Lightfoot and Nacewicz, 2006). Evidently, these features form the absolute minimal dimensions and are not advisable. With respect to provision of adequate opportunity for locomotion and exercise, the proportions of the enclosure are even more crucial than its absolute volume (Dilger, 1982a). A rectangular shape, for instance, facilitates flight (Hawkins, 2001; Luescher and Wilson, 2006). This feature is crucial, keeping in mind that free-ranging parrots may fly considerable distances between feeding and roosting sites on a daily basis (Gilardi and Munn, 1998; Meyers and Vaughan, 2004). In addition, wild parrots spend large portions of their active time climbing among trees during play or foraging activities (Forshaw and Cooper, 1989). Therefore, box-type cages with solid walls are unsuitable for housing psittacine birds, because such enclosures deprive the birds of opportunities to climb. In contrast, cages made of stout wire stimulate climbing, provided that mesh or horizontal wiring is used rather than vertical wiring (Hawkins, 2001). Double wiring and sufficient space between cages should be present to prevent neighbouring birds from biting each other's toes (Stoodley *et al.*, 1992; Harcourt-Brown, 2000).

Aside from the construction materials and cage dimensions, the internal design of the aviary or cage as well should maximize the usable space (Hawkins, 2001). Optimal use of all 3 dimensions can be attained by well-placed perching or swinging devices. In general, perches should be placed as far apart as possible to stimulate flight (Dilger, 1982a; Turner, 1992). Furthermore, as parrots are prey animals that roost in trees, the presence and height of perches is important to ensure their sense of safety and comfort. Therefore, perch height should approximate human shoulder height (Luescher and Wilson, 2006; Wilson, 2006). Other measures to promote a sense of security in parrots include the provision of a hiding place, such as a dark, cavity-like wooden box, or visual barriers in, on top of, or adjacent to the cage (Evans, 2001; Lawton, 2005). Additionally, a minority of parrot species, such as many cockatoos, spend much of their active time on terrestrial activity (Wilson, 2006). Cockatiels, for instance, display a distinctive running behaviour when given the opportunity. Standing aviaries or cages with a solid, non-slippery bottom are therefore advised for this species; whereas suspended wire cages are discouraged (Dilger, 1982a; Hawkins, 2001; Wilson, 2006).

## PERCHES

Appropriate perches are a must in a psittacine enclosure. Perching devices are commercially available in a wide range of materials, each having its own advantages and disadvantages. First of all, its diameter should allow the bird's feet to grip the perch firmly, without resulting in toes overlapping each other (Turner, 1992). Other favourable perch traits include:

- good grip texture
- a variable diameter to promote foot health
- flexibility to stimulate balance and exercise
- chewable, but non-toxic
- neither slippery, nor abrasive
- not cold in nature, such as aluminium

Concrete grooming perches offer good grip texture, help abrade nail tips, and give parrots an opportunity to trim their continuously growing beaks. However, the rough texture might cause skin lesions on the soles of the feet (EC, 1986b), especially if no other perch materials are provided. Woody branches of the cholla cactus (*Opuntia subspecies*) have natural holes that can be filled with a hardened clay-based mixture of minerals, yielding a source of occupation as well as essential nutrients. Cuttlebone perches are a good source of calcium, especially for parrots fed seed mixtures, which are severely deficient in calcium (Ullrey *et al.*, 1991). Perches made of branches of untreated, non-toxic wood have the advantage of providing good gripping surface, chewing opportunities, and some flexibility, which stimulates balancing exercises. Moreover, branches and twigs have natural curvatures, vary in diameter, and are soft. All of these features promote pedal blood circulation and thus foot health (Dilger, 1982a; Evans, 2001).

Hardwoods, such as manzanita woods, are relatively indestructible, but they are not recommended because their hardness deprives birds of chewing opportunities and can induce foot lesions (Harcourt-Brown, 2000; Evans, 2001). In addition, manzanita wood becomes very slippery when wet, which might cause the birds to fall and injure themselves (Wilson, 2005a). Another alternative is the use of electrically heated polymer perches. These perches provide comfortable footing through their texture, variable diameter, and agreeable temperature, unlike, for example, the cold, slippery surface of aluminium bars.

Other types of perches frequently seen in avian enclosures include plastic or compressed wood, sometimes covered with sandpaper. None of these perches are recommended, because they are all uniform in thickness, forcing the birds to continuously bend their toes the same way, which contributes to poor foot health. Moreover, they are often too slippery to provide secure footing, resulting in birds falling when startled. If covered with sandpaper, these perches are abrasive to the plantar surface of the foot, inflicting skin lesions, which may become infected and can cause bumblefoot, an inflammatory condition recognizable by the appearance of erythema, local swelling, and the presence of abscesses (Evans, 2001; Harcourt-Brown, 2000). Aside from faulty perches, other factors contributing to the development of bumblefoot include obesity, inactivity, and nutritional imbalances (Gill, 2005).

### **ENVIRONMENTAL ENRICHMENT**

Free-ranging parrots are accustomed to an environment in which they have been evolving for centuries and where they have developed a species-specific behavioural repertoire. In the wild, psittacine species have been reported to forage for 4 to 6 hours per day and to spend most of their active time in close proximity to conspecifics (Snyder *et al.*, 1987). Except for the solitary kakapo, parrots usually live in social groups. Amazon parrots, for instance, live in social units composed of a pair together with their offspring; whereas African greys are known to roost in groups of hundreds (Forshaw and Cooper, 1989). In captivity, in contrast, they are often deprived of social contact and have limited opportunities for exploration and foraging. The constraint of these and other highly motivated behaviours may lead to frustration, which results in chronic stress after prolonged exposure (Ödberg, 1978). In turn, this can lead to the development of abnormal behaviours such as stereotypies, which are invariant, apparently useless behaviours that are repeated regularly over time (Ödberg, 1978)

A broad definition of environmental enrichment is “an animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being” (Shepherdson *et al.*, 1998). Sources of environmental enrichment can be subdivided into animate and inanimate enrichment (Evans, 2001, Meehan and Mench, 2006). Animate enrichment includes for instance group-housing and social contact with humans; whereas inanimate enrichment can be categorized further into those that stimulate physical activity and those that enable natural behaviours (Meehan *et al.*, 2003a and 2003b).

### **3.3. THE USE OF PARROTS AS LABORATORY ANIMALS**

Because birds possess relatively fewer peripheral nociceptors, they had been thought to have a higher pain threshold than mammals. However, birds show comparable behavioural and physiologic responses to painful stimuli (Gentle, 1992). Hence, unless scientifically refuted, they should be assumed to experience pain to the same degree as mammals do. Painful conditions in birds, which may inevitably be associated with certain laboratory procedures, should therefore be avoided or alleviated whenever possible. Further, although most parrots are highly social species, laboratory confinement during research trials often requires solitary housing. Nonetheless, the periods in which the birds are involved in trials are often a minor share of the duration of their laboratory confinement. Hence, an ethical approach includes the presence of two types of enclosures, one that complies with the restrictive requirements of the experiment and another that accommodates the expression of the animal's natural behaviour as much as possible. In addition, appropriate confinement construction and design should safeguard the health and well-being of both the birds and their caretakers. Depending on the nature and anticipated duration of the research project, it may be prudent to consider the use of animals at an off-site setting, such as a zoo or ornithological collection, instead of establishing on-site capacity in the research institution. Consideration of alternatives is particularly relevant in light of the substantial investments involved in the acquisition of birds, appropriate housing and daily care associated with the setup of an on-site parrot facility, and the discrepancy between the lifespan of the research subjects and the projected duration of laboratory confinement.

#### **LEGISLATION**

The acquisition, transport, housing, and care of animals kept for scientific purposes, and the reporting thereof, are subject to national legislation, which varies greatly between countries. The member states of the European Community, for instance, prohibit use of CITES I species in animal research, unless the objectives are preservation of the species or whenever the examined species is the only available model in fundamental biologic studies. However, European legislation only regards procedures that inflict pain, suffering, distress, or lasting harm as animal experiments (EC, 1986a and 1986b). Nevertheless, the scientific use of CITES-listed, wild-caught birds or their eggs is permissible only if they are essential to the research and all necessary licenses have been granted by the appropriate authorities (EC, 1979).

In the United States (US), in comparison, any non-human vertebrate animal use in research, teaching, or testing, is considered experimental and therefore subject to legal requirements (Cohen, 2006). Herein, any non-human member of the animal kingdom kept in captivity for experimental or observational purposes is regarded as a laboratory animal (UFAW, 2010). Then again, coverage of birds in the Animal Welfare Act (AWA), which regulates the treatment of laboratory animals in the US, has been a subject of debate, as the Act's original definition of "animal" did not include birds. Subsequent amendments only partly addressed this exclusion. To date, the AWA explicitly excludes purpose-bred birds, but covers birds that are not bred for research purposes (Cohen, 2006). However, US research facilities that receive federal funding also have to comply with the Public Health Service policy, which is outlined in the Guide for the Care and Use of Laboratory Animals (NRC, 1996). The stipulations of the latter, in contrast to the AWA, also include purpose-bred birds, but lacks species-specific guidelines on housing and management (Federal Register, 2004).

Additionally, the import, export, and interstate trade of species listed as endangered or threatened in the US or elsewhere under the Endangered Species Act are governed through a permit system by the US Fish and Wildlife, which is also responsible for CITES administration (NRC, 2006). Moreover, importation of CITES-listed avians - with some exceptions, including 37 captive-bred parrot species - requires a Wild Bird Conservation Act permit. The Lacey Act, on the other hand, which concerns importation of birds that cause damage to indigenous flora and fauna, defined as injurious birds, does not apply to either birds imported for scientific research or psittacines imported for any purpose. Finally, import or export of Appendix I specimens is in the US only allowed under very strict permitting requirements for scientific purposes, education, or conservation, but not for commercial trade (ARENA/OLAW, 2002; NRC, 2006). Irrespective of purpose, US importation of birds requires a 30-day quarantine period (except for imports from Canada); if a single bird in the lot is diagnosed with highly pathogenic avian influenza or Newcastle disease during the quarantine period, the whole lot must be destroyed (Paul, 2005). Further information about legislation is available in NRC (2006) and Paul (2005).



### EXTENT OF USE OF PARROTS FOR RESEARCH PURPOSES

Official statistics of laboratory animal use is subject to varying national legislations as well. Yet, accurate information on the extent of psittacine bird use in laboratory settings is largely lacking because of deficient specificity in official statistics. Of the 10.7 million non-human vertebrate laboratory animals registered in Europe in 2005, for instance, fewer than 5% were birds. But specificity with regard to birds only included classification into 3 broad categories: domestic fowl, quail, and “other birds” (Evans, 2001). Moreover, in the US, statistics for fiscal year 2004 reported only 1.1 million animals, but these did not include purpose-bred birds, rats of the genus *Rattus* and mice of the genus *Mus*, and neither specified psittacine bird use (ARENA/OLAW, 2002; USDA, 2006). In contrast, annual statistics of various Australian states are highly detailed and include all scientific use of vertebrate and higher invertebrate animals of the order Cephalopoda (octopus, squid, cuttlefish, nautilus). The 2004 annual report of the Queensland Department of Primary Industries and Fisheries, for instance, categorizes birds into exotic captive (575), exotic wild (492), native captive (124), native non-endemic (243), native wild (22413), and other (3294) birds (QGDPIF, 2006).

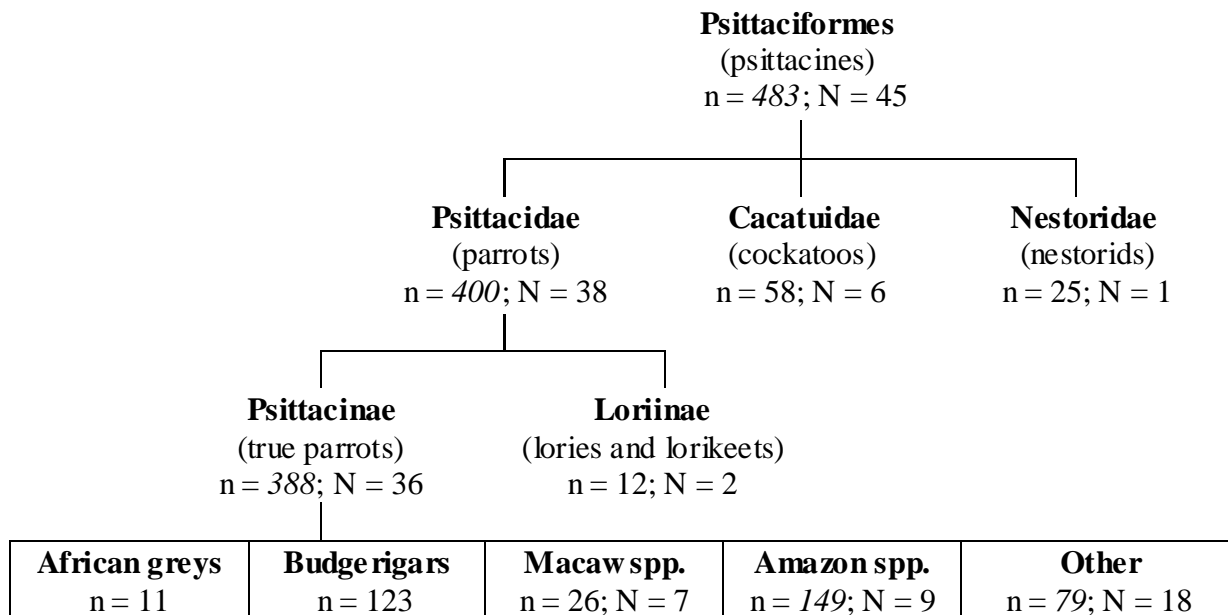
Despite an overall lack in specificity of official statistics, the number of psittacine birds used for scientific purposes is presumed to be quite limited (Hargis, 1989). In order to verify this, we performed an ISI Web of Knowledge literature search in January 2010, using the search string “Topic=(psittaci\*) OR Topic=(parrot\*)” and including all 2009 references. This search yielded 141 database entries, which we categorized into type of research and research field as shown in **table 3**. The main research fields in laboratory experiments were veterinary medicine and diagnostics (bacteriology, haematology, morphology, and reproduction; 45%) and behaviour and sensory studies (behaviour, acoustics, and vision; 17%).

**Table 3.** Number of peer-reviewed studies published during 2009 involving Psittaciformes as subjects sorted by research type (rows) and research fields (columns).

	VMD	BS	EC	GEN	NUTR	Total (%)
Field study	4	6	32	4	1	47 (33%)
Laboratory experiment	11	16	0	2	2	31 (22%)
Opportunistic sampling	17	1	0	10	0	28 (20%)
Case report	23	1	0	0	0	24 (17%)
Undetermined	8	0	1	2	0	11 (8%)
Total	63	24	33	18	3	141 (100%)

VMD: veterinary medicine and diagnostics; BS: behaviour and sensory; EC: ecology; GEN: genetics; NUTR: nutrition.

Only 31 of 141 publications that cited the use of parrots for research purposes involved captive confinement. As shown in **figure 1**, these included 483 individuals of 45 different psittaciform species. Because it was not possible to determine the number of animals used in three studies, the total number shown is a slight underrepresentation of the actual count of laboratory Psittaciformes. Most animals (83%) belonged to the Psittacidae family, and 12% and 5% of individuals belonged to the families Cacatuidae and Nestoridae, respectively. Within the Psittacidae, most of the subjects (97%) were true parrots (only 12 individuals were lorikeets), of which amazons (38%) and budgerigars (32%) were by far the most represented species. The type of housing was in 42% ( $n = 13$ ) of the parrots located at on-site facilities, 23% ( $n = 7$ ) at off-site facilities, and in 35% of cases ( $n = 11$ ) housing information was unavailable. The off-site facilities were all outdoor aviaries. In contrast, most studies conducted at on-site facilities used indoor cages (59% or  $n = 4$ ), whereas outdoor confinement comprised only 23% ( $n = 2$ ), and in 18% of the studies ( $n = 1$ ) this information was unavailable. Information on group housing was provided in 61% ( $n = 19$ ) of the studies: 19% ( $n = 6$ ) used individual housing, 13% ( $n = 4$ ) pair housing, and 29% ( $n = 9$ ) group housing.



**Figure 1.** Total number of Psittaciform individuals ( $n$ ) and species ( $N$ ) included as study subjects in peer-reviewed studies published during 2009, which involved laboratory confinement. For one study using amazon parrots and for two from the ‘other’ category the exact sample size could not be derived. The resulting underrepresentation of the actual count is presented in italic font.

## FACTORS TO CONSIDER IN THE SELECTION OF LABORATORY PARROTS

Instead of opting for availability or proximity to a retailer, the acquisition of animals that will be confined in laboratory facilities for research purposes should always be based on careful consideration. Most importantly, whenever possible, the least endangered and least cognitively developed species should be the species of choice consistent with achieving the experimental goals.

In addition to age and gender, several other factors should be taken into consideration. First, a heterogeneous group of laboratory animals is likely to increase variability of research or test results and thus decreases the strength of experimental set-ups, which possibly requires the use of additional animals. Second, good knowledge of the animals' background improves interpretation of research data and promotes sound decisions on necessary health measures, which may considerably reduce the likelihood of introducing infectious agents into the resident colony. Third, poor socialization may influence the animals' aptitude to undergo laboratory confinement or may hamper the ease of handling. Appropriate socialization of birds is thus of utmost importance in their laboratory confinement. Socialization facilitates handling by animal caretakers, enhances the performance of experimental procedures, and reduces the risk of development or aggravation of welfare issues.

Next, it is advisable to consider the following species traits when acquiring laboratory parrots: body size, ecology and behaviour, anticipated longevity, and suitability for the intended research. Bird size, ecology and behaviour can affect the ethics of a decision about the housing of certain species in the available space; solitary housing, which is necessary for some research purposes, can be deleterious to the well-being of certain species. Further, the loudness of normal vocalization varies by species: African grey parrots, for instance, are quieter than most amazons and cockatoos (Wilson, 2006). This may require thoughtful consideration if the animal housing area is near research offices. In addition, it is especially critical to bear in mind that all parrots are long-lived animals (Carpenter *et al.*, 2001), but some species are more so than others. Budgerigars, for instance, have a relatively short lifespan (8 to 10 years) in contrast to the larger parrots (50 to 80 years). Longevity may therefore be an ethical consideration when choosing a parrot species, as most are very likely to outlive the duration of a research project. Finally, the selection of an appropriate parrot species for laboratory use can depend on their susceptibility to a particular infectious or metabolic disease. For example, African grey parrots, in particular, develop hypocalcaemic

tetanic syndrome in response to an absolute or relative calcium deficiency. Other parrot species rather maintain a normal blood calcium level, but instead develop metabolic bone disease as a result of bone-calcium mobilization in response to such a deficiency (Kollias, 1995; Levine, 2003). Therefore, the peculiar calcium metabolism in African greys, for instance, makes this species an outstanding model to investigate its underlying mechanisms.

#### **LONG-TERM FATE OF LABORATORY PARROTS**

Given their considerable attainable lifespan, research projects involving parrots are likely to comprise merely a brief episode in these birds' lives. The fate of, in particular such long-lived animals, after research projects have ended, is thus essential to consider (Hawkins, 2001). Therefore, the long-term fate of laboratory parrots must be carefully planned prior to the start of research projects. Three options are available for parrots upon the completion of their use in research: they can be placed under private care, humanely euthanized, or reintroduced into their natural habitat. However, performing euthanasia on healthy animals often is considered ethically questionable, particularly when dealing with endangered species or those with such long lifespan.

Release of the birds into their natural habitat implies an expensive and long-term commitment and should only be considered for species that have become globally or locally critically endangered according to the CITES criteria (IUCN, 1995). Furthermore, reintroduction of previously captive parrots into the wild encompasses two main drawbacks. First, despite inclusion of a preconditioning period to the local environment and food supply, the survival rate of released captive-reared parrots remains poor due to persisting deficits in basic survival skills, such as foraging behaviour, social interacting, and predator-avoidance (Snyder *et al.*, 1994). A second drawback includes the potential hazard of dispersal of infectious diseases to wild populations. Therefore, a thorough veterinary examination and quarantine period should always precede the release of birds (Sanz and Grajal, 1998; AGDEH, 2005). Then again, although successful release into the wild is difficult to achieve and involves infection hazards, the survival of critically endangered species may be enhanced, and inbreeding abated, through the introduction of new individuals to very small populations.

With respect to the above, placing the birds in private care or in zoological collections after research projects have ended, can be considered the most feasible option. For this purpose, and thus in order to avoid the need for euthanasia of healthy animals, all possible strategies that facilitate future placement should be employed during their stay in the laboratory (Hawkins, 2001). More specifically, encouraging acceptable behaviour will enhance the willingness of people to become involved in the lasting care of these birds (Meehan and Mench, 2002; Jenkins, 2005; Wilson, 2005a). Hereto, environmental stimulation and behavioural training reduce the incidence of undesirable behaviour, such as excessive screaming, feather destructive behaviour, aggression, and extreme fearfulness (Meehan and Mench, 2002; Meehan *et al.*, 2003a and 2003b). For example, enrichment of barren enclosures significantly reduced the fear of amazon parrots towards both novel objects and unfamiliar human handlers (Meehan and Mench, 2002). Moreover, when parrots are used for studies that involve intensive human interaction, such as studies involving avian cognition, parrots develop the need for a consistent amount of human attention, regardless of the continuation of the project (Hawkins, 2001).

#### **REFINEMENTS IN MANUAL RESTRAINT**

Some aspects of animal experimentation require catching and restraint of birds. These manipulations inevitably induce distress to the birds and may provoke panic flights, which can result in feather damage or more serious injuries, such as fractures (Dilger, 1982b; Turner, 1992). However, ease of catching and restraint may be promoted and distress diminished by regular handling and appropriate techniques. This way, the fear the bird experiences, chasing-capture time, as well as the risk of injury to bird and handler can be reduced (Turner, 1992).

Often parrots are considered dangerous to handle because they have large and powerful beaks. However, biting mainly occurs after it has been conditioned through inappropriate handling. In contrast, wild birds use their beaks almost exclusively for social interaction and to clasp onto or grasp objects. Because parrots are curious animals, they will often attempt to climb approaching objects, for instance a human hand that comes into close proximity. However, inexperienced or fearful caretakers may respond with hesitation or quick withdrawal, hereby teaching the bird to grab the hand with their beak. As a result, these birds might learn how they can use their beaks as a forceful method to manipulate humans (Wilson, 2005a).

Hence, animal caretakers and all other personnel responsible for laboratory parrots should be instructed in appropriate handling techniques. In addition, when attempting to capture birds, loud noises and other disturbing elements should be avoided, the environment is preferably dimly lit, and if possible the inside of the cage should be dark, as this situation is known to calm parrots (Turner, 1992).

When using a towel to catch a parrot, the animal must be approached from the front and never attacked from the rear or above, as this stance may incite the parrot to react as it would to an assault from a predator (Wilson, 2005b). To restrain the parrot safely, the lower jaw of the beak, the feet, and the wings should be immobilized. Both the legs and the long primary feathers of both wings can be held safely in a single hand (**Figure 2**), preventing scratching and wing flapping. The other hand is used to prevent biting, which can be done by gently placing the fingers around the neck of the bird, with the thumb firmly but gently pressing towards the lower jaw. The bird's sternum must be kept unrestricted at all times, as pressure would compromise respiration (Turner, 1992).



**Figure 2.** Manual restraint of an African grey parrot. Note that the sternum is left unrestricted, one hand is used to immobilize feet and wings, and the thumb of the other hand presses towards the lower jaw, preventing the bird from biting.

In case assistance of a second person is unavailable to help in research procedures or provision of veterinary care, the bird can be fixed by a single hand using a towel-wrap. A right-handed handler is best seated with legs crossed, left leg over right, holding the parrot between body and left forearm while the left hand clutches the parrot's legs through the towel. This way of restraint does not completely immobilise the bird, but biting or scratching is prevented, whilst one hand remains free. In addition, the towel serves as a type of collar in which the bird can bite or nibble, hereby providing a substrate for redirection of any reactions during the restraint (**Figure 3**). Stroking the head and talking to the parrot can also help to calm the bird and habituate it to being touched and handled by humans. Using the right hand, the handler can manipulate the legs, cloaca, or head. However, in hot environments, the temperature inside the towel can rise quickly; thin, cotton towels are thus preferred in those circumstances. A disadvantage to this way of holding is that the body and wings are inaccessible.

Limiting the time of restraint is important to avoid excessive distress, which could potentially induce a life-threatening increase in the parrot's body temperature. Greenacre and Lusby (2004), for instance, monitored rectal temperature, heart rate and respiratory rate every minute during a 15-minute manual restraint in 12 healthy amazon parrots (6 blue-fronted amazons [*Amazona aestiva*] and 11 Hispaniolan amazons [*A. ventralis*]). Respiratory rate and temperature increased significantly within 4 minutes. Both parameters continued to increase, with 1 bird's body temperature reaching lethal levels (46.1 to 47.2 °C) at the end of the 15-minute restraint. Hence, these authors concluded from their data that manual restraint of psittacines should preferably be restricted to 4 minutes. Prolonged restraint being only carried out when absolutely necessary, and in this case, overheating should be monitored by measuring respiratory rate (Greenacre and Lusby, 2004). The use of digital thermistor thermometers is preferred over mercury thermometers to assess avian rectal temperature for 2 reasons. First, the upper sensory limit of mercury thermometers (approximately 42.2 °C) does not cover normal avian body temperature, which ranges from 41.7 to 44.4 °C. Second, mercury thermometers require a longer time to reach a stable reading than digital thermometers do (Harrison and Richie, 1994; Greenacre and Lusby, 2004).



**Figure 3.** Towel-wrap method to safely restrain a parrot using a single hand. (a) A right-handed handler wraps the towel around the neck of the parrot using the right hand. Subsequently, the wings have to be folded tightly along the body by using the left hand, so that wing flapping is prevented. (b) Next, the left hand folds the left flap of the towel firmly from the upper left to the lower right of the parrot's body, and the right flap is folded in the other direction. (c) Then, the upper edge of the towel has to be shaped into a large collar that forms a barrier between the handler and the beak of the parrot. (d) Having wrapped the bird in the towel, the handler can use the other hand to perform procedures on the parrot without the assistance of a second person.



## ANIMAL HEALTH AND ZOOSES

### *Disease*

Most diseases and conditions are accompanied by discomfort, distress, and eventually severe pain or even death. Animal caretakers and research personnel should therefore carefully monitor all individual birds on a regular basis, actively looking for signs of compromised health, which are often subtle and go unnoticed to the inattentive eye. These precautions are necessary to protect both the animals' welfare and the quality of the research data. The experimental use of healthy animals may reduce inter-individual variability and improve the statistical power of experimental designs (Garner, 2005). This way, the number of animal trials and animal numbers can be reduced, or is at least not unnecessarily increased.

A healthy bird colony requires quarantine units, separate housing and infectious disease monitoring for newly acquired birds, and thorough health checks of all birds at regular intervals. Aside from obvious signs of trauma or infectious disease, animal care and research personnel should also be attentive to more subtle indications of impaired health, such as signs of malnutrition. These include, for instance, abnormal plumage<sup>4</sup>, bone dimorphism, emaciation, clinical signs of metabolic disturbances and infectious diseases arising from impaired immunological function. Next, inadequate management or housing conditions, such as abrasive perches, a suboptimal environmental temperature, or unsatisfactory hygiene, can also lead to lesions or other indications of impaired health.

### *Zoonoses*

Like many laboratory animals psittacine birds can be carriers of infectious agents with zoonotic potential (i.e., transmissible to humans). Hence, all personnel should be well informed of the possible risks of zoonotic infections, which can be contracted either directly from the birds or indirectly from their excreta or contaminated materials and air. In addition, adequate personal and environmental hygiene measures should be in place at the animal facilities. These also include control of vectors of diseases, such as vermin. Furthermore, personnel with impaired immunity, such as pregnant women, should strictly avoid any direct or indirect contact with the birds. Besides these general guidelines, newly acquired parrots should be subject to appropriate quarantine and screening procedures; as such measures enable early identification of possibly zoonotic agents and may prevent the transmission of

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<sup>4</sup> Examples of abnormal plumage include stress bars, weakened feathers, broken or damaged feathers, and depigmentation (reviewed in Koski, 2002).

infectious agents to resident birds. In addition, regular animal health checks and routine diagnostic screening for important zoonotic agents further contribute to the protection of both personnel and bird health.

**Table 4** provides a partial list of zoonotic agents that can be contracted from parrots (Scott 1995). Of particular importance is *Chlamydophila psittaci*, the etiologic agent of the human disease psittacosis, which is also referred to as parrot fever or ornithosis (Turner, 1987; Scott, 1995). In birds, infection with *C. Psittaci* usually passes subclinical, but it also may be associated with non-specific clinical signs such as dyspnea, anorexia, lethargy, diarrhoea, biliverdinuria - yellow to green urates -, and oculo-nasal discharge (Turner, 1987; Gould, 1995; Greenacre, 2003). Parrots can thus be asymptomatic carriers and shedders of *C. psittaci*. The strains found in amazons seem to be particularly virulent to humans (Turner, 1987).

The incubation period is 1 to 4 weeks and diagnostic tests for *C. psittaci* in parrots include culture, cytology, polymerase chain reaction, and the enzyme-linked immune-sorbent assay, but none of these are 100% accurate (Turner, 1987; Gould, 1995). In humans, the infection also usually remains subclinical. However, clinical signs may vary from mild respiratory disease to pneumonia, nausea, vomiting, hepatitis, myocarditis, disorientation, mental depression, delirium, and even death. Possible transmission routes from birds to humans include, in order of decreasing importance:

1. inhalation of contaminated air
2. direct contact with birds, feathers, excreta, oculo-nasal discharge, or infected tissue
3. bites or other open wounds

**Table 4.** A partial list of potential zoonotic diseases that can be contracted from contact with psittacine birds (after Scott, 1995).

Disease	causal organism	predominant transmission	Effect on host		control measures
			Bird	Human	
Salmonellosis	<i>Salmonella spp.</i>	oro-faecal	subclinical to acute disease	GI fever	hygiene RHC
Psittacosis (Ornithosis)	<i>Chlamydophila psittaci</i>	inhalation	subclinical to acute disease	subclinical respiratory signs (mortality)	screening hygiene RHC
Yersiniosis	<i>Y. pseudotuberculosis</i> <i>Y. enterocolitica</i>	oro-faecal	subclinical to acute disease	GI	hygiene rodent control
Tuberculosis	<i>Mycobacterium spp.</i>	oro-faecal	skin lesions systemic signs	local respiratory urinary tract	hygiene RHC
Ectoparasite infestation	<i>Dermanyssus gallinae</i>	direct contact	subclinical pruritus anaemia	pruritus skin lesions	hygiene RHC

GI: gastro-intestinal signs, RHC: routine health checks

## 1.4. NUTRITION AND DIGESTIVE CHARACTERISTICS

### NUTRITIONAL ECOLOGY AND NATURAL DIET

The majority of parrot species is generally considered to be seed eating or granivorous birds. This idea on this nutritional ecology is largely based on the typical beak morphology, sightings of foraging parrots, and apparent food preferences when kept in captivity. Moreover, they survived extended periods of seed and grain diets during the long boat travels necessary for their introduction into Europe well, this type of diet being excellently suited for storage and preservation during these travels (Sylva, 1991; Collar, 1997; Forshaw, 1998). Subsequently, they seemed to thrive on this type of diet, reaching long lifespans in which they apparently maintained good health. The husbandry practice of feeding parrots a mainly, or even exclusively, seed and grain diet has thus historically grown. The commonness of this practice and their granivory classification, however, has created the assumption that it fully describes their nutritional requirements (Nott and Taylor, 1994).

Most neotropical parrots, however, inhabit closed-canopy forests, which renders research on their nutritional ecology very difficult and incomplete (Gilardi and Munn, 1998). Nevertheless, extensive field studies have revealed a huge variety in ingredients of the dietary habits of wild parrots. This indeed includes a wide diversity of seeds and nuts, but also comprises other parts of plants, such as flowers, buds, leaves, fruit and bark, insects and larvae and even meat of carcasses (Ullrey *et al.*, 1991; Collar, 1997). In addition, seasonal differences in abundance and availability of food sources, as well as in nutritional needs and consequently foraging behaviour, are innate to the natural diet of parrots (Long, 1984; Wermundsen, 1997). Finally, the great diversity within the large order of psittacine birds is also reflected in a distinct dietary specialization between species (Klasing, 2000).

Many species that inhabit drier regions, such as cockatiels and budgerigars, are mainly seed predators (Macwhirter, 2003). Within the neotropical parrots, in contrast, amazons and many macaw species are rather frugivorous-granivorous (Martuscelli, 1995; Berg *et al.*, 2007). However, Berg *et al.* (2007) noted in fruit foraging great green macaws (*Ara ambiguous*) a much higher consumption of seeds compared to the surrounding pulp. The pesquet's parrot (*Psittichas fulgidus*), on the other hand, is highly specialized frugivorous, its natural diet being almost exclusively comprised of figs. The featherless facial zone in pesquet's parrots, which is also present in macaws, is therefore thought to function at least in part as an anatomical adaptation to prevent feather contamination with dried fruit pulp (Collar, 1997).

Morphological adaptations to a highly specialized diet are perhaps most evident in lories and lorikeets. Their slender beak and long, narrow brush-tipped tongue facilitate consumption of nectar and pollen, this dietary specialisation being also reflected in a less muscular and shorter gastrointestinal tract compared to other parrots species (Churchill and Christenson, 1970; Ziswiler and Farner, 1972; Richardson and Wooller, 1990; Roszkopf, 2003; Häbich, 2004). Yet, although these birds predominantly feed on nectar and pollen, their natural diet comprises also other minor components, such as fruit, flowers and invertebrates (Cannon, 1984; Collar, 1997; Gartrell, 2000).

The natural diet of the three Nestoridae species reflects yet other dietary specialisations. The nocturnal and flightless kakapo is entirely herbivorous (Eason *et al.*, 2006). The non-breeding adults feed mostly on tree leaves, grasses, and herbs, ingesting only the plant juices and spitting out squeezed pellets of coarse fibre. However, this diet is nutritionally inadequate to rear their young, who depend on the availability of the fruit of pink pine (*Halocarpus biformis*) and rimu trees (*Dacrydium cupressinum*). In consequence, kakapos only breed in years of superabundant fruiting of these trees, which is every 2 to 6 years (Houston *et al.*, 2007). Keas, in contrast, are omnivorous parrots that feed on nearly anything that is edible, their diet comprising a wide variety of plant material and invertebrates, but also eggs, nestlings and carrion (reviewed in Diamond and Bond, 2004). The natural diet of kakas, on the other hand, includes large amounts of bark dwelling invertebrates in North Island Kakas (*Nestor m septentrionalis*), and more specifically, the larvae and honeydew excretions of particular scale insects in South Island kakas (*Nestor m meridionalis*), next to seeds, nectar, pollen and fruit (Beggs and Wilson, 1987 and 1991; Moorehouse, 1997). The nesting behaviour of kakas, similar to kakapos, is also synchronised to the abundance of specific food items. In the population of North Island kakas studied by Wilson *et al.* (1998), for instance, breeding attempts were restricted to years in which the red beech (*Nothofagus fusca*) produced abundant quantities of seeds. In addition, feeding from the sap of these trees seems to be restricted to females, which is thought to affect nesting behaviour through predictive, nutritional cues with regard to the extent of seed yield (Moorehouse, 1997).

### **DETOXIFICATION OF DIETARY INGREDIENTS**

Parrots inherently tend to peel foodstuffs, which is thought to be a method to safely extend their dietary repertoire to items that are toxic to other species, as the outer layers of certain plant products often contain the highest concentration of toxins (Gilardi *et al.*, 1999). Yet, as parrots lack functional caeca and only have a small large intestine, dehusking of seeds is also suggested to comprise a strategy to reduce fibre intake (Ullrey *et al.*, 1991). Other natural methods of detoxification in parrots include ingestion of clay or charcoal, which adsorb potential dietary toxins and may confer cytoprotection of the gastrointestinal lining (Collar, 1997; Gilardi *et al.*, 1999). Geophagy in parrots typically occurs before foraging, pointing at a deliberate defense mechanism against secondary toxic plant compounds (Burger and Gochfeld, 2003; Mee *et al.* 2005). Brightsmith and Muñoz-Najar (2004) posit the provision of additional sodium and other minerals when the dietary supply is insufficient as a further function of geophagy, suggesting a certain degree of innate nutritional wisdom in parrots.

### **DIETARY CONSTITUENTS AND FEATHER COLOURATION**

In contrast to several other bird orders, where red, yellow and orange feather pigment is a function of dietary-derived carotenoids or metabolites thereof, the normal feather colouration in psittacine birds cannot be influenced by dietary supplements (McGraw *et al.*, 2004). Over 80% of psittaciform species display red in their feathers, which is generated by unique red pigments (polyenal lipochromes or psittacofulvins) that are locally synthesized *de novo* at maturing feather follicles (Forshaw and Cooper, 1978; Stradi *et al.*, 2001). Blues, on the other hand, are formed by feather nanostructures (Dyck texture) while greens evolve from a mixture of the blue structural colour and endogenously produced yellow pigments (Dyck, 1971a and 1971b; Rowley, 1997; McGraw and Nogare, 2005). Black, brown, and grey shades result from melanin pigmentation, whereas unpigmented feather keratin is white (Prum *et al.*, 1999; McGraw *et al.*, 2005). Despite lack of effects of dietary carotenoids on normal feather colouration, chronic malnutrition can induce aberrant feather pigmentation (Koski, 2002). Moreover, dietary inadequacies in general can often be first noted at the integument (Harrison and McDonald, 2006). A typical indication of malnutrition, or even a period of impaired health, includes the presence of stress marks at the feathers. These horizontal, linear defects across the vane indicate cortisone release at the time of feather formation in response to a nutritional deficit or a period of disrupted feed intake (Harcourt-Brown, 2000).

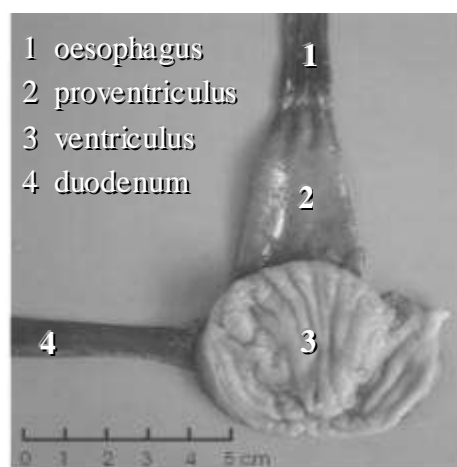
## CHARACTERISTICS OF DIGESTIVE ANATOMY AND PHYSIOLOGY

As discussed before, distinct natural feeding habits between psittacine species are largely reflected by characteristic adaptations in tongue and beak morphology, and sometimes in facial feathering. In parrots, the salivary glands are well developed and produce mucinous saliva that mainly serves as a lubricant (Farner, 1960; Hagen, 1992). Based on the number of taste buds, birds are generally considered to have poorer taste perception compared to mammals. Still, parrots have on average a much greater number of taste buds (350) compared to for instance chickens (24). Nevertheless, this is negligible compared to humans (9,000) or calves (25,000) (reviewed by Kare and Rogers, 1976). Hence, psittacine birds are thought to select food items based on size, shape, texture and colour, rather than on taste (Ullrey *et al.*, 1991; Kollias, 1995). The identification and selection of edible compounds is hereby facilitated by the tactile bill-tip organ, which is present in parrots and many other birds (Gottschaldt, 1985).

The presence, morphology and function of the crop, which is a unilateral, bilateral or tubular dilatation of the oesophagus, vary greatly between bird species (Stevens and Hume, 1995). In parrots, the crop is transversally orientated and possesses folds through which it can dilate to a considerable extent (Hagen, 1992). The crop mainly functions as a temporary reservoir in which ingested food is moistened and gradually passed to the stomach (Hueser, 1944; Hagen, 1992). Additionally, endogenous and bacterial enzymatic hydrolysis of carbohydrates, such as starch, starts in the crop (Hagen, 1992). However, the typical crop acidity of 4.5 to 6.7 favours a high activity of bacterial amylase from Lactobacilli, and suggests only a minimal role of saliva gland and crop wall amylase (Ziswiler and Farner, 1972). Finally, parrots regurgitate crop contents to feed their young, but do not secrete crop milk like pigeons do. The regurgitated food of parakeets, however, is also labelled crop milk, but is in fact glandular stomach content (Thomson, 1965).

The avian stomach is composed of at least two parts: the glandular stomach or proventriculus, and the muscular stomach or ventriculus. The glandular stomach equivalents the stomach of non-ruminating mammals, and secretes mucus, gastric acid (HCl) and pepsinogen. In parrots, in contrast to for instance birds of prey, the proteolytic pro-enzyme pepsinogen is not yet activated to pepsin in the glandular stomach, but in the muscular stomach (Farner, 1960). The much higher level of basal gastric juice secretion is thought to be indicative of a more efficient digestion in birds compared to mammals (Hagen, 1992). Its main function is particle

reduction of ingested food, which thus resembles that of mammalian teeth. This grinding effect is facilitated through two cooperating anatomic specialisations: the muscular wall and the corneous koilin layer, which is formed from a carbohydrate and protein rich muscular stomach secretion (Stevens and Hume, 1995; Bartels *et al*, 1997). The development of both muscular wall and koilin layer reflect the natural dietary habits of the species. The extent of these structures is more developed in poultry and pigeons, which ingest hard foodstuffs as a whole, compared to granivorous parrots, which as well forage on hard foodstuffs, but ingest only its much softer kernel (Hagen, 1992). In addition, as granivorous parrots use their beaks for removal of the hardest plant parts, optimal digestive efficiency does not require ingestion of insoluble grit, unlike for instance in pigeons (Harrison and McDonald, 2006). However, *ad libitum* access to insoluble grit may lead to excessive intake and impaction (Donoghue and Stahl, 1997). Finally, the soft structure of the natural diet of frugivorous parrots, and to a lesser extent nectarivorous parrots, is reflected by a further reduction in the development of the gizzard musculature and koilin layer, as compared to granivorous parrots (Stevens and Hume, 1995; Orosz, 1997; Macwhirter, 2000).



**Figure 4.** Stomach of a blue and yellow macaw (*Ara ararauna*)

The avian small intestine is comprised of the duodenal loop and ileum (Thomson, 1965). The primary site of enzymatic digestion and absorption of macronutrients, minerals and vitamins is localized in this intestinal segment (Stevens and Hume, 1995). In general, its length is relatively long in herbivorous and granivorous birds, but relatively short in insectivorous, carnivorous and frugivorous birds (Ziswiler and Farner, 1972). Caeca are absent and the large intestine is very short in psittacine birds (Ullrey *et al.*, 1991). Hence, hindgut fermentation of poorly digestible carbohydrates is presumed very limited in parrots (Koutsos *et al.*, 2001). As the intestinal tract, ureters and exit ducts of the reproductive system all end in the cloaca, birds, except for ostriches (*Struthio camelus*), void faeces (solid fraction), urates (chalky white fraction) and urine (clear, liquid fraction) together (Skadhauge and Dawson, 1999). The resulting urinary contamination of faeces complicates digestibility studies in birds.



Although the presence of gram negative bacteria in the intestinal flora of healthy parrots has been a matter of debate, their normal intestinal flora is currently considered to be comprised of an entirely gram positive bacterial community of non-spore forming, aerobic and anaerobic rods and cocci (reviewed by Harrison and McDonald, 2006).

### **CAPTIVE DIETS**

Most adult, true parrots in captivity are fed a commercial parrot seed mixture, which is eventually added with fruit, vegetables, egg food, supplements or table food (Werquin *et al.*, 2005). Lories and lorikeets, in contrast, are usually fed a powdered nectar food that is diluted in water as principal nutrition, which is often added with fruit.

#### *Seed based diets*

Whole-seed diets are readily ingested by parrots and are often perceived to be a natural diet. However, notwithstanding parrots being classified as seed eaters, their natural diet comprises a much greater variety in ingredients (Ullrey *et al.*, 1991; Collar, 1997). Moreover, the typical cultivated seeds and nuts used in commercial seed mixtures do not originate from the native habitats of parrots (Kollias, 1995). In addition, although seeds and nuts show variety in size, shape and colour, lack of variety in chemical composition results in several nutritional constraints. First, the edible part of seeds is strongly imbalanced in calcium to phosphorus (Ca:P) ratio (Wolf *et al.*, 1998). Moreover, some wild cultivars of preferred seeds on which parrots forage are much higher in calcium content compared to the cultivated seed types in seed mixtures for parrots (Youssef *et al.*, 2007). Other common nutritional deficiencies in the edible part of seeds include vitamins A, D, K and biotin, vitamins of the B-complex, and a number of trace-minerals, such as selenium, copper, zinc and available manganese (Forbes and Altman, 1998; Harrison, 1998; Schoemaker *et al.*, 1999; Wolf, 2002). Manifestation of resulting deficiencies remains subclinical for prolonged periods of time, and when clinical symptoms do emerge they are often unspecific and remain undiagnosed (Wolf *et al.*, 1998).

Formulation of species-specific seed mixtures is largely based on the size of seeds in relation to the beak size and on experience as regards dietary preferences of the species, rather than on species-specific nutrient requirements. Seed mixtures targeted for small-sized parrots, such as budgerigars, contain high proportions of millet and canary seed. Sunflower seed, in contrast, is highly represented in seed diets targeted for larger sized parrots, in which the proportion and size of nuts usually increases with the size of the targeted parrot.

Overall, seed mixtures are extremely high in energy and fat content, which is further increased by the typical feeding behaviour of parrots to dehusk seeds and nuts, ingesting only the kernel. Data of Werquin *et al.* (2005) showed an average of  $16.4 \pm 0.9$  MJ ME  $\text{kg}^{-1}$  and  $19.7 \pm 6.3$  % fat in 30 analysed commercial seed mixtures; the edible, dehusked fraction containing  $22.4 \pm 2.9$  MJ ME  $\text{kg}^{-1}$  and  $31.7 \pm 13.1$  % fat (mean  $\pm$  standard deviation). In comparison, diets for chickens for fattening usually contain only 12 to 13 MJ ME  $\text{kg}^{-1}$  and considerably less than 10% fat (NRC, 1994).

### *Pellets*

Pellets form an alternative to seed-based diets. The ingredients are ground to a meal and either compressed or extruded. The process of extrusion involves a multi-step process in which the meal is first moistened and heated through steam injection. Next, an Archimedes screw forces the mash through a tube, which greatly increases pressure, temperature and shear forces. At the end of the tube, a rotating die cuts the mash into small pieces, which expand following the abrupt pressure drop, resulting in an up to 400% increase in volume. Afterwards, the pellets are dried to prevent spoilage, and coated with vegetal oil to seal the pores. The latter not only diminishes the surface area for bacterial colonisation, but also increases the energetic density of the pellets and provides a means to supplement the pellets with essential nutrients that are sensitive to exposure to the extrusion process (Ghysels, 2003; Werquin, 2003). Nutritional benefits of extrusion include gelatinisation of starch<sup>5</sup>, destruction of certain anti-nutritional factors<sup>6</sup>, and sterilisation (Tait en Beams, 1988; Werquin, 2003). However, it also leads to Maillard-reactions<sup>7</sup> and to destruction of heat-sensitive vitamins. Nevertheless, expected losses of essential nutrients can be accounted for by compensatory supplementation prior to or after extrusion, by including stabilised forms of sensitive nutrients (Werquin, 2003; Singh *et al.*, 2007). Moreover, the extent of these undesirable destructive effects can be reduced through modulation of the extrusion conditions: lower temperature, higher moisture content and lower residence time (Singh *et al.*, 2007).

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<sup>5</sup> Gelatinisation of starch is a disruption of the tight structure of amylose (linear chain of glucose units) and amylopectin (branched chain of glucose units) within starch granules, through which its solubility in water, and hence its availability to the action of digestive enzymes is increased (Werquin, 2003).

<sup>6</sup> e.g. a reduction in phytate, which increases the bioavailability of certain minerals (Singh *et al.*, 2007)

<sup>7</sup> Maillard-reactions occur between the free amino-end of amino acids and the carbonyl groups of reducing sugars, which renders the involved amino acids nutritionally unavailable (Singh *et al.*, 2007). Lysine is the most reactive amino acid in Maillard-reactions as it has two available amino groups (O'Brien and Morrissey, 1989).

A main advantage of pelleted diets over seed-based diets includes prevention of selective feeding behaviour and the possibility of an accurate, computerized formulation according to available nutritional guidelines (Ghysels, 2003). Then again, nutritive value is not the only issue in dietary strategies. Feed cost, for instance, is usually higher in pelleted diets compared to seed mixtures. Still, published data on resulting health benefits are largely lacking. An exception hereto is the publication of Ullrey *et al.* (1991), who demonstrated a significant increase in fledging percentage in eight tested parrot species after a dietary change from a seed mixture to extruded pellets, both diets being added with vegetables and fruit *ad libitum*. Then again, a persisting criticism is that the outcome of such trials is, although indicative, not to be generalized to such diet types as a whole.

Another important constraint against feeding pellets instead of seeds is a presumed reduction in time spent on feed intake, resulting in boredom, overfeeding and behavioural disturbances. However, Wolf *et al.* (2002) demonstrated similar time budgets for feeding behaviour when parrots are fed pellets compared to seed mixtures. Bauck (1998) describes the often perceived higher variety and behavioural enrichment qualities of multi-component seed diets compared to uniform pellets as an anthropomorphisation. A general criticism of a reduced time budget spent on feeding behaviour in captive parrots is justified, but does not specifically apply to pelleted diets. Foraging is the appetitive phase of feeding behaviour and precedes the actual consumption of food, which is the consummatory phase. Under natural conditions, time spent performing behaviours from the appetitive phase amounts to several hours per day. In captive confinement, in contrast, food is usually provided in a dish, which denies foraging activities and drastically reduces the overall time spent on feeding behaviour (reviewed in Meehan *et al.*, 2003b). Furthermore, the reduction in, or even omission of the appetitive phase reduces the diversity of the parrots' behavioural repertoire. From a behavioural point of view, provision of foraging opportunities may be more important than the appearance of the offered food (Bauck, 1998). However, over-sized pellets, which resemble the size of the typical ingredients consumed in the wild, in contrast to regular pellets or most ingredients in commercial seed mixes, offer the opportunity to engage in extensive manipulation with feet and beak. To this respect, orange-winged amazons (*Amazona amazonica*) showed a pronounced preference for over-sized pellets compared to regular pellets, which were 20 to 30 times smaller, but identical in shape, nutritional density and surface texture ( Rozek *et al.*, 2010; Rozek and Millam, 2011). These over-sized pellets were intermediate in size between the two most preferred native foods of orange-winged amazons, being *Mauritatia* (3 to 5.5 cm length and 4 to 7 cm diameter) and *Roystonea* (1.2 to 1.7 cm length, and 1 cm diameter) palm

fruit (reviewed in Rozek *et al.*, 2010). Time spent feeding was also vastly increased when offered over-sized pellets (Rozek *et al.* 2010). Moreover, the amount of work the parrots were prepared to do to get hold on over-sized pellets when regular pellets were freely available, suggests a strong motivation for ingestive behaviour (Rozek and Millam, 2011). Besides larger-sized food items, barriers to chew through, feeder puzzles, and other devices that require the bird's manipulation to obtain food, are also indicated (Bauck, 1998; Meehan and Mench, 2006).

Another drawback of pelleted diets is the initial reluctance by parrots accustomed to seed-based diets. However, most parrots can be safely converted to pelleted diets provided a gradual transition period, and good monitoring of food intake, or preferably body weight of the individual birds (Ghysels, 1997). Yey, parrots are habitual animals, but the likelihood of neophobia or reluctance to novel food items is influenced by the extent of environmental enrichment in early life (Fox and Millam, 2007).

Finally, excreta firmness can be lower when fed pelleted diets compared to seed mixtures, which is particularly undesirable in pet birds that are housed indoors. This can be due to an increase in excreta moisture content or by a decrease in water binding capacity. A generally higher sodium content in pelleted diets might possibly increase excreta moisture content, as demonstrated in laying hens (Smith *et al.*, 2000), whereas artificial particle size reduction in the manufacturing of pellets might negatively influence excreta water holding capacity (Yasar, 2003).

#### *Vitamins and mineral supplements*

Concentrated mineral and vitamin solutions can be used to supplement the drinking water in an attempt to compensate for dietary deficits. However, this practice is strongly discouraged because vitamins are unstable in aqueous solutions and reduce water palatability, possibly decreasing water intake and introducing a risk of dehydration (Donoghue and Stahl, 1997; Hawley, 1997). Moreover, a precise dosing is difficult to achieve as water intake can vary widely between individuals, species, diets and environmental temperatures (McDonald, 2006). In addition to liquid supplementation, various methods to increase the vitamin and mineral content of seed based diets have been developed. Their efficiency, however, is questionable as this requires sufficient voluntary intake. Topical applications on seeds and nuts, for instance, do not achieve their prospected goals because parrots only ingest the kernels and discard the supplemented husks.

### COMMON NUTRITIONAL INADEQUACIES IN CAPTIVE PARROTS

Malnutrition comprises the primary cause of the majority of all clinical problems in pet birds presented to veterinarians (Harrison, 1998). A primary seed and nut diet is generally considered the most prevalent cause of nutritional disorders in parrots. In addition, inappropriately formulated pelleted diets and excessive vitamin or mineral supplementation comprise two other frequently encountered dietary grounds for malnourishment (Stahl and Kronfeld, 1998).

Obesity, which can be defined as excessive accumulation of bodyfat to the extent that health is impaired, is one of the most prevalent health problems in captive parrots (La Bonde, 1997; WHO, 2000). Estimation of the amount of subcutaneous and abdominal adipose tissue is a subjective criterion and requires manual restraint. An objective working definition for obesity in pet birds is an overweight that exceeds 15% of the optimal bodyweight of the species (La Bonde, 1997). Although this criterion is unambiguous and does not necessitate prolonged manual restraint, it greatly relies on available species-specific guide weights of healthy individuals. Restricted physical activity concomitant to excessive energy intake forms the main underlying basis (Levine, 2003). Yet, its development is often multi-factorial in origin, and is not limited to obvious factors such as an inadequate diet, species predilection and lack of exercise (La Bonde, 1997).

The most prevalent nutritional deficiencies in captive parrots include calcium and vitamins A and D<sub>3</sub> (Donoghue and Stahl, 1997). Imprudent supplementation, on the other hand, may incite toxicity symptoms or secondary effects on the supply of other nutrients. Excess vitamin C, for instance, reduces the bioavailability of copper but improves the absorption of iron, which may increase the incidence of aortic rupture and iron storage disease, respectively. Likewise, adequate absorption of the fat-soluble vitamins A, D, E and K is hampered if one of the others is provided in excess. Moreover, excessive supplementation with vitamins A and D readily results in toxicity (MacDonald, 2006). Previously, toxic effects of dietary supplementation with menadione or vitamin K<sub>3</sub>, the synthetic form of vitamin K, have been suggested in parrots. However, these concerns have been refuted by study results of Wolf *et al.* (2005), who demonstrated a wide safety range of oral vitamin K<sub>3</sub> in lovebirds. Excess supply of water-soluble vitamins, in contrast to fat-soluble vitamins, can be excreted through the urine and are less likely to cause toxicity symptoms.

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# CHAPTER 2

## **SCIENTIFIC AIMS**





Malnutrition comprises a principal source of health problems in captive psittacine birds. Manifestation of malnourishment often remains subclinical for a prolonged period, but over time can become life threatening. In addition, inadequate nutrition may impair natural defence barriers and suppress the immune system, which renders the birds more susceptible to infectious diseases. Moreover, the recovery from unrelated illnesses may be prolonged and reproductive performances reduced. Therefore, adaptations to the captive diets and feeding strategies of parrots are highly indicated.

A high incidence of obesity and its health-impairing consequences result largely from restricted physical activity and inappropriate dietary strategies in captive parrots. However, restricted feeding as a method to lower daily energy intake entails important drawbacks, including the fact that most parrot species are highly social birds that are often group-housed. The first aim of this thesis addressed this issue through the development of a suitable feeding strategy in which voluntary energy intake is reduced in *ad libitum* fed parrots, without compromising an adequate supply of essential nutrients. The majority of parrot species that are regularly confined in captivity fall into two highly different, broad categories of natural and captive diets, namely nectarivorous and granivorous. Therefore, species of both dietary groups were considered.

A generally recognized drawback of multi-component seed diets is its unpredictable nutritive value and questionable effectiveness of supplements when fed to parrots. The underlying reason hereof is the distinctive feeding behaviour of parrots to dehusk seeds and selectively feed on separate ingredients when offered a multi-component diet. Still, it remains common practice to feed such diets as principal nutrition to granivorous parrots. A second aim was then to assess the actual impact of management and animal factors on the nutrient supply of this type of diet when fed to granivorous parrots, taking into account common storage and feeding methods.

Finally, in contrast to seed-based diets, pellets can be accurately formulated according to available nutritional recommendations without their nutritional value being deteriorated through selective behaviour or segregation effects due to inappropriate storage. Despite these advantages from a nutritional point of view, the less desirable features of droppings when fed pellets might impair the owner appreciation towards this type of diet, especially when birds

are housed indoors. Therefore, a final aim was to assess effects of food processing on excreta characteristics in granivorous parrots.

**THE SPECIFIC SCIENTIFIC AIMS WERE TO EVALUATE:**

1. The effect of dilution degree of *ad libitum* fed artificial nectar and availability of fresh fruit on voluntary energy intake and supply of essential nutrients in two lorikeet subspecies: green-naped lorikeets (*Trichoglossus haematodus haematodus*) and red-breasted lorikeets (*Trichoglossus haematodus mitchellii*).
2. The effect of fresh fruit next to an *ad libitum* fed seed-based diet on voluntary energy intake and supply of essential nutrients in yellow-shouldered amazons (*Amazona barbadensis*).
3. The effect of segregation during storage and feeding behaviour of yellow-shouldered amazons (*Amazona barbadensis*) on the ingredient and nutrient composition of a multi-component seed-based diet. Further, the effectiveness of dietary supplements in multi-component seed diets was evaluated.
4. The effect of pellet particle size on excreta characteristics of African grey parrots (*Psittacus erithacus erithacus*) fed an extruded pellet diet *ad libitum*.

# CHAPTER 3

## **Effect of Dilution Degree of Commercial Nectar and Provision of Fruit on Food, Energy and Nutrient Intake in Two Rainbow Lorikeet Subspecies**

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*Modified from*  
**Zoo Biology (2009)**  
**Volume 28, 98-106**

## **ABSTRACT**

*A feeding trial was performed on adult rainbow lorikeets of two subspecies: six green-naped lorikeets (*Trichoglossus haematodus haematodus*) and six red-breasted lorikeets (*Trichoglossus haematodus mitchellii*). Throughout the entire trial, half of the birds from each subspecies had ad libitum access to water-diluted commercial nectar powder and drinking water only, whereas the other half also received ad libitum pieces of apple. During three consecutive 14-d periods, the nectar powder was diluted to a different degree: 1:3 (low), 1:5 (high) and 1:4 (medium) (v:v). Diluting nectar to a higher degree resulted in both subspecies in a significant decrease in dry matter intake of nectar, but intake of apple increased when offered next to higher diluted nectar. Besides a higher degree of dilution of nectar, availability of apple also resulted in lower intake of nectar. Intake of apple was significantly higher in red-breasted lorikeets compared to green-naped lorikeets. Neither degree of dilution, nor availability of fruit had a clear-cut effect on total energy intake, but significant interactions between dilution degree and subspecies, and between dilution degree and availability of fruit were present. In green-naped lorikeets fed solely nectar, daily intake of energy showed a noticeable numerical decrease when the nectar was diluted to a higher degree. An opposite effect of the degree of dilution on voluntary energy intake was noted in red-breasted lorikeets when fed nectar added with fruit. In green-naped and red-breasted lorikeets, lowest voluntary energy intake was attained when fed high-dilution nectar and low-dilution nectar added with fruit, respectively. Overall, protein and thiamine intake as well as Ca:P ratio of the ingested ration were lowest when fed highly diluted nectar added with fruit. Yet, minimal requirements were still covered. Because energy content of fruit can be higher than liquid diets, in this case medium-or high-dilution nectar, ad libitum provision of fruit as a means to lower voluntary energy intake in lorikeets, for instance in case of obesity, needs to be considered with care.*

## INTRODUCTION

Lorikeets belong to the subfamily Loriinae within the order Psittaciformes and originate from Indonesia, Papua New Guinea, the Solomon isles, the Philippines and Australia (Juniper and Parr, 1998; Cornejo and Clubb, 2005). Natural diets of most parrot subfamilies such as Cacatuine and Psittacinae (parrots, parakeets, macaws and relatives) consist mainly of fruits and seeds. Yet, lorikeets predominantly feed on nectar and pollen, whereas fruit, flowers and some invertebrates are other minor constituents of their diet (Cannon, 1984; Collar, 1997; Gartrell, 2000). Their specialized tongue structure enables lorikeets to harvest both nectar and pollen, which are attractants that plants use to entice pollinators (Gartrell, 2000; Roskopf, 2003).

Nectar mainly provides energy through simple sugars, predominantly glucose, fructose and sucrose, which constitute most of the dry matter. Nectar is thus high in gross energy content, but extremely low in crude protein, vitamin and mineral levels (Gartrell, 2000). Hence, nectar-eaters need to forage for alternative protein sources such as pollen or insects. This has been demonstrated by Cannon (1979), who showed that lorikeets lose weight when fed only artificial nectar (bread and honey), but maintain weight when this diet was supplemented with protein.

Pollen contain 16 to 30% protein on a dry matter base (DM) and are rich in minerals and vitamins B, C, E and precursors of vitamin A (Vogel, 1983). Furthermore, the amino acid profile of pollen protoplasm meets the currently presumed psittacine requirements for dietary essential amino acids, except for methionine (Gartrell, 2000). In contrast, mealworm haemolymph for instance, is next to methionine, also deficient in arginine, histidine and threonine (Gartrell, 2000). Yet, although pollen protoplasm is a potentially valuable protein source, feeding trials in lorikeets revealed a very low apparent protein digestibility of pollen (Brice *et al.*, 1989). Prerequisite to protoplasm digestion is the efficient emptying of pollen in the alimentary tract, because the outer exine shell is indigestible. Brice *et al.* (1989) used bee-collected and frozen stored pollen, which might have influenced its emptying according to Richardson and Wooller (1990). Then again, Gartrell and Jones (2001) demonstrated equal pollen emptying between fresh and frozen stored pollen, but speculated upon a possible negative effect of bee secretions.

The distinct feeding behaviour between granivorous and nectarivorous parrots is reflected in the morphology of the alimentary tract. Firstly, lorikeets have a slender beak and a long, narrow, brush tipped tongue with a rough dorsal surface, enabling them to harvest nectar and pollen from flowers (Churchill and Christenson, 1970; Richardson and Wooller, 1990; Roskopf, 2003). Granivorous parrots, on the other hand, dehusk seeds and nuts before ingestion, which is enabled by a peculiar tongue musculature, powerful, mobile beaks and a rippled inner surface of the upper beak, as described in Kalmar *et al.* (2007b). Other striking anatomical features indicating the distinct feeding behaviour of lorikeets include the much less developed gizzard wall musculature and koilin thickness, and shorter intestinal tract compared to seed-eating parrots (Ziswiler and Farner, 1972; Richardson and Wooller, 1990; Roskopf, 2003; Häbich, 2004).

Lorikeets kept in captivity are usually fed dilutions of commercially available powdered nectar food as principal nutrition. Because these foods are sold as a powder that has to be diluted before use, the degree of dilution might be determining for overall nutrient and energy intake. An improper feeding strategy might therefore contribute to either weight loss or obesity. Captive-kept lorikeets form no exception to other psittacine species in having a high prevalence of obesity and fatty liver, which is considered an important factor in reproductive disturbances next to shortening lifespan (Doneley, 2002; McDonald, 2003). Additionally, the diet of captive lorikeets is often supplemented with fruit and, consequently, this has to be taken into account. The present trial aimed to investigate the effect of dilution degree of an artificial nectar powder and availability of supplemental fruit on water, food, energy and nutrient intake in two lorikeet subspecies differing in body size.

## **MATERIALS AND METHODS**

### *Animals, Location and Housing*

The trial was performed during the summer months at the parrot breeding centre of the Loro Parque Fundación in Puerto de la Cruz (Tenerife, Spain). Twelve adult rainbow lorikeets were used, of which six green-naped lorikeets (*Trichoglossus haematodus haematodus*) and six red-breasted lorikeets (*Trichoglossus haematodus mitchellii*). Bodyweight (BW) averaged  $149 \pm 4$  g and  $96 \pm 6$  g in green-naped and red-breasted lorikeets, respectively. A thorough veterinary inspection was performed before the start of the trial to check the general health status of all participating birds. Birds were housed individually in outdoor hanging, wire mesh aviaries with dimensions 100 cm x 100 cm x 250 cm (height x width x length), that were

equipped with two perches at different heights. Cage design and size conformed to species-specific housing recommendations (Hawkins *et al.*, 2001; Kalmar *et al.*, 2007a). Plastic sheets were installed between the cages to prevent spilled food or excreta of one bird falling underneath another cage.

### *Diets*

Throughout the entire trial, half of the birds from each subspecies had *ad libitum* access to water-diluted, commercially available, artificial nectar powder and drinking water only, whereas the other half also received *ad libitum* apple pieces. Apple was chosen to be the type of additional fruit next to the diluted commercial nectar, in agreement with data of Cornejo and Clubb (2005) who tested voluntary intake of different kinds of fruit and vegetables in lorikeets and found a much higher intake of apple compared to other kinds of fruit or vegetables, including orange, carrot, pear, watercress and pepper. During three consecutive 14-d periods, the nectar powder was diluted to a different degree: 1:3 (low), 1:5 (high) and 1:4 (medium) (volume powder:volume water). The density of the nectar powder was 0.635 g/ml. Drinking and feeding bowls were covered with a wired surface in which a hole was cut to enable the birds to drink, but prevented them from bathing in their drinking water or spilling food. The wired surface could be removed for cleaning purposes. Nectar, fruit and drinking water were provided daily at 9:00 and 14:00 h.

The ingredients of the nectar powder (Orlux Lori, Versele-Laga Ltd., Deinze, Belgium) were, in decreasing order: sugars, vegetable protein, vegetal by-products, bakery products, oils and fats, minerals, yeast, methionine, lysine, threonine, vitamins and trace elements. Nutrient content of nectar powder was provided by Versele-Laga Ltd. and nutrient content of apple was retrieved from literature (Souci *et al.*, 2000; Wolf, 2002). Caloric density of diets was calculated using the equation of Larbier and Leclercq (1992). Dry matter content as well as energy content of apple on an as fed basis were higher compared to medium- and high-dilution nectar, but were lower compared to low-dilution nectar. Protein content as well as Ca:P ratio were lowest in apple (**Table 1**).

**Table 1.** Nutrient and energy content of apple expressed on dry matter basis (DM) and as-fed (AF) and of commercial nectar powder and nectar at the tested dilution degrees (D) (vol. powder:vol. water). Preparation of artificial nectar at the tested dilution degrees expressed in gram and in volume parts of water to powder (powder density = 635 g/l).

		Apple <sup>1</sup>		Commercial Nectar			
		DM	AF	Powder <sup>2</sup>	D 1:3	D 1:4	D 1:5
Moisture	(%)	0	84.9	4.2	83.4	86.8	89.0
Dry Matter	(%)	100	15.1	95.8	16.6	13.2	11.0
Crude Protein (CP)	(%)	2.3	0.3	15.0	2.6	2.1	1.7
Ether Extract (EE)	(%)	3.8	0.6	5.0	0.9	0.7	0.6
Crude Fibre (CF)	(%)	13.4	2.0	0.8	0.1	0.1	0.1
Sugar	(Su) (%)	68.3	10.3	46.9	8.1	6.5	5.4
Starch	(St)	4.0	0.6	7.9	1.4	1.1	0.9
Calcium	(Ca) (g kg <sup>-1</sup> )	0.38	0.06	9.86	1.71	1.36	1.13
Phosphorous	(P)	0.73	0.11	5.50	0.95	0.76	0.63
Ca:P		0.52	0.52	1.79	1.79	1.79	1.79
Thiamine	(mg kg <sup>-1</sup> )	2.38 <sup>†</sup>	0.36	7.20	1.25	0.99	0.83
Energy <sup>3</sup>	(MJ ME kg <sup>-1</sup> )	11.21	1.69	1.46	1.99	1.58	1.32
Nectar	(g g <sup>-1</sup> )				21-100	16-100	13-100

<sup>1</sup>Souci et al. (2000), <sup>2</sup>Versele-Laga Ltd., <sup>†</sup>Wolf (2002)

<sup>3</sup>Metabolisable Energy = 0.1551 CP + 0.3431 EE + 0.1301 Su + 0.1669 St (Larbier and Leclercq, 1992)

### Measurements

Each period started with an adaptation period of 7 days. Subsequently, during day 8-14, the individual intakes of nectar, apple and drinking water were measured twice daily. Bowls with water and the tested diets were placed in the same environment as the cages, enabling the correction of daily intakes for evaporative moisture losses. The birds were weighed at the beginning of the trial and at day 8 and day 14 of each feeding period. Daily water, food and nutrient intake are expressed as ml or g per kg metabolic bodyweight (MBW).

### Statistical Analysis

Data were subject to repeated measures analysis of variance with dilution degree as within-subject variable and provision of apple and subspecies as between-subject variables. All analyses were performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA).



## RESULTS

All birds were healthy and in able-bodied condition, neither obese nor thin, at the beginning of the trial. They remained healthy during the trial and showed normal eating and drinking behaviour. BWs did not significantly change during the trial (**Table 2, upper panel**). Intake of drinking water was significantly higher in the smaller subspecies compared to the larger subspecies ( $p < 0.001$ ). Neither diluting the nectar to a higher degree, nor provision of fruit resulted in a substantial change in intake of drinking water. However, because of increased dietary water intake, there was a significant increase in total water intake when fed higher diluted nectar ( $p < 0.001$ ) (**Table 2, middle panel**).

Diluting the nectar to a higher degree significantly increased both nectar and apple intake on an as fed basis ( $p < 0.001$ ). However, this increase in nectar intake was not sufficiently high to maintain its dry matter intake, which significantly decreased when fed higher diluted nectar ( $p < 0.001$ ). Availability of apple further led to a decrease in dry matter intake of the nectar compared to feeding the same dilution degree of nectar without addition of apple ( $p < 0.001$ ). The latter was most evident in red-breasted lorikeets, in which apple intake relative to MBW, taking into account dilution degree of nectar, was approximately 5.5 times higher compared to green-naped lorikeets ( $p < 0.05$ ) (**Table 2, middle panel**).

Total energy intake relative to MBW was significantly higher in the smaller subspecies compared to the larger subspecies ( $p < 0.05$ ). Further, neither degree of dilution, nor availability of fruit had a clear-cut effect on total energy intake, but significant interactions between dilution degree and subspecies ( $p < 0.05$ ) and between dilution degree and availability of fruit ( $p < 0.001$ ) were present. Green-naped lorikeets fed nectar and apple maintained a similar energy intake, independent of degree of dilution of the nectar. In contrast, availability of apple resulted in red-breasted lorikeets in a substantial increase in energy intake when fed high-dilution nectar compared to low-dilution nectar. When fed solely nectar, energy intake decreased only slightly in red-breasted lorikeets, but decreased considerably in green-naped lorikeets when the nectar was diluted to a higher degree. Finally, irrespective of subspecies, both a higher dilution degree and availability of apple significantly decreased daily protein intake and thiamine or vitamin B<sub>1</sub> intake. The Ca:P ratio was 1.79 in nectar but only 0.52 in apple. Providing apple and diluting the nectar to the highest degree resulted in a decrease in the Ca:P ratio of the total ingested ration down to 1.56 and 1.75 in red-breasted and green-naped lorikeets, respectively (**Table 2, lower panel**).

**Table 2.** Metabolic bodyweight (MBW) of birds, average daily water, food, energy, protein and thiamine intake relative to MBW and Ca:P-ratio of ingested ration in two lorikeet subspecies (S) fed artificial nectar at three degrees of dilution (D): low, medium (med) or high, with or without provision of fruit (F).

Dilution Degree	No Fruit			Fruit			Statistics		
	low	med	high	low	med	high	SEM	effects	
<b>Average MBW</b> (kg <sup>0.75</sup> )									
H	0.24	0.24	0.25	0.24	0.24	0.23	0.006	<b>S</b>	
M	0.17	0.17	0.18	0.17	0.17	0.17			
<b>Water intake</b> (ml kg <sup>-0.75</sup> d <sup>-1</sup> )									
Drinking water	H	44	42	36	41	42	35	2.8	<b>S</b>
	M	78	73	57	66	66	59		
Total moisture	H	385	472	523	366	457	535	16.6	<b>D, S</b>
	M	449	558	635	431	569	645		
<b>Food Intake - As Fed</b> (g kg <sup>-0.75</sup> d <sup>-1</sup> )									
Nectar	H	409	496	547	366	430	509	17.9	<b>D, F, DxS</b> <i>SxF, DxSxF</i>
	M	445	559	649	315	349	389		
Fruit	H	-	-	-	24	50	55	27.6	<b>D, S, DxS</b>
	M	-	-	-	121	236	282		
<b>Food Intake - Dry Matter basis</b> (g kg <sup>-0.75</sup> d <sup>-1</sup> )									
Nectar	H	68	66	60	61	57	56	2.0	<b>D, F, SxF</b>
	M	74	74	72	52	46	43		
Fruit	H	-	-	-	4	7	8	4.2	<b>D, S, DxS</b>
	M	-	-	-	18	36	43		
<b>Energy Intake</b> (kJ ME kg <sup>-0.75</sup> d <sup>-1</sup> )									
H	813	784	721	769	763	765	18.7	<i>S, DxS, DxS</i>	
M	885	883	856	831	951	990			
<b>Protein Intake</b> (g kg <sup>-0.75</sup> d <sup>-1</sup> )									
H	11	10	9	10	9	9	0.3	<b>D, F</b>	
M	12	12	11	9	8	8			
<b>Ca:P</b>									
H	1.79	1.79	1.79	1.78	1.75	1.75	0.014	<b>D, S, DxS</b>	
M	1.79	1.79	1.79	1.70	1.61	1.56			
<b>Thiamine (Vit B<sub>1</sub>) intake</b> (mg kg <sup>-0.75</sup> d <sup>-1</sup> )									
H	0.51	0.49	0.45	0.47	0.44	0.44	0.011	<i>D, F</i>	
M	0.56	0.55	0.54	0.44	0.43	0.42			

data include one-week measurements after one week of adaptation

H: green-naped lorikeet, M: red-breasted lorikeet

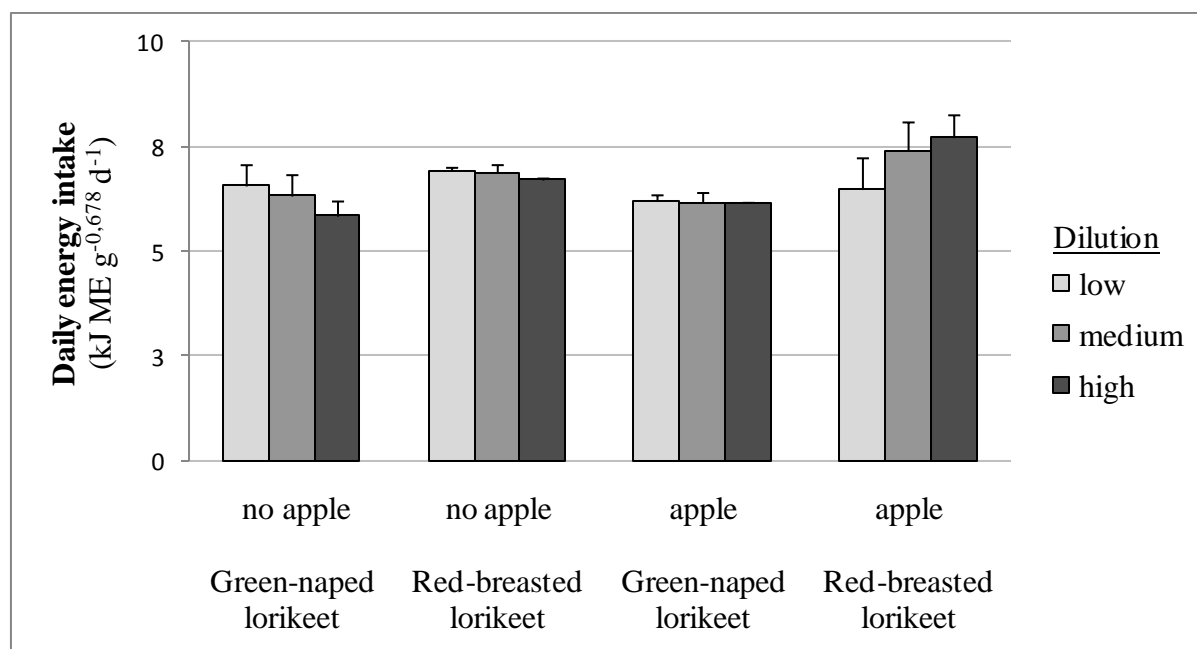
significance: **bold typing** :  $p \leq 0.001$ , regular typing :  $p \leq 0.01$ , *italic typing* :  $p < 0.05$

## DISCUSSION

Daily energy intake relative to MBW was significantly higher in the smaller subspecies compared to the larger subspecies. This finding is interesting, as both subspecies were in maintenance metabolic state. Wolf *et al.* (2007) also found higher energy requirements relative to MBW in smaller compared to larger lorikeets, whereas expression of energy requirement per unit MBW already accounts for allometric scaling. In dogs, for instance, Rubner demonstrated empirically in 1883 that basal metabolic rate is proportional to body surface area and not to BW (White, 2010). Krogh (1916) first suggested that the relationship between BW and body surface area in mammals and birds is best described by BW raised to some power  $p$ , which is close to 0.67. The following decades, researchers searched for and debated about the value of a universal exponent  $p$ . Brody and Procter (1932), Kleiber (1932) and others suggested that in mammals, metabolic rate scales with an exponent  $p$  close to 0.75, for which the most prominent mechanistic explanation is that this  $p$ -value scales with the geometry of nutrient supply networks or the ‘fractional distribution network’ (West *et al.*, 1997). Already in 1932, Brody and Procter (1932) advocated a distinct  $p$ -value (0.64) for birds. King and Farner (1961), however, computed a  $p$ -value of 0.744 applicable for birds weighing more than 0.1 kg. Lasiewski and Dawson (1967), in turn, proposed  $p$ -values regardless of BW of 0.724 and 0.723 for passerine and non-passerine birds, respectively, and advocated a fundamental similarity of the metabolism-weight relationships between mammals and birds. More recently, McNab (2009)<sup>8</sup> fitted curves for basal metabolic rate of various bird orders, in which the  $p$ -value for psittacine birds was 0.678. McNab (2009) also fitted coefficients to compute basal metabolic rate in birds, based on ecological factors of the species, such as native food sources, habitats used and climates occupied. In contrast to daily energy intake expressed on a  $\text{kJ ME kg}^{-0.75} \text{ d}^{-1}$  basis, in which red-breasted lorikeets showed a significantly higher intake compared to green-naped lorikeets, recalculation of the data on a  $\text{kJ ME g}^{-0.678} \text{ d}^{-1}$  basis revealed similar intakes between both subspecies. The same effect was seen on daily water intake. Voluntary daily energy intake ( $\text{kJ ME g}^{-0.678} \text{ d}^{-1}$ ) of current data is represented in **figure 1**.

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<sup>8</sup> In the equations of McNab (2009), body mass is expressed in g instead of kg



**Figure 1.** Daily energy intake (mean  $\pm$  sem) in two lorikeets subspecies (S) fed artificial nectar at three degrees of dilution (D) with or without provision of fruit. Statistical analysis revealed a significant D $\times$ S interaction ( $p = 0.015$ ).

As the birds in the current trial were all in maintenance conditions and both subspecies share the same familiar affiliation and ecological factors, it was unexpected to find a significant effect of species on voluntary energy intake. Therefore, it is more likely that scaling BW (kg) to the power 0.75 was less accurate compared to scaling BW (g) to the power 0.678. Apart from the choice of exponent value to compute MBW, other factors such as a difference in temperament between species, might have contributed to the apparent higher energy intakes and requirements of smaller lorikeets observed in the current data and by Wolf *et al.* (2007), respectively (Careau *et al.*, 2008).

The present trial revealed that both diluting commercial nectar to a higher degree and providing apple as a supplement to nectar significantly reduce voluntary dry matter intake of nectar. Daily energy intake in green-naped lorikeets was highest when fed solely low-dilution nectar and lowest when fed solely high-dilution nectar. Availability of apple resulted in this subspecies in intermediate energy intakes, regardless of dilution degree of nectar. Nevertheless, voluntary energy intake for all tested rations remained within the ranges of minimum requirements for maintenance as determined in this subspecies by Wolf *et al.* (2007) [ $0.65 \text{ MJ ME kg}^{-0.75} \text{ d}^{-1}$ ] and by Cannon (1979) [ $0.85 \text{ MJ ME kg}^{-0.75} \text{ d}^{-1}$ ]. In red-breasted lorikeets, on the other hand, provision of apple next to medium- or high-dilution

nectar resulted in a higher energy intake compared to feeding solely nectar of any degree. Hence, at low dilution degree of a liquid diet, availability of fruit might dilute the energy content of the total ration and decrease voluntary energy intake. However, *ad libitum* provision of fruit is not a clear-cut means to decrease voluntary energy intake in birds fed liquid diets, as energy content of fruit can be higher compared to liquid diets. This was the case with the medium (1:4) and high (1:5) diluted nectar in this study. Seed diets, which are commonly offered to granivorous parrots, on the contrary, are much higher in energy content compared to fruit; hence availability of fruit next to seed-based diets will likely more unambiguously lower voluntary energy intake. Kalmar *et al.* (2006), for instance, showed a 13.5% decline in energy intake in yellow-shouldered amazons (*Amazona barbadensis*) fed a seed mixture (15.13 MJ ME kg<sup>-1</sup> offered seed mixture *in toto*; 19.28 MJ ME kg<sup>-1</sup> ingested dehusked fraction) and fruit (0.21 MJ ME kg<sup>-1</sup>) compared to solely the seed mixture.

Häbich (2004) estimated daily maintenance protein requirements in green-naped lorikeets and Goldie's lorikeets (*T. Goldiei*), a smaller subspecies (30-40 g BW), at 2.1 g kg<sup>-0.75</sup> d<sup>1</sup> and 2.3 g kg<sup>-0.75</sup> d<sup>1</sup>, respectively. Assuming an apparent protein digestibility of 62.9% in commercial nectar and 43.6% in apple (Häbich, 2004), it is clear that neither diluting a high protein commercial nectar to a high degree, nor additional provision of apple, which is extremely low in protein content, compromises protein intake.

Finally, diluting a liquid diet to a higher degree and provision of other food items such as fruit, both have diluting effects on vitamins and minerals, potentially leading to deficiencies or even mortality. Mortality caused by thiamine deficiency, for instance, is well described in avian species including lorikeets, and is therefore important to be evaluated (Holz *et al.*, 2002). Thiamine supply in the current trial ranged between 0.72 and 1.01 mg per kg BW, which is within the range recommended for lorikeets (0.5 - 1.25 mg thiamine per kg BW) (Holz *et al.*, 2002). Next to a diluting effect, some fruits also contain anti-nutritional factors, such as anti-thiamine in blueberries, which further deteriorates vitamin supply (Somogyi, 1973). Further, bacterial contamination of the food with thiaminase-producing bacteria, *Bacillus thiaminolyticus* and *Clostridium sporogenes ssp.*, might compromise thiamine supply as well, which should be anticipated by hygienic measures (Princewill, 1980; Holz *et al.*, 2002). In addition, *ad libitum* availability of other food items next to a complete diet might deteriorate mineral balances such as the Ca:P ratio. In this study, the Ca:P ratio significantly decreased down to 1.56 at high apple intake, owing to a high phosphorous content in apple

relative to calcium, but remained at all times within the optimal range of 2:1 - 1.5:1 (Scott *et al.*, 1982; Wolf, 2002). Considering provision of fruit or vegetables to lorikeets, it should be clearly stated that not all plants are suitable treats for birds. Avocados, for instance, are toxic to parrots and its consumption can even result in mortality (Hargis *et al.*, 1989).

It should be noted that the current trial was conducted in outdoor aviaries during the summer months in northern Tenerife, and the different environmental conditions could have resulted in distinct intake behaviour (e.g. MacLeod and Dabutha, 1997; Ayala-Berdon *et al.*, 2009). Particularly in conditions of increased energetic demands, such as when kept in cold ambient temperatures, feeding an overdiluted diet can lead to inadequate food intake or overhydration, as suggested by Köhler *et al.* (2010) for other nectar feeders. Therefore, it is warranted to determine the safe upper limit of degree of dilution of nectar in lorikeets maintained under a range of environmental conditions and at different metabolic states.

## CONCLUSIONS

In both subspecies, diluting nectar to a higher degree as well as additional provision of fruit decreased dry matter intake of nectar, resulting on the one hand in lower protein and thiamine intake and on the other hand in a lower Ca:P ratio. Nevertheless, minimum requirements for maintenance were still met. Neither degree of dilution, nor availability of fruit had a clear-cut effect on total energy intake, but significant interactions between dilution degree and subspecies, and between dilution degree and availability of fruit were present. In green-naped lorikeets fed solely nectar, daily intake of energy showed a noticeable numerical decrease when the nectar was diluted to a higher degree. An opposite effect of the degree of dilution on voluntary energy intake was noted in red-breasted lorikeets when fed nectar added with fruit. In green-naped and red-breasted lorikeets, lowest voluntary energy intake was attained when fed high-dilution nectar and low-dilution nectar added with fruit, respectively.

#### **ACKNOWLEDGEMENTS**

The technical staff of the Laboratory of Animal Nutrition and Loro Parque Fundación are kindly acknowledged. The authors thank both the reviewers, and Stephanie Van Weyenberg and Christel Moons for their constructive contribution to this manuscript.

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# CHAPTER 4

## **Effects of segregation and impact of specific feeding behaviour and additional fruit on voluntary nutrient and energy intake in yellow-shouldered amazons (*Amazona barbadensis*) when fed a multi-component seed diet *ad libitum***

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*Modified from*

**Journal of Animal Physiology and Animal Nutrition (2010)**

**Volume 94, e383-e392**

## **ABSTRACT**

*Parrots are commonly fed multi-component seed diets. However, both segregation and feeding behaviour might alter ingredient and nutrient composition of the offered diet. First, the nutritional impact of segregation was assessed as it occurs when multi-component diets are temporarily stored in food containers that are replenished before completely emptied and birds being fed from the upper layer. The most detrimental effect hereof was a vast decrease in mineral supplements, leading to a decrease in Ca:P ratio in the offered food in relation to the formulated diet. Next, caloric distribution shifted towards more energy in the form of ether extract (EE) at the expense of energy in the form of nitrogen-free extract (NfE), as proportion of oilseeds increased and NfE-rich seeds decreased. Next, a feeding trial was performed on six yellow-shouldered amazons (*Amazona barbadensis*) in which the nutritional impact of parrot-specific feeding behaviour was assessed as well as the influence of additional provision of fruit next to the seed mixture. Profound selective feeding behaviour and dehiscing of seeds resulted in a vast increase in energetic density by up to 64% in the ingested fraction in relation to the offered mixture in toto. Furthermore, the already suboptimal Ca:P ratio further deteriorated and caloric distribution shifted towards an over twofold EE-energy and a vast reduction NfE-energy, whilst CP-energy remained similar. Finally, provision of fruit next to the seed diet significantly lowered voluntary energy intake from  $936 \pm 71 \text{ kJ ME kg}^{-0.75} \text{ d}^{-1}$  to  $809 \pm 109 \text{ kJ ME kg}^{-0.75} \text{ d}^{-1}$ , without compromising adequate protein intake. In conclusion, despite efforts by nutritionists to formulate diets to approximate estimated species-specific requirements, nutritional composition of the actually consumed fraction of multi-component seed diets can be vastly deteriorated by both animal and management factors. Furthermore, offering of fruit next to a seed-based diet effectively reduces voluntary energy intake and can hence be applied to abate obesity.*

## INTRODUCTION

Captive parrots are often fed multi-component seed diets. This type of food is commonly perceived as a natural diet for true parrots (Psittacinae), which enables performance of the birds' normal ethogram, including manipulation of food with feet and beak. However, although Psittacidae are classified as seed eaters, the natural diet of wild parrots consists of a wide variety of ingredients which include indeed seeds and nuts, but also other parts of plants, such as flowers, buds, leaves, fruit and bark, insects and larvae and even meat of carcasses (Ullrey *et al.*, 1991; Collar, 1997). Moreover, typical cultivated seeds and nuts used in commercial parrot diets do not originate from the native habitats of parrots or are impossible to harvest by parrots because of physical constraints (Kollias, 1995). Peanuts, pumpkins and sunflowers, for instance, originate from the American continent (Harter *et al.*, 2004; Paris *et al.*, 2006; Burow *et al.*, 2008) and are thus non-indigenous to the natural habitat of African or Australian parrot species. Furthermore, it would be very difficult for wild parrots to get hold of peanuts as they grow underground or pumpkin seeds as they are surrounded by a vast amount of pumpkin flesh.

On top of the misconception that commercial seed mixtures offer a natural food source to parrots, these diets are inherently deficient in several essential nutrients. Common nutritional deficiencies in the edible part of seeds include vitamins A, D, K and biotin, vitamins of the B-complex, and a number of trace-minerals, such as selenium, copper, zinc and available manganese (Forbes and Altman, 1998; Harrison, 1998; Schoemaker *et al.*, 1999; Wolf, 2002). Besides these deficiencies, seeds are also strongly imbalanced in Ca and P supply, leading to a suboptimal (too low) Ca:P ratio (Wolf *et al.*, 1997).

According to Harrison (1998), the primary cause of up to 90% of all clinical problems in pet birds presented to veterinarians is the consequence of malnutrition. The following commonly encountered disorders in parrots are examples in which inadequate nutrition plays a major role in the aetiology. Murphy (1992) and Kollias (1995) for instance, designate psittacine fatty liver syndrome in large parrot species to a biochemical malfunction within the liver lipid metabolism because of a deficient supply of biotin and choline in fat-rich, seed based diets and in some cases to mycotoxins present in moldy seeds. Additionally, next to relative inactivity and genetic predisposition, a high fat diet contributes to development of atherosclerosis, for which reported incidences in parrots vary widely but are at least over 10% in African grey parrots and amazons (Bavelaar and Beynen, 2004). Vitamin D<sub>3</sub> and calcium

deficiency or a suboptimal Ca:P ratio may lead to tetanic hypocalcaemia syndrome in African grey and Timneh parrots, nutritional secondary hyperparathyroidism, rachitis or osteomalacia (Kollias, 1995; Schoemaker *et al.*, 1999). Vitamin A deficiency, on the other hand, results in hyperkeratotic lesions which compromise the barrier function of epithelia, resulting in a decreased defence against infections (Roskopf and Woerpel, 1991).

The aims of the current study were to assess disrupting factors which compromise the effect of formulated complete, multi-component seed-based diets and to evaluate the effect of provision of additional fruit in an attempt to lower voluntary energy intake in parrots. First, seed diets are often stored in containers which are replenished before completely emptied, the birds always being fed from the upper layer. However, as a result of differences in particle size and density, ingredients in multi-component diets tend to segregate when stored, which likely results in a distinct ingredient profile of formulated and actually offered diet in the above-mentioned management conditions. Therefore, nutritional impact of segregation in a seed diet is quantitatively assessed. Second, the specific feeding behaviour of parrots, being selective consumption and dehusking of seeds, aggravates nutritional inadequacies inherent to seed diets. For instance, seed kernels contain higher amounts of phosphorus but lower amounts of calcium in relation to whole seeds, which further worsens the already suboptimal Ca:P ratio (Wolf *et al.*, 1998). Moreover, even if mineral and vitamin supplements in form of pellets or a calcium source such as oyster shells are added to a seed mixture, these are only effective if the birds actually consume them. Therefore, the second aim of the current study is to assess the impact of feeding behaviour on actual intake of ingredients and macronutrients when yellow-shouldered amazons (*Amazona barbadensis*) are fed a multi-component, pellet-enriched seed diet *ad libitum*. Third, as obesity is one of the most common health compromising conditions in parrots, the effect of additional fruit next to the seed diet on energy and nutrient intake is evaluated in these granivorous parrots. In lorikeets, which are nectarivorous parrots, Kalmar *et al.* (2009) demonstrated a significant reduction in voluntary intake of nectar when fruit was additionally provided. Daily energy intake showed a significant interaction between degree of dilution of the nectar and availability of fruit. Although not significant, availability of apple resulted in lower energy intake when added to low-dilution nectar but in higher energy intake when added to high-dilution nectar compared to feeding solely nectar of the respective degree of dilution (Kalmar *et al.*, 2009). In contrast to nectar, seed-based diets intended for granivorous parrots do not entail a possibility to contain less energy compared to supposedly energy-diluting supplements such as fruit.



## MATERIALS AND METHODS

### *Nutritional impact of segregation and dehusking*

A 100 g sub-sample of a seed mixture (Prestige Premium Large Parakeet Mix; Versele-Laga Ltd., Deinze, Belgium) was taken from the top layer of a feed storage container at the breeding centre of the Loro Parque Fundación in La Vera, Tenerife. All seed types were separated, weighed and relative proportions calculated in relation to the intended formulation. Next, nutrient contents of intended formulation and the sub-sample *in toto*, as well as its dehusked fraction were calculated. Nutrient contents of seeds *in toto* and seed kernels were provided by Versele-Laga Ltd. Energetic density was assessed using the formula of Schoemaker and Beynen (2001):

$$\text{Energy content (MJ ME kg}^{-1}\text{)} = 0.18 \text{ CP} + 0.39 \text{ EE} + 0.17 \text{ NfE}$$

Where: CP = crude protein (%), EE = ether extract (%) and NfE = N-free extract (%).

### *Feeding trial*

#### Animals and housing

Six 1-year-old yellow-shouldered amazons (*Amazona barbadensis*), four females and two males, with an average bodyweight of  $275 \pm 13$  g were individually housed in indoor wire cages with dimensions 55 cm x 55 cm x 55 cm (height x length x width) equipped with two perches ( $\varnothing$  2.5 cm) at different heights. As the birds originated from an equatorial area in which day length is 12 h year-round (Dilger, 1982), a natural diurnal cycle of 12 h light and 12 h dark was applied. Room temperature and relative humidity were  $20.6 \pm 0.7$  °C and  $70 \pm 3\%$ , respectively. The trial was performed at the breeding centre of the Loro Parque Fundación in La Vera, Tenerife.

#### Experimental design and measurements

The parrots were subjected to a 2 x 2 cross-over design with 8-day periods, in which two feeding strategies were tested. Fresh drinking water was available at all times and based on a pilot trial, the seed mixture was provided daily in amounts in which all components were available *ad libitum*. In strategy A, a daily amount of 175 g of a seed mixture (Amazone Mix, Versele-Laga Ltd.) was provided in two meals, at 8:00 am and 14:00 pm, whereas in strategy B, fruit (apple) was given at 8:00 am, and removed and replaced by an amount of 100 g of the seed mixture at 14:00 pm. The birds were weighed after an overnight fast at the beginning of

the trial and at the end of both feeding periods. Each feeding period consisted of a 4-day adaptation period followed by a 4-day period in which intakes of water, fruit and seeds were recorded per bird, per day. The birds were all acquainted with the seed mixture and the fruit as their normal ration. Daily intake of drinking water and fruit was corrected for evaporative losses. Intake of seeds per seed type was calculated based on the difference between offered and refused whole seeds. Then ingredient composition of offered and ingested seed mixture was calculated per bird per feeding strategy and expressed as seeds *in toto* per 100 g mixture *in toto*, and as seed kernels per 100 g dehusked mixture, respectively. Seeds and nuts were also classified into oilseeds and carbohydrate rich seeds based on their respective contents of ether extract (EE) and N-free extract (NfE). Herein, oilseed kernels contain more than 40% EE and less than 15% NfE, whereas kernels of carbohydrate or NfE-rich seeds contain less than 15% EE and more than 40% NfE. Finally, nutrient and energy intake were calculated based on the nutrient content of apple and seed kernels of the ingested fraction of the seed mixture. Nutrient content of ingredients were provided by Versele-Laga Ltd.

### Statistics

All data were pooled per bird, per diet and statistically analysed with bird as experimental unit. Normality and homoscedasticity were tested with the Kolmogorov-Smirnov and modified Levene test, respectively. Then, data were subject to the general linear model repeated measures analysis with diet as within-subject variable. All results are expressed as mean  $\pm$  standard deviation (SD) and significance was set at  $p < 0.05$ . All statistics were carried out in S-PLUS 8.0 (TIBCO Software, Palo Alto, CA, USA) and SPSS 16.0 (SPSS, Chicago, IL, USA).

## RESULTS

### *Nutritional impact of segregation and dehusking*

Segregation resulted in a distinct ingredient partition in relation to the intended formulation of the multi-component seed diet. The proportion of small particles, such as millet, canary seed and oyster shells, steeply decreased through segregation; whereas the proportion of the larger seeds, such as peeled oats and sunflower seed, sharply increased (**Table 1**). With this, the proportion of oilseeds increased from 24.0 to 33.5% and NfE-rich seeds decreased from 66.0 to 59.4%. Moreover, supplements in form of oyster shells and extruded pellets supplemented with vitamins, amino acids and minerals (VAM-pellets) decreased from 10.0 to 7.2%.

**Table 1.** Ingredient composition (%) of a formulated seed mixture before and after segregation as well as the relative difference between both samples (%).

	Formulated	Sub-sample <sup>1</sup>	Difference
Millet	38.0	28.5	- 25.0
Canary seed	10.0	6.4	- 36.0
Sunflower seed	11.0	17.4	+ 58.2
- striped	8.0	11.8	+ 47.5
- white	3.0	5.6	+ 86.7
VAM-pellets <sup>2</sup>	8.0	6.4	- 20.0
Peeled oats	7.0	11.2	+ 60.0
Cardy	6.0	8.4	+ 40.0
Buckwheat	5.0	6.6	+ 32.0
Hempseed	3.0	3.8	+ 26.7
Linseed	3.0	3.2	+ 6.7
Paddy rice	3.0	3.0	0.0
Wheat	3.0	3.8	+ 26.7
Oyster shells	2.0	0.8	- 60.0
Niger seed	1.0	0.8	- 20.0
Sum	100.0	100.3	

<sup>1</sup>100 g sub-sample of the top layer of a feeding container at the breeding centre of Loro Parque Fundación, Tenerife

<sup>2</sup>VAM-pellets: extruded pellets supplemented with vitamins, amino acids and minerals

Aside from a distinct ingredient profile, segregation vastly deteriorated the Ca:P ratio from 2.61 to 1.36. Macronutrients, in contrast, were only modestly affected: crude fibre (CF), EE and crude protein slightly increased at the expense of NfE. Dehusking of seeds further increased CP content from 14.2 to 18.5% and EE from 13.3 to 18.4%, whereas CF content decreased profoundly from 11.7 to 3.1% and NfE re-increased from 45.5 to 51.9%. Despite changes in nutrient contents by segregation, the energy content was fairly similar (+3%) in relation to the formulated mixture. However, the partitioning of energy shifted towards more EE-energy and less NfE-energy, while the CP-energy remained similar. Next, energetic density in the sub-sample would increase by 25% when seeds would be dehusked and the above mentioned shifts in energy partition of fat and carbohydrate would further extend (**Table 2**). Without supplementation of the seed mixture with oyster shells and VAM-pellets, the Ca:P ratio would be as low as 0.25 in the offered diet, segregation would slightly increase the Ca:P ratio to 0.30 and dehusking would result in a Ca:P ratio of merely 0.15.

**Table 2.** Effect of segregation and dehusking on nutrient content, Ca:P ratio and energy density in a multi-component seed diet as fed.

		Formulated mixture	Sub-sample	
			<i>In toto</i>	Dehusked
Crude Protein	(%)	13.9	14.2	18.5
	(% <sub>ME</sub> )	16.7	16.6	17.3
Ether Extract	(%)	10.9	13.3	18.4
	(% <sub>ME</sub> )	28.3	33.6	37.1
N-Free Extract	(%)	48.6	45.5	51.9
	(% <sub>ME</sub> )	55.0	49.9	45.6
Crude Fibre	(%)	9.8	11.7	3.1
Ca:P ratio		2.61	1.36	1.12
ME	(2)	15.03	15.49	19.32

<sup>1</sup>100 g sub-sample of the top layer of a feeding container at the breeding centre of the Loro Parque Fundación, Tenerife

<sup>2</sup>ME: metabolisable energy (MJ ME kg<sup>-0.75</sup>)

*Feeding trial*

All birds were clinically healthy and in able-bodied condition at the start of the trial. The birds remained healthy and bodyweight change was minimal and not significantly different between both feeding strategies. The birds ate a considerable amount of fruit of  $39.4 \pm 12.1 \text{ g kg}^{-0.75} \text{ d}^{-1}$  on fresh matter basis and consumed significantly less seed kernels when additional fruit was provided:  $30.1 \pm 5.4 \text{ g kg}^{-0.75} \text{ d}^{-1}$  compared to  $38.0 \pm 3.2 \text{ g kg}^{-0.75} \text{ d}^{-1}$ . On the other hand, daily intake of drinking water was not influenced by availability of fruit next to the seed mixture. Nevertheless, the combined intake from both drinking water and fruit moisture resulted in a significantly higher water intake in relation to feeding only seeds (**Table 3**).

**Table 3.** Effect of feeding strategy on bodyweight (BW) and daily intake of food and water in amazons fed either a seed mixture or a smaller amount of the mixture with fruit added *ad libitum* (n = 6; mean  $\pm$  SD).

		Only Seeds	Seeds & Fruit	<i>p</i> -value
BW change	(%)	0 $\pm$ 2	-1 $\pm$ 2	0.825
Seed intake				
seeds <i>in toto</i>	( $\text{g kg}^{-0.75} \text{ d}^{-1}$ )	54.7 $\pm$ 5.2	43.7 $\pm$ 8.1	0.004
seed kernels	( $\text{g kg}^{-0.75} \text{ d}^{-1}$ )	38.0 $\pm$ 3.2	30.4 $\pm$ 5.4	0.009
Fruit intake				
fresh matter	( $\text{g kg}^{-0.75} \text{ d}^{-1}$ )	-	39.4 $\pm$ 12.1	-
dry matter	( $\text{g kg}^{-0.75} \text{ d}^{-1}$ )	-	6.0 $\pm$ 1.8	-
Water intake				
Drinking	( $\text{ml kg}^{-0.75} \text{ d}^{-1}$ )	36.6 $\pm$ 7.2	29.3 $\pm$ 15.3	0.161
Fruit	( $\text{ml kg}^{-0.75} \text{ d}^{-1}$ )	-	33.4 $\pm$ 10.3	-
Sum	( $\text{ml kg}^{-0.75} \text{ d}^{-1}$ )	36.6 $\pm$ 7.2	62.7 $\pm$ 13.9	0.002

The daily amount of seed mixture was in both feeding strategies sufficiently high to allow *ad libitum* feeding of all separate ingredients. Seed selection resulted in a vastly distinct ingredient profile between offered and ingested mixture. Birds strongly preferred oilseeds above NfE-rich seeds. Despite the offered seed mixture containing 67% NfE-rich seeds and only 23% oilseeds, the self-chosen mixture out of the offered diet contained only  $14 \pm 5\%$  and  $19 \pm 6\%$  NfE-rich seeds, but  $82 \pm 7\%$  and  $78 \pm 5\%$  oilseeds in strategies A and B, respectively. Next, VAM-pellets were consumed by the birds, but in much smaller amounts in relation to their presence in the formulated mixture. Feeding strategy did not significantly alter preferences for oilseeds, NfE-rich seeds or VAM-pellets (**Table 4**).

**Table 4.** Proportion of NfE-rich seeds, oilseeds and VAM in offered and actually consumed seed mixture in amazons fed either the seed mixture at an amount of 175 g/d or at an amount of 100 g/d with fruit added *ad libitum* (n = 6; mean  $\pm$  SD).

		Offered mixture	Strategy A (only seeds)	Strategy B (seeds and fruit)	p-value
<u>NfE-rich seeds</u>					
<i>in toto</i>	(%)	67	$14 \pm 5$	$19 \pm 6$	0.180
kernels	(%)	71	$17 \pm 5$	$23 \pm 6$	0.132
<u>Oilseeds</u>					
<i>in toto</i>	(%)	23	$82 \pm 7$	$78 \pm 5$	0.324
kernels	(%)	17	$77 \pm 8$	$72 \pm 6$	0.281
<u>VAM-pellets</u>					
<i>in toto</i>	(%)	8	$2 \pm 2$	$1 \pm 1$	0.478
kernels	(%)	10	$2 \pm 3$	$1 \pm 2$	0.499

VAM-pellets: extruded pellets supplemented with vitamins, amino acids and minerals

Next to a profound difference in EE and NfE content, kernels of oilseeds were also richer in CP (>20%) in relation to kernels of NfE-rich seeds (<20%, except for canary seed: 23%) and were higher in energetic density. Proportion of husks was also higher in oilseeds, except for peeled peanuts. The Ca:P ratio was suboptimal in oilseed kernels ( $0.16 \pm 0.09$ ), as well as in NfE-rich seed kernels ( $0.22 \pm 0.38$ ). The worst Ca:P ratios were found in corn and buckwheat kernels, in which the Ca:P ratio was only 0.05 and 0.06, respectively. Finally, although the protein content was on average over twofold in oilseed kernels compared to NfE-rich seed kernels when expressed on weight basis, this difference was much smaller when protein supply was expressed on energy basis (**Table 5**).

**Table 5.** Proportion of husks in NfE-rich seeds and oilseeds, and nutrient content, Ca:P ratio, energetic density and nutrient content relative to energetic density in respective seed kernels.

		NfE-rich seeds <sup>1</sup> (n=11)		Oilseeds <sup>2</sup> (n=7)	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
Husks	(%)	10 $\pm$ 9	0 - 23	37 $\pm$ 20	0 - 57
CP	(%)	13 $\pm$ 4	8 - 23	29 $\pm$ 6	22 - 40
EE	(%)	4 $\pm$ 3	2 - 11	54 $\pm$ 6	46 - 59
NfE	(%)	72 $\pm$ 8	57 - 83	6 $\pm$ 3	2 - 11
ME	(MJ ME kg <sup>-1</sup> )	16.2 $\pm$ 1.3	14.5 - 18.5	27.1 $\pm$ 1.8	24.3 - 29.2
CP	(g MJ <sup>-1</sup> )	7.9 $\pm$ 2.0	5.1 - 12.4	10.7 $\pm$ 2.6	7.7 - 15.7
EE	(g MJ <sup>-1</sup> )	2.6 $\pm$ 1.6	1.0 - 5.9	19.8 $\pm$ 1.2	17.9 - 21.1
NFE	(g MJ <sup>-1</sup> )	44.5 $\pm$ 5.6	32.2 - 51.2	2.1 $\pm$ 1.1	0.7 - 4.1
Ca:P		0.22 $\pm$ 0.38	0.05 - 1.37	0.16 $\pm$ 0.09	0.02 - 0.26

EE: ether extract, NfE: N-free extract, CP: crude protein, ME: metabolisable energy

<sup>1</sup>oats, wheat, corn, buckwheat, paddy rice, milo, dari, canary seed, millet, popped wheat, popped corn

<sup>2</sup>hemp seed, white sunflower seed, striped sunflower seed, pumpkin seed, safflower, peeled peanuts, cedar nuts

Most types of NfE-rich seed had hardly been touched, whereas buckwheat and to a lesser degree corn and paddy rice were consumed in small amounts. Popped corn, however, had also hardly been eaten. Sunflower seed, pumpkin seed, safflower and peeled peanuts were the most preferred oilseeds. Interestingly, the parrots preferred striped sunflower seeds over white sunflower seeds or safflower. Within oilseeds, they did not show a clear preference depending on proportion of husks or EE, NfE or CP content of kernels. Finally, seed preferences were not largely different between birds and remained similar irrespective to the amount of offered seed mixture or availability of fruit. The only significant difference in proportional intake of individual seed types between both feeding strategies was noticed with peeled peanuts (Table 6).

**Table 6.** Ingredient profile (%) per seed type *in toto* of offered and actually consumed seed mixture in amazons fed either the seed mixture at an amount of 175 g/d or at an amount of 100 g/d with fruit added *ad libitum* (n = 6; mean  $\pm$  SD). Proportion of husks and ether extract (EE), N-free extract (NfE) and crude protein (CP) content of kernels per seed type are indicated with symbols.

	Offered mixture	Ingested fraction		Husks	EE kernel	NfE kernel	CP kernel
		Only seeds	Seeds & Fruit				
<b><u>NfE-rich seeds</u></b>							
Oats	12.2	0.3 $\pm$ 0.7	0.6 $\pm$ 0.6	-	--	++	-
Wheat	12.2	1.4 $\pm$ 1.1	0.7 $\pm$ 0.6	-	--	++	-
Milo/Dari	9.2	0.1 $\pm$ 0.1	1.1 $\pm$ 1.1	0	--	++	-
Canary seed/Millet	8.2	1.4 $\pm$ 3.3	0.4 $\pm$ 0.6	-	-/--	++	-
Corn	8.2	2.9 $\pm$ 0.7	3.2 $\pm$ 2.4	--	--	++	--
Buckwheat	8.2	5.2 $\pm$ 5.3	11.0 $\pm$ 7.8	-	--	++	-
Paddy rice	6.1	2.4 $\pm$ 1.6	1.5 $\pm$ 1.4	-	--	++	-
Popped wheat/Corn	3.1	0.2 $\pm$ 0.5	0.1 $\pm$ 0.2	--	--	++	--
<b><u>Oilseeds and nuts</u></b>							
Sunflower seed (striped)	6.1	28.4 $\pm$ 7.4	27.3 $\pm$ 2.0	+/-	++	--	+/-
Hempseed	3.1	4.3 $\pm$ 1.5	4.9 $\pm$ 3.0	+	+	--	+/-
Pumpkin seed	3.1	13.2 $\pm$ 6.4	14.8 $\pm$ 3.3	-	+	--	+
Safflower	3.1	10.1 $\pm$ 3.5	9.0 $\pm$ 2.0	++	++	--	+/-
Sunflower seed (white)	3.1	16.0 $\pm$ 3.2	14.4 $\pm$ 2.0	+	++	--	-
Peeled peanuts	2.0	9.4 $\pm$ 4.3 <sup>a</sup>	6.6 $\pm$ 3.5 <sup>b</sup>	0	+	-	+/-
Cedar nuts	2.0	0.3 $\pm$ 0.4	0.5 $\pm$ 0.7	+	+	--	-
<b><u>Other</u></b>							
VAM-pellets	8.2	1.7 $\pm$ 2.1	0.7 $\pm$ 1.2	0	-	+	+/-
Red peppers	1.0	0.7 $\pm$ 0.4	1.1 $\pm$ 1.1	0	-	+/-	-
Rose hip	1.0	2.1 $\pm$ 2.4	2.0 $\pm$ 0.4	0	0	+	--

<sup>a,b</sup> different letters in the same row indicate a significant difference at  $p < 0.05$

VAM-pellets: extruded pellets supplemented with vitamins, amino acids and minerals

Symbols: -- : < 10%, - : < 25%, +/- : 25 to 40%, + : > 40%, ++ : > 55%



Dehusking of seeds considerably increased caloric density of the offered mixture by 18% from 15.1 to 17.9 MJ ME kg<sup>-1</sup>. The combined effect of dehusking and seed selection increased caloric density to 24.7 ± 1.6 MJ ME kg<sup>-1</sup> and 24.0 ± 0.9 MJ ME kg<sup>-1</sup> in strategies A and B, respectively. Feeding behaviour also affected partition of energy. More specifically, energy derived from EE vastly increased at the expense of energy derived from NfE, whereas CP-energy only slightly increased. However, neither the amount of offered seed mixture, nor provision of fruit had a significant effect on caloric density or on energy partition in the actually ingested fraction of the seed mixture. Nevertheless, as provision of fruit resulted in a significantly lower seed intake, total energy intake decreased significantly by 13.6% when fruit was available next to the seed mixture. Provision of fruit also significantly increased NfE-energy and significantly lowered EE and CP-energy (**Table 7**).

**Table 7.** Caloric density and distribution in offered and ingested diet, and daily energy intake of amazons when fed either a seed mixture at an amount of 175 g d<sup>-1</sup> or at an amount of 100 g d<sup>-1</sup> with fruit added *ad libitum* (n = 6; mean ± SD).

		Offered Mixture		Strategy A	Strategy B		
		In toto	Kernels	Seeds	Total	Seeds	Fruit
ME <sup>1</sup> (as fed)	(1)	15.1	17.9	24.7 ± 1.6 <sup>a</sup>	14.4 ± 1.6 <sup>b</sup>	24.0 ± 0.9	2.1
Energy intake	(2)	-	-	956 ± 71 <sup>ax</sup>	809 ± 109 <sup>b</sup>	728 ± 115 <sup>y</sup>	82 ± 25
Caloric distribution							
CP <sub>energy</sub>	(%ME)	15.7	16.8	18.6 ± 1.0 <sup>a</sup>	13.7 ± 1.2 <sup>b</sup>	18.3 ± 0.3	3.5
EE <sub>energy</sub>	(%ME)	26.6	29.1	67.4 ± 5.3 <sup>a</sup>	46.9 ± 2.8 <sup>b</sup>	63.9 ± 4.8	6.5
NfE <sub>energy</sub>	(%ME)	57.7	54.0	14.0 ± 4.4 <sup>a</sup>	39.4 ± 3.6 <sup>b</sup>	17.8 ± 4.9	90.0

<sup>a,b</sup> different letters in the same row indicate a significant effect on total ration at  $p < 0.05$

<sup>x,y</sup> different letters in the same row indicate a significant effect on seeds at  $p < 0.05$

(<sup>1</sup>) ME: metabolisable energy (MJ ME kg<sup>-1</sup>), (<sup>2</sup>) kJ ME kg<sup>-0.75</sup> d<sup>-1</sup>

CP: crude protein, EE: ether extract, NfE: nitrogen free extract

Crude fibre content was lowest in fruit with 2.6% CF on an as fed basis, whereas seeds *in toto* contained 10.4% CF, seed kernels 3.1% CF and the ingested fraction of the seed mixture 4.4 ± 0.3% CF and 4.3 ± 0.5% CF in strategies A and B, respectively. Nevertheless, CF content relatively to energetic density was by far higher in apple in relation to ingested seed kernels: 12.6 g MJ<sup>-1</sup> in apple, compared to 1.8 g MJ<sup>-1</sup> in ingested seed kernels.

Furthermore, despite a significant decrease in CF content of total ration as fed, availability of fruit tended to increase daily intake of CF. Next, provision of fruit supplementary to the seed mixture resulted in a significantly lower intake of CP and EE, but in a significantly higher intake of NfE. Finally, the Ca:P ratio was suboptimal in the offered seed mixture despite inclusion of VAM-pellets, and was further aggravated both by dehusking and seed selection. However, if not supplemented, the Ca:P ratio would have lowered more steeply from 0.23 to 0.14 through dehusking, entailing a decline by 39% instead of a 12% decline from 0.43 to 0.38 in the supplemented mixture (**Table 8**). Calcium and phosphorous content on dry matter basis were 0.19% Ca and 0.43% P in the mixture *in toto*, and 0.16% Ca and 0.42% P in the offered kernels. The ingested kernels, however, contained  $0.12 \pm 0.02\%$  Ca and  $0.56 \pm 0.01\%$  P when fed only the seed mixture and  $0.10 \pm 0.01\%$  Ca and  $0.56 \pm 0.01\%$  P when fed the same seed mixture added with fruit.

**Table 8:** Nutrient content (%) and Ca:P ratio of offered and ingested diet (as fed) and daily nutrient intake ( $\text{g kg}^{-0.75} \text{d}^{-1}$ ) of amazons when fed either a seed mixture at an amount of 175 g/d or at an amount of 100 g/d with fruit added *ad libitum* (n = 6; mean  $\pm$  SD).

	Offered Mixture		Strategy A	Strategy B		
	In toto	Kernels	Seeds	Total	Seeds	Fruit
Nutrient Content						
CP	13.2	16.7	$25.4 \pm 0.6^a$	$11.0 \pm 2.1^b$	$24.5 \pm 1.1$	0.4
EE	10.3	13.3	$42.9 \pm 6.1^a$	$17.3 \pm 2.7^b$	$39.4 \pm 4.3$	0.3
NfE	51.1	56.8	$20.0 \pm 5.1$	$17.6 \pm 4.0$	$24.9 \pm 5.7$	11.1
CF	10.4	3.1	$4.4 \pm 0.3^a$	$3.3 \pm 0.1^b$	$4.3 \pm 0.5$	2.6
Daily nutrient Intake						
CP	-	-	$13.9 \pm 1.3^{ax}$	$10.8 \pm 1.7^b$	$10.6 \pm 1.7^y$	$0.2 \pm 0.0$
EE	-	-	$23.5 \pm 3.9^{ax}$	$17.2 \pm 2.6^b$	$17.0 \pm 2.6^y$	$0.1 \pm 0.0$
NFE	-	-	$10.9 \pm 2.9^a$	$15.5 \pm 3.5^b$	$11.2 \pm 4.3$	$4.3 \pm 1.3$
CF	-	-	$2.4 \pm 0.3^{ax}$	$2.8 \pm 0.4^a$	$1.8 \pm 0.2^y$	$1.0 \pm 0.3$
Ca:P ratio	0.43	0.38	$0.21 \pm 0.04^{ax}$	$0.19 \pm 0.02^a$	$0.18 \pm 0.02^y$	0.63

<sup>a,b</sup> different letters in the same row indicate a significant effect on total ration at  $p < 0.05$

<sup>x,y</sup> different letters in the same row indicate a significant effect on seeds at  $p < 0.05$

<sup>aa</sup> differently underscored letters in the same row indicate a tendency at  $0.100 < p < 0.05$

CP: crude protein, EE: ether extract, NfE: N-free extract, CF: crude fibre

## DISCUSSION

Despite incontestable data from the literature revealing seed mixtures to be incomplete diets for psittacine birds, parrots are still commonly fed seed diets, both by bird fanciers and in ornithological collections. Also, whereas seed-based diets can be fortified with oyster shells or VAM-pellets, this attempt to abate deficiencies and imbalances inherent to seeds is only effective when the birds actually consume these supplements (Hawley, 1977). The current data revealed three interfering factors that even further deteriorated adequate nutrient supply: segregation, seed dehusking and selective feeding.

First, diets are often stored in closed containers instead of in the original bags, these containers being regularly replenished before completely emptied. Animal caretakers scoop feed from the container, feeding the upper layer to the birds. However, seeds tend to segregate according to size and density when stored: small seeds settle to the bottom, while larger seeds remain on top. The first trial demonstrates the detrimental effect of segregation on the Ca:P ratio. The original mixture was supplemented with oyster shells and VAM-pellets, hence the Ca:P ratio was increased from 0.25 to 2.61, which is abundantly above the recommended ratio of 1.5:1 described by Wolf (2002). However, segregation resulted in a relative decrease in oyster-shells and VAM-pellets by 60% and 20%, respectively, in relation to the formulated mixture. This resulted in a decline in the Ca:P ratio to 1.36, which is below the recommended ratio. It should be noted that the current trial did only sampled the upper layer of a feeding container, and did not involve sampling of lower layers.

In addition to effects of storage of multi-component seed diets, parrots dehusk seeds and nuts prior to ingestion, consuming only the kernel. With an unselective ingestion of seed kernels, the unequal distribution of calcium and phosphorus in kernels and husks would further deteriorate the Ca:P ratio to 1.12, which corresponds to a decline by 18%. In adult birds under maintenance condition, internal mechanisms may conceal this reversed mineral balance between calcium and phosphorus when fed seed-based diets for a long period of time. Still, clinical signs will eventually develop, although these are often unspecific and difficult to diagnose (Wolf *et al.*, 1998). The effect of dehusking on the Ca:P ratio would be of even greater extent if the seed mixture was not supplemented with calcium sources, in which case the Ca:P ratio would decline by 50% from 0.30 to 0.15. The lesser extent of effect in the supplemented diet is the result of a relative increase in supplements through dehusking, which diminishes the effect of unequal distribution in kernels and husks.

Segregation did not result in a vast change in energetic density of the diet, but the increase of large oilseeds and decrease of small NfE-rich seeds altered caloric distribution towards a higher proportion of EE-energy on account of NfE-energy, CP-energy remaining similar. Seed dehusking and unselective consumption of kernels, on the contrary, would greatly increase caloric density and further shift caloric distribution towards EE-energy. Because of lack of repetitions, the described nutritional effects of segregation in stored multi-component seed diets are only to be interpreted as an indication of magnitude. However, these data strongly suggest that improper management of multi-component diets may result in a difference between the chemical composition of the manufacturer's formulation and the actually offered ration.

An additional source of dissimilarity between intended and actually consumed ration includes the profound selective feeding behaviour of parrots, next to dehusking of seeds and consumption of only the seed kernels. The current trial clearly demonstrated the strong preference for oilseeds above NfE-rich seeds in amazons, whereas NfE-rich seeds were predominant in the offered mixture. Furthermore, VAM-pellets were ingested in a much lower proportion in relation to their inclusion in the formulated mixture. However, neither amount and timespan of offered food, nor availability of fruit, notably affected selective feeding of separate ingredients when amounts permitted *ad libitum* feeding. Despite the relatively poor sense of taste in birds compared to mammals, taste still might have at least in part directed selective intake. However, psittacine birds are thought to rather select food items based on size, shape, texture and colour, than on taste (Ullrey *et al.*, 1991; Kollias, 1995). The identification and selection of edible compounds is hereby facilitated by the tactile bill-tip organ, which is present in parrots and many other birds (Gottschaltdt, 1985).

As previously mentioned, calcium and phosphorus are unequally distributed in kernels and husks, resulting in an aggravation of an already inverse Ca:P ratio in seeds when consuming only seed kernels. Notwithstanding inclusion of VAM-pellets, the offered seed mixture already showed a suboptimal Ca:P ratio as for practical reasons concomitant inclusion of oyster shells was omitted in this diet. Nevertheless, in agreement with the first trial, inclusion of mineral supplements in the seed-based diet resulted in a more blunted decline of the Ca:P ratio through dehusking, in relation to the unsupplemented mixture. In addition to the suboptimal Ca:P ratio, the calcium content of the ingested fraction as well was below the minimum requirements for maintenance in pet birds, which is 0.15% Ca on a dry matter basis,

whereas the phosphorous content was over fivefold the minimum requirements (Wolf *et al.*, 1998).

Furthermore, caloric density in the edible portion of the offered diet was 19% higher than expected based on total analysis of the ration, which includes the husks that are not ingested by parrots. Moreover, selective feeding behaviour further increased caloric density of consumed fraction of the seed diet by 60% when fed seeds and fruit, and by 64% when fed only seeds, in relation to the offered mixture *in toto*. Next, both seed dehusking and selective feeding resulted in a higher protein and, more pronouncedly, in a higher fat content. Carbohydrate content increased as a result of dehusking, but vastly decreased by seed selection. Finally, seed dehusking strongly lowered CF content.

A decrease in voluntary energy intake, in an attempt to abate obesity, is difficult to accomplish in parrots as these birds are - or definitely should be - housed in pairs or groups (Kalmar *et al.*, 2010). Therefore, a limited amount of offered feed would only result in restricted feeding or even emaciation of the weakest birds, the most dominant birds still feeding *ad libitum*. Moreover, when feeding multi-component diets, the most dominant birds will still be able to feed on the preferred food items, leaving the less desirable items for the others. Restricting the amount of food, but placing several food bowls in the aviary is also complicated in these highly intelligent birds, as illustrated by following anecdotal observation. In an aviary with macaws, several food bowls were placed at distinct locations in an attempt to diminish competition for food. However, three dominant macaws worked in shifts to alternately protect and eat from the food bowls. Two birds chased off the other macaws, whilst the third bird could undisturbedly select preferred food items until, in this case, all nuts and large oilseeds were finished.

Current data show that provision of fruit, next to *ad libitum* feeding of seeds, considerably reduced voluntary energy intake from  $936 \pm 71$  to  $809 \pm 109$  kJ ME kg<sup>-0.75</sup> d<sup>-1</sup>. The observed reduction in voluntary energy intake could be explained by a saturation effect of CF or the low-energetic, watery volume of apples. Apple provides for sevenfold as much CF per MJ ingested ME compared to seed kernels. However, apple contains less CF when expressed on weight basis. But even though consumption of drinking water was not affected by availability of fruit, birds ingested more fluid and thus more volume when fruit was available. Therefore, dilution of energetic density of seed kernels through additional intake of moisture-rich fruit

might have predominantly affected voluntary energy intake, CF supply being of lesser importance. A third explanation of lower energy intake in the amazons when fed apple next to seeds is the shorter amount of time the parrots were allowed to eat from the energy dense seed mixture. Still, with this feeding strategy the birds did not compensate energy intake through increased seed consumption in the afternoon.

Although feeding fruit next to the seed mixture significantly reduced daily protein intake, protein supply still remained well above minimum requirements for adult amazons in maintenance conditions, which is  $1.9 \text{ g kg}^{-0.75} \text{ d}^{-1}$  with the prerequisite of a balanced amino acid profile (Westfahl *et al.*, 2008). As daily protein intake of amazons in the current trial was  $13.9 \pm 1.3 \text{ g kg}^{-0.75} \text{ d}^{-1}$  when fed seeds, and  $10.8 \pm 1.7 \text{ g kg}^{-0.75} \text{ d}^{-1}$  when fed seeds and fruit, minimal amino acid requirements will have been fulfilled, even though certain amino acids, such as lysine and methionine, might be limited in seed kernels. Yet, protein requirements might be increased when feeding a high energy diet in which most of the energy is derived from EE, as is when feeding a seed-based diet. This can be hypothesized based on the critical role of amino acids as anaplerotic intermediates in the citric acid cycle, permitting the continued function of the latter (Owen *et al.*, 2002).

In conclusion, notwithstanding efforts of nutritionists to formulate seed-based diets to approximate estimated species-specific requirements, both management and animal factors contribute to a vastly distinct nutrient composition between formulated and actually consumed fraction. Therefore, it should be advised that psittacine diets that contain whole seeds and nuts are formulated to meet the available nutritional guidelines according to the nutrient content of the kernels instead of on the ingredients *in toto*. Hence, nutritional effects of dehusking are accounted for. Furthermore, the label of such diets should ideally provide the nutrient content of the dehusked, consumable fraction, next to the obligatory indicative values of the total diet. In addition, although not specifically studied in this thesis, incorporation of larger-sized supplements and regular turning of storage containers might reduce the adverse effects of segregation in multi-component diets. As parrots show a much higher preference for over-sized pellets (Rozek *et al.*, 2010; Rozek and Millam, 2011), increasing the size of supplements might also beneficially affect the intake of essential nutrients.

The most unfavourable consequence of segregation is a negating effect on added supplements intended to rectify deficiencies or imbalances inherent to seeds. Although the Ca:P ratio of the formulated diet was well above recommended guidelines, it was demonstrated that the proportion of calcium sources in form of oyster shells and VAM-pellets profoundly decreased through segregation, which resulted in a deterioration of the Ca:P ratio. Selective feeding, on the other hand, is peculiar to parrots and only avoidable when feeding monocomponent diets such as pellets. The current trial demonstrated the profound preference of parrots for oilseeds, resulting in a vast increase in EE and energy content of the actual consumed diet compared to the offered mixture. However, independently to the amount of offered seed diet, parrots also consume a limited fraction of NfE-rich seeds next to oilseeds, but in inverse proportion to usual formulations. The most detrimental nutritional imbalance of the seed mixture was the suboptimal Ca:P ratio, which was further deteriorated as a result of seed dehusking and selective feeding. Finally, voluntary energy intake in granivorous parrots can be effectively lowered by provision of fruit next to a seed diet, without compromising adequate protein intake.

#### **ACKNOWLEDGEMENTS**

An Cools is kindly acknowledged for her constructive contribution to this manuscript.

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# CHAPTER 5

## **Apparent nutrient digestibility and excreta quality in African grey parrots fed two pelleted diets based on coarsely or finely ground ingredients**

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*Modified from*

**Journal of Animal Physiology and Animal Nutrition (2007)**

**Volume 91, 210-216**

## **ABSTRACT**

*A feeding trial was performed to study the influence of particle size in extruded parrot pellets on apparent digestibility, excreta consistency and excreta pH-value. Two test diets were alternately provided to eight African grey parrots according to a 2 x 2 cross-over design. Both diets were similar in nutrient content and ingredient composition but differed in particle size of the composing particles of individual pellets. Apparent digestibility of macronutrients was studied using the total collection method. Next, the appearance of the excreta was studied by calculation of surface area to weight ratio of individual excrements as an objective measurement of consistency. Last, excreta pH was measured directly on fresh excrements and on homogenized 10% excreta solutions. Neither apparent digestibility coefficients nor excreta pH values were significantly different between both pellet diets. However, excreta consistency was significantly ( $p < 0.05$ ) more solid when fed the coarse diet than when fed the finely ground diet. The results of this study suggest that excreta consistency can be improved through larger pellet particle size, without adverse effects on nutritive value of the diet.*

## INTRODUCTION

Despite evidence of pronounced nutritional imbalances and deficiencies inherent to seed diets, captive parrots are still commonly fed such diets. Major concerns include an extremely low calcium:phosphorus ratio and content of vitamin A precursors in the edible part of seeds (Forbes and Altman, 1998; Wolf, 2002). Pelleted diets offer the advantage that they can be formulated to meet the energy and nutrient requirements according to available guidelines.

Of course, nutritionally well-balanced diets are effective only when bird owners actually decide to provide these to their birds. Therefore, nutritive value is not the only issue in formulated diets, but factors influencing the owners' willingness to purchase the feed also have to be considered. Bird owners often perceive that excreta are less firm when parrots are fed pellets. A lower firmness of excreta is the result of either higher excreta moisture content or a lower water holding capacity of the excreta. Two feed characteristics, which are highly different between seed diets and pellets, can be proposed to contribute to this observed difference in excreta consistency: sodium content and particle size of ingredients. Sodium content is, in general, much lower in seed diets [ $0.054 \pm 0.24\%$  Na in kernels on dry matter basis) compared to pelleted diets ( $0.321 \pm 0.19\%$  Na on dry matter basis] (Wolf *et al.*, 1997). This might explain less firm excreta when fed pelleted diets due to an increase in excreta moisture content, as demonstrated by Smith *et al.* (2000) in laying hens. Next, particle size reduction has been shown to decrease water holding capacity of excreta in broiler chickens (Yasar, 2003). Our hypothesis is that a different grinding level of pellet ingredients can be used to improve excreta consistency in parrots fed pelleted diets. Another physical trait of excreta is the pH-value. A low intestinal pH in the cranial part of the digestive tract can contribute to protection against the acid-intolerant gram-negative pathogens such as *Salmonella* spp. (e.g. pigs: Naughton and Jensen, 2001), therefore excreta pH might also be an indicator of clinical importance. In the author's experience, psittacine excreta have pH-values of 6 when fed seed diets whereas this is 8 when fed pelleted diets (unpublished data). The present trial was aimed at investigating the influence of particle size in pelleted parrot feed on feed and water intake, apparent digestibility coefficients, excreta consistency and pH in African grey parrots under maintenance conditions.

## MATERIAL AND METHODS

### *Animals and housing*

Four female and four male 3-year-old African grey parrots (*Psittacus erithacus erithacus*), with an average bodyweight of  $490 \pm 21$  g, were housed individually in wire metal cages (mesh size 2 x 2 cm, Ø 1 mm). Feed and water were provided *ad libitum* in inox food bowls, which were easily attached to and detached from a semicircular rotating platform located at the front of the cage. The inner dimensions of the cages were 0.70 m long, 0.75 m wide and 1 m high, which resulted in a space of 0.525 m<sup>3</sup>. Two metal perches (Ø 3 cm) were placed horizontally at 0.40 and 0.65 m height. The bottom of the cage consisted of a removable, metal drawer with a depth of 3.5 cm, which facilitated collection of excreta and feed refusals as well as perpendicular photographing of excrements with minimal disturbance to the birds. Temperature fluctuated between 19.4 °C and 20.9 °C. Relative humidity ranged from 37.9% to 46.5%. The photoperiod was determined by outdoor sunlight, which consisted approximately of 14.5 h of light a day. Efforts were made to relieve boredom by providing an acrylic, commercially available bird toy to each parrot and placing a timer controlled radio inside the animal house.

### *Diets*

The experimental diets were similar in metabolisable energy, nutrient composition and mineral content (**Table 1**), but differed in grinding level of ingredients. The ingredients of both diets were in decreasing order: cereals, seeds (minimum 10% groundnut kernels), fruit (minimum 5% fresh fruit), vegetable protein extracts, derivatives of vegetable origin, sugars, minerals, l-lysine, methionine, yucca schidigera extracts, fructo-oligosaccharides, vitamins and trace elements.

Metabolisable energy (ME) was calculated according to the equation of Schoemaker and Beynen (2001) (Equation [1]):

$$\text{ME (MJ ME kg}^{-1}\text{)} = 0.18 \text{ CP} + 0.39 \text{ EE} + 0.17 \text{ NfE} \quad [1]$$

Where: CP = crude protein (%)

EE = ether extract (%)

NfE = nitrogen free extract (%)

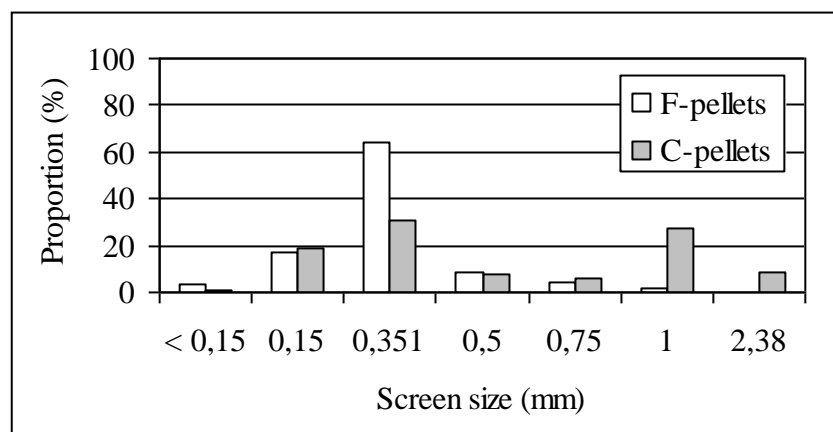
**Table 1.** Nutrient composition (%) and energy content (MJ ME kg<sup>-1</sup>) of the test diets (on fresh matter basis).

	F-pellets	C-pellets
Crude protein	15.6	15.4
Ether extract	14.8	14.6
Crude Fibre	3.5	3.5
Crude ash	4.6	4.2
N-Free Extract	53.3	55.7
Sodium	0.16	0.16
Potassium	0.52	0.52
Ca:P	1.5	1.5
Metabolizable energy	17.6	17.9

F-pellets : fine particle size pellets

C-pellets : coarse particle size pellets.

Particle size profiles of feed components of both diets were gathered prior to extrusion (**Figure 1**). This was performed by weighing the remainders after sieving the ground ingredients on a series of seven successive sieves, decreasing in screen size. In the following, the diets will be referred to as F-pellets (fine) and C-pellets (coarse).

**Figure 1.** Particle size distribution of the ground ingredients for both test diets (F = fine particle size, C = coarse particle size).

*Experimental design*

The birds were divided into two groups of four individuals each, to which both diets were provided according to a cross-over design with feeding periods of 9 days. Each experimental period started with a 4-day adaptation period, followed by a 4-day collection period in which consistency of excrements was assessed prior to total collection of excreta. Additionally, feed and water intake was determined to compute apparent nutrient digestibility coefficients. The excreta were pooled per bird per diet and weighed (precision 0.1 g), frozen (-20 °C), freeze-dried, homogenized in a commercial blender and stored until proximate analysis. Finally, excreta pH was measured the day after the 4-day collection period.

Feed and water intake

Water and feed intake were calculated with an accuracy of 1 and 0.1 g, respectively, using Equations [2] and [3]:

$$W_i = W_g - W_r - F_w + W_e \quad [2]$$

$$F_i = F_g - F_r - F_w; \quad [3]$$

Where:  $W_i$  and  $F_i$  are daily water and feed intake

$W_g$  and  $F_g$  are water and feed provided

$W_e$  is daily amount of evaporation from the water bowls

$F_w$  is dried amount of feed spilled in the water bowls

Apparent digestibility coefficients

Proximate analysis was performed on homogenised feed and excrement samples according to standard methods of the AOAC (1980). Next, freeze dried excreta samples were analysed spectrophotometrically to determine uric acid content according to Terpstra and De Hart (1974). Uric acid derived nitrogen (N), which constitutes one third of the uric acid content, was subtracted from the excreta N content as determined by Kjeldahl analysis. In addition, uric acid content was subtracted from excreta dry matter (DM) content.



Apparent digestibility coefficients (aD<sub>X</sub>) of DM, organic matter (OM), crude protein (CP), ether extract (EE) and nitrogen free extract (NfE) were calculated using the total collection method as used in Sales and Janssens (2004) (Equation [4]):

$$aD_X = 100 \times \frac{F_i * [X]_f - E * [X]_e}{F_i * [X]_f} \quad [4]$$

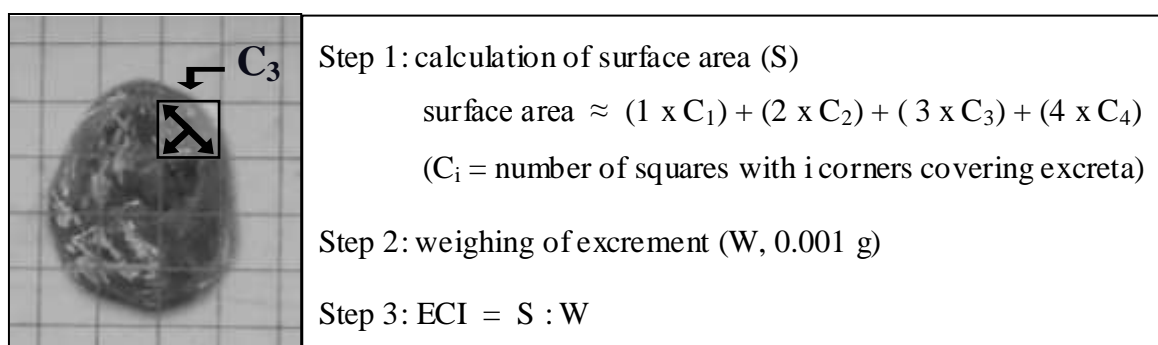
Where: aD<sub>X</sub> is the apparent nutrient digestibility coefficient of nutrient X

[X]<sub>f</sub> and [X]<sub>e</sub> are nutrient concentration in feed and excreta

F<sub>i</sub> and E are feed intake and excreta production

### Excreta consistency

Consistency of excreta was objectively evaluated by calculation of surface area to weight ratio. A lower score of the resulting excreta consistency index (ECI) indicates greater firmness of the dropping. This method was used to evaluate at least five excrements per bird, per diet. To determine surface area, digital photographs were taken perpendicular to a grid with mesh size 7 x 7 mm, placed over fresh excrements. Excrements were photographed and weighed within 15 min after defecation with the intention to minimise weight decrease through moisture evaporation. In addition, only excrements underneath the highest perch were considered to ensure a fixed distance between defecation point and cage bottom, excluding influences on surface area that do not originate from differences in excreta consistency. The surface area of excrements was estimated through manual analysis of enlarged photographs on a computer screen. For this, summation of grid corners covering the excrement was used as a proximate measure (**Figure 2**).



**Figure 2.** Measurement of excreta consistency index (ECI). Calculation of excrement surface area was done through summation of grid corners covering the excrement. The marked square covers the excrement with 3 of its corners (C<sub>3</sub>).

### Excreta pH

Excreta pH was measured electrochemically using a digital glass electrode probe (accuracy 0.1). The first measurement was done directly on fresh excrements within 15 min after defecation. Each of the excrements was diluted to 10% with distilled water, homogenized with a small, rod-shaped mixer and a second measurement was carried out. At least three excrements per bird per diet were examined this way.

### *Statistics*

All data were pooled per bird per diet and effects were statistically evaluated using the paired t-test in Microsoft Excel 2003 (Microsoft Corporation, Redmont, WA, USA). A value of 0.05 ( $p < 0.05$ ) was considered to be significant. All results in this study are expressed as mean  $\pm$  standard deviation.

## **RESULTS**

Tables 2 and 3 provide a summary of the results. Water and feed intake were not significantly altered by the particle size of the test diets. No significant effects were observed on apparent digestibility coefficients of dry matter, crude protein, ether extract or nitrogen free extract (**Table 2**).

**Table 2.** Feed intake ( $\text{g kg}^{-0.75} \text{d}^{-1}$ ), water intake ( $\text{ml kg}^{-0.75} \text{d}^{-1}$ ) and apparent nutrient digestibility (%) of African grey parrots fed fine (F) and coarse (C) particle size pellets (mean  $\pm$  SD; n = 8).

	F-pellets	C-pellets	<i>p</i> -value
Food intake	49.3 $\pm$ 5.5	50.3 $\pm$ 8.8	0.790
Water intake	104.6 $\pm$ 25.1	118.0 $\pm$ 35.4	0.212
aD_DM	75.2 $\pm$ 3.1	75.0 $\pm$ 3.0	0.912
aD_CP	50.4 $\pm$ 6.0	49.0 $\pm$ 6.1	0.719
aD_EE	94.3 $\pm$ 1.1	94.2 $\pm$ 1.1	0.932
aD_NfE	84.8 $\pm$ 2.3	84.7 $\pm$ 2.7	0.958

F: fine particle size, C: coarse particle size

aD\_X: apparent digestibility coefficient of nutrient X DM: dry matter, CP: crude protein, EE: ether extract, NfE: nitrogen-free extract

Daily wet excreta output and wet excreta output expressed per gram of ingested diet were not affected by pellet particle size. Moisture content of the excreta was also not changed by diet type. However, ECI was significantly lower when fed the coarse pellets compared to the fine pellets, which represents firmer excreta when fed coarse particle pellets. Yet, consistency of excreta was not correlated to excreta moisture content ( $p > 0.05$ ).

Finally, the test diets did not induce differences in excreta pH. Moreover, pH directly measured on fresh excreta ( $\text{pH}_1$ ) was not different from measurement after homogenization in a 10% solution ( $\text{pH}_2$ ). The variability in measurement was also independent of the applied protocol (**Table 3**).

**Table 3.** Excreta characteristics of African grey parrots fed fine (F) and coarse (C) particle size pellets (mean  $\pm$  SD;  $n = 8$ ).

	F-pellets	C-pellets	<i>p</i> -value
Wet faecal output ( $\text{g d}^{-1}$ )	27.1 $\pm$ 6.2	30.2 $\pm$ 7.1	0.269
Wet faeces: food intake ( $\text{g g}^{-1}$ )	0.9 $\pm$ 0.2	1.0 $\pm$ 0.2	0.077
Faecal water content (%)	71.4 $\pm$ 2.8	72.8 $\pm$ 3.9	0.445
Excreta consistency index	35 $\pm$ 8	23 $\pm$ 4	0.009
Faecal $\text{pH}_1$	8.2 $\pm$ 0.1	8.2 $\pm$ 0.1	0.383
Faecal $\text{pH}_2$	8.2 $\pm$ 0.1	8.2 $\pm$ 0.1	0.456

F: fine particle size, C; coarse particle size

$\text{pH}_1$  : pH-level measured in fresh excreta

$\text{pH}_2$  : pH-level measured after homogenization in a 10% solution

## DISCUSSION

Ingested pellets disintegrate at crop level, the resulting particles continuing down the digestive tract (Nir *et al.*, 1995). Hence, particles in pellets can be considered to constitute the actual feed particle size. The impact of particle size can be classified into long-term and short-term effects. The latter include the impact on intestinal motility and efficiency of enzymatic digestion and absorption, whereas the former include effects on the alimentary tract itself (see below; Nir *et al.*, 1994; Carré, 2000).

The present study examined short-term effects of pellet particle size in African grey parrots. Feed and water intake were similar for both diets, as were the apparent digestibility coefficients. The current results on apparent organic matter digestibility, being  $77.6 \pm 2.8\%$  and  $77.8 \pm 2.8\%$ , respectively, when fed fine and coarse pellets are in accordance with data of Graubohm (1998) in which apparent organic matter digestibility of pellets varied between 75.2% and 81.8% in African grey parrots. Next, although variability in apparent protein digestibility was similar to that in other trials, absolute figures were lower. The reason for this remains unclear. A study of Cornejo and Wolf (2005), for instance, in which purple-bellied parrots (*Triclarina malachitacea*) were housed in aviaries and fed a multi-compound diet containing fruits, vegetables, small seeds and pellets, resulted in an average apparent protein digestibility of  $78.4 \pm 5.7\%$ . Whilst apparent protein digestibility in our study was only  $50.4 \pm 6.0\%$  and  $49.0 \pm 6.1\%$  in F-pellets and C-pellets, respectively.

The absence of effects of particle size on apparent digestibility coefficients in the current study is in contrast with studies performed on poultry. Data of Carré and Melcion (1995) showed a higher apparent protein digestibility when chickens were fed the fraction of ground peas remaining on a 3-mm sieve than when fed the fraction on a 0.5-mm sieve. Crevieu *et al.* (1997) and Yasar (2003) reported an increase in pea and wheat protein digestibility, respectively, in broilers when fed a coarsely ground mash compared to a finely ground mash. In contrast, Peron *et al.* (2005) observed no significant effect of wheat particle size for protein and lipid digestibility values in chickens. In addition, Carré *et al.* (1998) demonstrated improved starch digestibility when peas were finely ground. Broilers fed a finely ground corn mash showed better feed conversion ratios than when fed a coarsely ground corn mash (Lott *et al.*, 1992). Finally, a study of Nir *et al.* (1994) resulted in better broiler performances when chickens were fed a mash intermediate in texture, independent of grain source being corn, wheat or sorghum (**Table 4**).

**Table 4.** Effect of grinding level of single ingredient diets in poultry

Grain	parameter	Effect of particle size	Reference
Pea	aD_CP	Coarse > Fine	Carré and Melcion (1995)
Pea	aD_CP	Coarse > Fine	Creveu et al. (1997)
Pea	aD_St	Coarse < Fine	Carré et al. (1998)
Wheat	Performance	Medium > Fine / Coarse	Nir et al. (1994)
Wheat	aD_CP	Coarse > Fine	Yasar (2003)
Wheat	aD_CP, aD_EE	No significant effect	Peron et al. (2005)
Corn	FCR	Coarse > Fine	Lott et al. (1992)
Corn	Performance	Medium > Fine / Coarse	Nir et al. (1994)
Sorghum	Performance	Medium > Fine / Coarse	Nir et al. (1994)

aD\_X: apparent digestibility coefficient of nutrient X

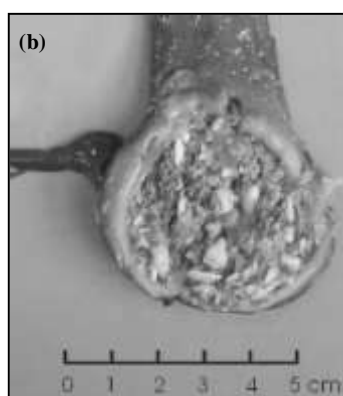
CP: crude protein, St: starch, EE: ether extract, FCR: feed conversion ratio

Impact of feed particle size on apparent digestibility coefficients can be explained by its influence on passage rate through the alimentary tract, which in turn results in altered digestion and absorption times of nutrients. Hence, a reduced action time of gizzard pepsins, resulting from a shorter transit time in the gizzard, might explain the lower bio-availability of protein observed in chickens fed finely ground feedstuffs (Svihus *et al.*, 2002). According to Carré (2000), the observed impact on protein digestibility could also originate from a lower ability of a less active gizzard to regulate intestinal transit. The lack of a significant impact on digestibility in the present investigation, in contrast to the majority of the poultry studies discussed above, could be resulting from factors related to feed, as well as animal factors.

Concerning feed factors, differences in ingredient composition of test diets might have contributed to the distinct results in other studies. First, the reviewed poultry studies used single ingredient mash diets, whereas the present study involved heat-extruded multi-ingredient pellets. Second, a literature review by Carré (2000) concluded a different sensitivity to particle size between grains and seeds. Particle size seems to have less impact on performance in monocotyledonous cereals, than in dicotyledonous seeds. The test diets used in the present study contained seeds, but also cereal grains and other feedstuffs. Third, Reece *et al.* (1985) noted a decreased influence of particle size when mash diets were pelleted. They noted a significant improvement in feed conversion when chickens were fed a coarsely ground mash rather than a finely ground mash, whereas after pelleting, this effect was reduced to only a statistical trend in better feed conversion. Therefore, Reece *et al.* (1985) suggested that grinding feed particles to optimal size in pelleted diets is of less importance, because of an already higher digestibility in pelleted diets than in mash diets.

Contributing animal factors may include anatomical as well as behavioural differences between parrots and poultry. First, these bird orders display marked differences in the anatomy of the alimentary tract. For example, the gizzard wall is strongly developed in poultry as opposed to parrots (**Figure 3**). Also, omnivorous birds, including poultry, possess large, glandular caeca, important to cellulose digestion (Ziswiler and Farner, 1972). In parrots, on the other hand, caeca are absent. Second, poultry ingest feedstuffs *in toto*, whereas psittacine birds dehusk seeds, grains or nuts and fractionise kernels prior to ingestion.

This typical feed processing behaviour of parrots is enabled by the peculiar tongue musculature, the powerful, mobile beaks, and a rippled inner surface of the upper beak (**Figure 4**). Hence, due to both anatomical and behavioural differences, artificial particle size reduction to different grades is likely to have less impact in parrots than in poultry.



**Figure 3.** Comparison of gizzard musculature in avian species. The left photograph depicts the strong gizzard wall of a chicken. The right picture illustrates the thin muscular wall of the gizzard of an African grey parrot.



**Figure 4.** Rippled inner surface of the upper beak of a hyacinth macaw.

Excreta of parrots fed the coarse particle diet had significantly firmer excreta. Changes in excreta consistency could be explained by differences in either moisture content or water binding capacity. Moisture content was not correlated to the ECI. This could be due to the excreta collection protocol, in which excreta were collected several times during light hours for a period of 4 days. Excreta produced at night, however, were collected the next morning. As a result, differences in evaporation rate could have caused a bias in excreta moisture content.

In poultry, excreta are also found to be softer when birds are fed a finely ground feed. Yasar (2003) attributes this effect to an increased availability of non-starch polysaccharides (NSP) due to particle size reduction in the diet. Water binding capacity is influenced by non-starch polysaccharides (NSP), in which available NSP form a water binding gel in the gut, increasing ileal content viscosity. In poultry, this gel is broken down by bacteria when excreta leave the gut. Consequently, that part of the water binding capacity is lost, resulting in excreta that are less firm (Yasar, 2003). The microbial flora of parrots is less extensive compared to poultry; still, this kind of bacterial breakdown might also occur in psittacine birds. Although not investigated, a difference in particle size in the excreta also might have contributed to differences in its water binding capacity. Lentle *et al.* (2008 and 2009), for instance, reviewed the influence of particle size on phase separation between the solid and liquid phase of ingesta.

The pH-value of the excreta showed little variability and was equally alkaline for both test diets, whereas an acidic pH is considered to be beneficial for the birds' health. In addition, the pH level measured in fresh excreta was not different from measurement after homogenization in a 10% solution. The variability in measurements was independent of the technique applied. These data indicate that the time-consuming procedure of preparing solutions of excreta prior to pH-measurement might not be necessary to improve precision.

Long-term effects of the extent of feedstuff grinding were not investigated in this study. Literature regarding this subject is limited to studies on poultry. Several authors observed a significant reduction in gizzard musculature and gizzard weight in broilers fed finely ground feed compared to broilers fed coarsely ground feed or whole grains (Nir *et al.*, 1994, 1995, Svihus *et al.*, 2002; Peron *et al.*, 2005). In addition, Bird *et al.* (1937) described an increase in incidence and severity of lesions at the gizzard lining of chickens when feed is finely ground. However, as mentioned above, unlike poultry, psittacine birds process and reduce the particle size of offered feedstuffs prior to ingestion. Hence, macroscopic anatomic changes or lesions due to feeding of pellets, in which feedstuffs are artificially reduced to small particles, are likely to be less pronounced in parrots, if occurring at all.

In conclusion, coarse grinding of ingredients in the production of extruded parrot pellets results in more firm droppings. This improves the perception of excrement appearance, which positively affects the customers' perception of pelleted diets. Moreover, this beneficial trait of coarse pellets is not accompanied by compromised food intake, digestibility of the diet, excreta pH or daily excreta output.

#### **ACKNOWLEDGEMENTS**

The authors would like to acknowledge Herman De Rycke for his technical assistance (proximate analysis of feed and excreta samples) and Christel Moons for revising the manuscript.



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# CHAPTER 6

## GENERAL DISCUSSION



Obesity, which is the end-result of higher energy intake compared to energy expenditure, is one of the most prevalent health problems in captive parrots (La Bonde, 1997; Levine, 2003). Activity has a considerable impact on daily energy expenditure (Harper, 2000). Stimulation of physical activity may hence contribute to lower the incidence of obesity. Free-ranging parrots, for instance, fly considerable distances between feeding and roosting sites on a daily basis (Gilardi and Munn, 1998; Meyers and Vaughan, 2004). Tucker (1969) measured the energetic cost of flight in budgerigars. Depending on speed and inclination, the energetic cost was between 11 and 20 times the basal metabolic rate (Tucker, 1969). Stimulation of flight in captive parrots may therefore substantially increase daily energetic expenditure. Limitation of energy intake embraces an alternative, or preferably complimentary, strategy. Group housing, however, renders restricted feeding less suitable as a method to reduce energy intake, as this allows dominant birds to continue to feed *ad libitum*, but may lead to emaciation of other birds. Diluting the dietary energetic density of the ration, in contrast, may form an effective means to lower voluntary energy intake. The first two aims of this thesis were to evaluate the effect of energetic dilution of the diet on voluntary energy intake in nectarivorous and granivorous parrots.

Lories and lorikeets are nectarivorous parrots. Nectar is high in energetic density, with most energy being derived from simple sugars, but is extremely low in crude protein, vitamin and mineral levels (Gartrell, 2000). Hence, nectar feeders also forage on pollen to supply them with adequate amounts of protein (Cannon, 1979). Yet, although these birds predominantly feed on nectar and pollen, their natural diet comprises also other minor components, such as fruit, flowers and invertebrates (Cannon, 1984; Collar, 1997; Gartrell, 2000). In captivity, lories and lorikeets are usually fed a powdered, artificial nectar food that is diluted in water as principal nutrition, to which often fruit is added. Diluting the nectar to a higher degree lowers the energetic density and might have a saturating bulk effect. *Ad libitum* provision of fresh fruit might add to this. However, diluting a liquid diet to a higher degree and provision of fruit also have diluting effects on protein, vitamins and minerals. Therefore, such a dietary strategy might entail nutritional constraints with respect to an adequate nutrient supply.

*The first research question was to evaluate the effect of degree of dilution of ad libitum fed artificial nectar and availability of fresh fruit on voluntary energy intake and supply of essential nutrients in two lorikeet subspecies: green-naped lorikeets (Trichoglossus haematodus haematodus) and red-breasted lorikeets (Trichoglossus haematodus mitchellii).*

The results showed that in both subspecies, diluting nectar to a higher degree as well as additional provision of fruit decreased dry matter intake of nectar. Neither degree of dilution, nor availability of fruit had a clear-cut effect on total energy intake, but significant interactions between dilution degree and subspecies, and between dilution degree and availability of fruit were present. In green-naped lorikeets fed solely nectar, daily intake of energy showed a noticeable numerical decrease when the nectar was diluted to a higher degree. An opposite effect of the degree of dilution on voluntary energy intake was noted in red-breasted lorikeets when fed nectar added with fruit. In green-naped and red-breasted lorikeets, lowest voluntary energy intake was attained when fed high-dilution nectar and low-dilution nectar added with fruit, respectively. The daily supply of protein and thiamine remained in the current trial above the species-specific minimal recommendations, but were significantly lowered by either dilution of the nectar to a higher degree or *ad libitum* availability of apple. Finally, the Ca:P ratio remained within the normal range as well, but was significantly lowered through provision of apple. However, although both feeding strategies in the current trial did not compromise adequate intake of protein, thiamine or Ca:P ratio, this might occur when fed nectar food with lower contents of essential nutrients on dry matter basis.

True parrots are commonly fed a multi-component whole-seed diet, which is perceived as a natural diet because parrots are classified as seed eaters. However, commercial parrot food mixes of cultivated seeds and nuts differ largely from the natural diet of wild parrots, which consume a wide range of plant parts as well as invertebrates and sometimes meat from animal carcasses. Their diet also changes based on seasonal availability (Ullrey *et al.*, 1991; Collar, 1997). The second aim was to evaluate the effect of fresh fruit next to an *ad libitum* fed seed-based diet on voluntary energy intake and supply of essential nutrients in yellow-shouldered amazons (*Amazona barbadensis*). Voluntary energy intake was significantly lowered by 13.5% through additional provision of apple, without compromising sufficient intakes of essential nutrients. Therefore, these results indicate that availability of fresh fruit can effectively and safely reduce voluntary energy intake in true parrots fed are fed seed diet *ad libitum*.



A generally recognized drawback of multi-component seed diets is its unpredictable nutritive value and questionable effectiveness of supplements when fed to parrots. First, the method of storage can vastly alter the nutritional composition of the manufacturer's formulation, because different-sized ingredients tend to segregate during storage. Second, parrots dehusk seeds and nuts, ingesting only the kernels and discarding the husks. Third, the supplements that attempt to correct imbalances or deficiencies inherent to seeds have to be consumed in sufficient proportions in order to be effective. However, parrots tend to show strong preferences for certain food items, which alter the intended nutrient composition of the diet. Therefore, the third aim was to evaluate the effect of segregation during storage and feeding behaviour of yellow-shouldered amazons (*Amazona barbadensis*) on the ingredient and nutrient composition of a multi-component seed-based diet.

Both segregation during storage and feeding behaviour of the birds resulted in a vastly distinct nutrient profile between the formulated and the ingested diet, which further aggravated the nutritional imbalances and deficiencies inherent to seeds. The most detrimental nutritional effect of segregation was a steep decline in the proportion of oyster shells and extruded pellets enriched in vitamins, amino acids and minerals, which were relatively small in size. This lowered Ca:P-ratio from 2.61 in the formulated mixture to 1.36, dehusking of seeds would further lower Ca:P-ratio to 0.82, which is far below the recommended Ca:P ratio of 1.5:1. Seed selection resulted in an increase of the fraction of oilseeds from 23% in the offered mixture to  $82 \pm 7\%$  and  $78 \pm 5\%$  in the ingested fraction, when feeding only seeds, respectively seeds and apple. Seed selection and dehusking increased energetic density of the diet from  $15.1 \text{ MJ ME kg}^{-1}$  to  $24.7 \pm 1.6 \text{ MJ ME kg}^{-1}$ , respectively  $24.0 \pm 0.9 \text{ MJ ME kg}^{-1}$  and lowered Ca:P-ratio of this seed mixture from 0.43 to  $0.24 \pm 0.07$ , respectively  $0.19 \pm 0.02$ , when fed only seeds or seeds and apple.

The results clearly demonstrated that, despite the efforts of nutritionists to formulate diets in order to approximate estimated, species-specific requirements, nutritional composition of the actually consumed fraction of multi-component seed diets can be vastly deteriorated by both animal and management factors. The specific feeding behaviour of parrots is inborn to the species. Therefore, formulation of psittacine diets that contain whole seeds and nuts should be done based on the nutrient content of the kernels instead of on the ingredients *in toto*. Hence, nutritional effects of dehusking are accounted for. Furthermore, the label of such diets should ideally provide the nutrient content of the dehusked, consumable fraction, next to the

obligatory indicative values of the total diet. In addition, although not specifically studied in this thesis, incorporation of larger-sized supplements and regular turning of storage containers might reduce the adverse effects of segregation in multi-component diets. As parrots show a much higher preference for over-sized pellets (Rozek *et al.*, 2010; Rozek and Millam, 2011), increasing the size of supplements might also beneficially affect its intake.

A primary seed and nut diet is generally considered the most prevalent cause of nutritional disorders in parrots. Pelleted diets offer the advantage that they can be precisely formulated to comply with available nutrient guidelines. In contrast to multi-component seed mixtures, pelleted diets offer a mono-component diet in which all pellets share the same nutritional content. As a result of this, effects of segregation or selective feeding on the nutrient profile of the ingested diet are excluded when fed pellets. Moreover, although many parrots peel pellets, consuming only the inner part, this does not entail a divergent nutrient profile between the dietary formulation and the dietary intake. However, as pet birds are often housed indoors, less desirable appearances of the droppings of pellet-fed parrots compared to seed-fed diets constitutes one of the drawbacks against pellets. A lower firmness of excrements, as observed when fed pellets can be due to an increase in excreta moisture content or by lower water binding capacity of excreta. A general higher sodium content in pelleted diets might possibly increase excreta moisture content, as demonstrated in laying hens (Smith *et al.*, 2000); whereas in chickens for fattening, artificial particle size reduction in the manufacturing of pellets has shown to negatively influence excreta water holding capacity (Yasar, 2003).

Feeding of pellets has been shown to inflict gastric lesions in chickens, which are thought to result from artificial particle size reduction through food processing (Bird *et al.*, 1937). However, parrots use their beaks to reduce the particle size of ingested foodstuffs. Chickens, in contrast, ingest grains whole and reduction of ingredient size normally occurs in the gizzard through its strong musculature and thick koilin layer, assisted by the mechanical action of ingested grit. Hence, it is likely that particle size reduction through food processing during the manufacturing of pellets has far less effects on the gizzard in parrots compared to chickens. Moreover, abnormal changes at the lining of the glandular or muscular stomach have not been described in pellet-fed parrots without access to insoluble grit for prolonged periods of time (Schoemaker *et al.*, 1999).

The last trial evaluated the effect of pellet particle size on excreta characteristics of African grey parrots (*Psittacus erithacus erithacus*) fed an extruded pellet diet *ad libitum*. Long-term effects of artificial particle size through food processing were not evaluated. Coarse grinding instead of fine grinding of ingredients in the production of extruded parrot pellets positively affected excreta consistency. Moreover, the excreta consistency index showed even a higher firmness when fed coarse particle pellets than when fed a parrot seed mixture (Kalmar *et al.*, 2007). In addition, this positive trait of coarse pellets was not accompanied by a compromised food intake, digestibility of the diet, excreta pH or by an increase in daily excreta output. Therefore, coarse grinding might beneficially affect the owner appreciation of pellets, whilst it does not imply adverse nutritive effects.

### **FUTURE PERSPECTIVES**

1. Overdiluted nectar may impose physical constraints to the birds, such as insufficient volumetric capacity of the gut to ensure adequate energy and nutrient intake or insufficient elimination of excess water (reviewed by Köhler *et al.*, 2010). Therefore, it is indicated to investigate the safe upper limit of degree of dilution of nectar at different physiological states and conditions of ambient temperature and relative humidity.
2. The results presented in chapter 4 warrant investigating different approaches to diminish segregation during storage of multi-component seed diets. These may include regular turning of feed containers or storage in the original bags, in which the surface area to height is smaller compared to common large-sized feed containers. Moreover, it should be investigated if incorporation of larger-sized supplements implies improved supply of micronutrients:
  - (I) Given the pronounced effect of segregation on small-sized supplements, incorporation of larger sized supplements might avoid that the actually offered diet contains a much lower proportion of supplements compared to the manufacturer's formulation.
  - (II) Because of the strong preference of parrots for over-sized pellets compared to regular pellets (Rozek *et al.* 2010; Rozek and Millam, 2011), larger sized supplements might improve its intake
3. The particle size of the natural diet of parrots is much higher compared to the particle size in the coarse pellets described in chapter 5 (Brightsmith *et al.*, 2010). Therefore, it would be interesting to investigate the effects of a much wider range of pellet particle size on intake behaviour, apparent nutrient digestibility and excreta characteristics.

**FINAL REMARKS**

Lack of consensus regarding the use of equations within the available literature on psittacine nutrition not only warrants the need for in depth investigation of the validity of the former, but also complicates comparison of research data. Further, the wide range in nutrient contents of commercially available ‘complete’ diets for granivorous (Werquin *et al.*, 2005) and nectarivorous parrots (McDonald, 2003), indicates remarkable gaps in the knowledge on the nutrient requirements of these two groups of birds as a whole. Specific nutritional guidelines at species level are currently even more remote. Moreover, most published recommendations originate from studies on commercial poultry, which are in many aspects highly dissimilar to parrots. These incomplete nutritional guidelines impede on the one hand the formulation of genuinely complete psittacine diets and on the other hand a careful evaluation of marketed diets. Hence, although a mono-component diet precludes segregation and selective feeding, this type of diet in itself allows but does not guarantee optimal nutrient supply to parrots, to date.

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# SUMMARY



Parrots have been kept in captivity for at least 2500 years, their popularity as highly intelligent pet animals that are admired for their vocal abilities and exotic appearance, increasing continuously. Partly as a result of their popularity, they have become the most endangered order of birds in the world. Therefore, optimisation of their husbandry is of utmost importance. This not only improves the health and well-being of our companion birds, but also enhances fertility and extends lifespan and thus reproductive period of rare specimens kept in ornithological collections for conservation purposes.

A major area that unquestionably requires improvement is the nutrition of captive parrots, as the inadequacy of captive diets leads to malnutrition and contributes to obesity, which comprise the primary cause of the majority of clinical problems in these species. Therefore, the objectives of this work were to identify and alleviate constraints with regard to the nutritional value of common so-called complete seed diets for parrots. Second, feeding strategies to reduce overweight by diminishing voluntary energy intake were evaluated in nectarivorous and granivorous parrots. Finally, attributes affecting owner perception towards pelleted parrot diets are considered, as provision of a more nutritionally balanced diet is more feasible through a mono-component diet.

Lorikeets are usually fed dilutions of commercially available powdered nectar as the principal source of nutrition. The first trial aimed to investigate the effect of the degree of dilution of an artificial nectar powder and availability of supplemental fruit (apple) on energy and nutrient intake in two lorikeet subspecies, green-naped lorikeets (*Trichoglossus haematodus haematodus*) and red-breasted lorikeets (*Trichoglossus haematodus mitchellii*). Diluting nectar to a higher degree resulted in both subspecies in a significant decrease in dry matter intake of nectar, but intake of apple increased when offered next to higher diluted nectar. Besides a higher degree of dilution of nectar, availability of apple also resulted in lower intake of nectar. Neither degree of dilution, nor availability of fruit had a clear-cut effect on total energy intake, but significant interactions between dilution degree and subspecies, and between dilution degree and availability of fruit were present. In green-naped lorikeets fed solely nectar, daily intake of energy showed a noticeable numerical decrease when the nectar was diluted to a higher degree. An opposite effect of the degree of dilution on voluntary energy intake was noted in red-breasted lorikeets when fed nectar added with fruit.

A second trial investigated the effect of management and animal factors on the nutritive value of multi-component seed-based diets, and the effect of additional fruit when fed to yellow-shouldered amazons (*Amazona barbadensis*). Besides nutritional deficiencies and imbalances inherent to seed diets, three interfering factors further deteriorated adequate nutrient supply: segregation, seed dehusking and selective feeding. Segregation of ingredients when temporarily stored in a food container vastly decreased the proportion of mineral, amino acid and vitamin supplements in the top-layer. As such containers are usually replenished before they completely emptied and daily portions are scooped from the top-layer, this inappropriate storage method negates the effect of nutritional supplements that are necessary to counter-balance nutrient deficiencies and imbalances inherent to seeds. Next, seed-dehusking and profound selective feeding behaviour substantially increased the fat content and energetic density of the fraction actually ingested compared to the offered diet. Furthermore, consumption of only the seed kernels and low ingestion of supplements additionally deteriorated the Ca:P ratio. However, with the prerequisite of the opportunity of *ad libitum* feeding from all ingredients, neither the availability of fruit nor increasing the daily amount of offered seed mixture altered the ingredient profile of the consumed fraction of the seed diet. Nonetheless, availability of fruit effectively lowered voluntary energy intake, although this also tended to decrease the Ca:P ratio.

Pelleted diets offer the advantage that they can be formulated according to available guidelines, whilst their nutritive content is not altered through segregation or feeding behaviour. However, several constraints remain with regard to this type of diet, which include less desirable excreta characteristics. Therefore, the aims of a third trial were to assess daily excreta output and its consistency and pH-value in African grey parrots (*Psittacus erithacus erithacus*) fed extruded pellets differing in particle size. Food and water intake, and apparent nutrient digestibility coefficients were not influenced by particle size of the pellets. A coarser grinding of ingredients prior to extrusion resulted in more firm droppings, whereas particle size did not affect daily excreta output or pH-value. The change in excreta consistency was not due to altered moisture content and therefore this must have originated from an effect on water binding capacity. Possibly, a decrease in availability of non-starch polysaccharides, resulting from lower particle size reduction, contributed to improved firmness of droppings when fed the coarse pellets compared to the fine particle size pellets.

In conclusion, provision of fruit effectively reduced voluntary energy intake in granivorous parrots without adverse effects on consumption of essential nutrients. This way, obesity can be safely abated even in group-housed parrots. In nectarivorous parrots, however, highly diluted nectar food can be lower in energetic density compared to fruit. Hence, *ad libitum* provision of fruit beside nectar does not necessarily lower the energy content of the ingested ration in nectar feeders. In green-naped and red-breasted lorikeets, lowest voluntary energy intake was attained when fed high dilution-nectar and low-dilution nectar added with fruit, respectively. Seed-based diets intended for granivorous parrots do not entail a possibility to contain less energy compared to supposedly energy-diluting supplements such as fruit. Yet, in order not to adversely affect essential nutrient supply, the contents of the latter in the principal diet should always be evaluated prior to implementation of feeding strategies that try to abate obesity.

Next, seed-based diets inherently entail a number of nutritional inadequacies that are not overcome through supplements. In contrast, segregation due to inappropriate storage negates supplementation efforts in the form of, for instance, oyster shells or extruded pellets enriched with vitamins, amino acids and minerals. Although not specifically studied in this thesis, incorporation of larger-sized supplements and regular turning of storage containers might reduce the adverse effects of segregation in multi-component diets. Another aspect that deteriorates the nutritional adequacy of supplemented seed-based diets includes the typical feeding behaviour of parrots. The most effective means to oppose both these management and animal factors is to offer mono-component pelleted diets, which precludes segregation as well as selective feeding and hereby ascertains ingestion of the intended formulation.

Finally, increasing the particle size in pelleted diets improved the appearance of parrot droppings without affecting food or water intake, apparent nutrient digestibility coefficients or other excreta characteristics. This may enhance the perception of parrot caretakers towards pelleted diets and contribute to persuade them to provide their birds with this nutritionally more adequate type of diet.



# SAMENVATTING





Papegaaien worden ruim 2500 jaar gehouden in gevangenschap. Dankzij hun exotische uiterlijk en hun vocale capaciteiten zijn deze intelligente vogels uitgegroeid tot frequent gehouden huisdieren. Deze populariteit heeft echter bijgedragen tot een sterke reductie van de in het wild levende populaties, welke ondermeer hierdoor bedreigd zijn in hun voortbestaan. Hieruit kan worden gesteld dat een optimalisatie van de huisvesting, verzorging en voeding van papegaaien van cruciaal belang is. Enerzijds komt dit de gezondheid en het welzijn van de gezelschapsdieren ten goede, anderzijds kan hierdoor de vruchtbaarheid, de levensduur en bijgevolg ook de reproductieve periode van zeldzame exemplaren in ornithologische collecties worden verhoogd.

Aangezien malnutritie en overgewicht de voornaamste primaire oorzaken zijn van klinische problemen bij papegaaien, is het verbeteren van hun voeding essentieel. Een eerste doelstelling in deze thesis was om zowel bij nectarivore als bij granivore papegaaiesoorten een aantal voederstrategieën te evalueren met het oog op een vermindering van de spontane energieopname, om zo obesitas tegen te gaan. Een tweede doelstelling was om een aantal nutritionele tekortkomingen te onderzoeken die voortvloeien uit voeding met zaden. Aangezien een gepelleteerd voeder beter is aangewezen om de vogels te voorzien van een uitgebalanceerd voeder, werd tot slot onderzocht hoe de perceptie ten opzichte van pellets kan worden verhoogd.

Het basisrantsoen voor nectarivore papegaaien gehouden in gevangenschap bestaat uit een oplossing van artificieel nectarpoeder in water, welke eventueel wordt aangevuld met vers fruit. De opzet van de eerste voederproef was om het effect na te gaan van enerzijds de verdunningsgraad van artificiële nectar en anderzijds de beschikbaarheid van vers fruit (appel) op de spontane voeder- en nutriëntopname in twee regenbooglori subspecies (*Trichoglossus spp.*). Consumptie van fruit was hoger bij Mitchell's lories (*T. haematodus mitchelli*) dan bij groenke lories (*T. haematodus haematodus*). In beide subspecies nam de opname van nectar toe bij hogere verdunning. Deze toename in verse stof opname was echter onvoldoende hoog om eenzelfde droge stof opname te behouden. Ook toevoeging van stukjes vers fruit resulteerde in een lagere droge stof opname van nectar. Bij een hogere verdunningsgraad van de nectar nam bovendien de opname van fruit toe. Het effect van voederstrategie op dagelijkse energieopname toonde significante interacties tussen subspecies en verdunningsgraad van de nectar en tussen beschikbaarheid van fruit en verdunningsgraad van de nectar. Bij groenke lories was de dagelijkse energieopname het laagst bij een rantsoen van hoog verdunde nectar zonder fruit, terwijl bij Mitchell's lories was dit bij een rantsoen van

sterk geconcentreerde nectar met toevoeging van fruit. Noch de Ca:P verhouding, noch de eiwit of thiamine voorziening, werden gecompromitteerd door een hogere verdunningsgraad van de nectar of door toevoeging van vers fruit.

De meest courante voedermethode voor granivore papegaaien is het *ad libitum* verstrekken van een zadenmengeling, welke eventueel wordt aangevuld met vers fruit en supplementen. In een tweede proef werd bij geelschouder amazones (*Amazona Barbadosensis*) het effect onderzocht van management- en dierfactoren op de nutritionele waarde van een zadenmengeling. In navolging van de lori-proef, werd ook in deze voederproef nagegaan of toevoeging van vers fruit de spontane energieopname reduceert. Naast de inherente deficiënties en nutritionele onevenwichtigheden van zaden, hadden drie bijkomende factoren een negatieve invloed op een adequate nutriëntvoorziening: segregatie, selectieve ingrediëntopname en het pellen van de zaden.

De bewaring van zadenmengelingen gebeurt frequent in grote voederbakken. Deze worden regelmatig bijgevuld met nieuw voeder en van de bovenste laag wordt telkens het dagelijkse rantsoen geschept. Een dergelijke bewaring induceert echter segregatie van de ingrediënten. Hierdoor wordt de concentratie van de kleinste partikels in het effectief verstrekte voeder beduidend gereduceerd ten opzichte van de formulatie. Onder de ingrediënten met de kleinste grootte vallen echter ook de mineraal-, aminozuur- en vitaminesupplementen, die worden toegevoegd aan zadenmengelingen om tekorten te compenseren. Vergelijking van de ingrediëntsamenstelling zoals aangegeven op het etiket met deze van een staal uit de top laag van één van de stockgebakken in het Loro Parque te Tenerife, duidde op een veel lager gehalte aan deze supplementen in de verstrekte voederfractie, dan zou worden verwacht op basis van de etiketwaarde. Dit effect werd nog versterkt door een te lage supplementopname door de vogels. Als een bijkomende dierfactor werd de Ca:P verhouding van de opgenomen fractie van het rantsoen verder verlaagd door het pellen van de zaden en consumptie van enkel de kernen. Dit voedergedrag zorgde ook voor een belangrijke toename in het vet- en energiegehalte, wat nog werd bekrachtigd door een sterke voorkeur van papegaaien voor oliezaden. Bij de gekozen hoeveelheden in deze proefopzet werden in beide behandelingen alle ingrediënten in het gegeven rantsoen *ad libitum* verstrekt. Maar noch de beschikbaarheid van vers fruit, noch een grotere hoeveelheid van de zadenmengeling beïnvloedde de percentuele samenstelling van de opgenomen zaden. Tot slot werd de spontane energieopname gevoelig verlaagd dankzij de beschikbaarheid van vers fruit. Dit leidde echter ook tot een tendens tot een lagere Ca:P verhouding in het opgenomen rantsoen.

Een gepelleteerd voeder biedt het voordeel dat segregatie en selectief eetgedrag geen effect hebben op de nutritionele samenstelling. Hierdoor heeft het opgenomen voeder dezelfde samenstelling als de formulering en de etiketwaarde. Dergelijke voeders laten dus beter toe om de vogels te voorzien van een uitgebalanceerd rantsoen. Er bestaan echter verschillende punten van kritiek op gepelleteerde voeders. Hieronder valt onder meer een grotere dagelijkse hoeveelheid mest, die bovendien minder vast is in consistentie, en een hogere zuurtegraad heeft in vergelijking tot voeding met zaden. Omwille van dit argument was de doelstelling van een derde voederproef om te evalueren of de partikelgrootte binnen pellets de mestkarakteristieken kan beïnvloeden. Een grotere partikelgrootte resulteerde in grijze roodstaartpapegaaien (*Psittacus erithacus erithacus*) in beduidend vastere excreta, zonder de dagelijkse hoeveelheid of de zuurtegraad ervan te beïnvloeden. De partikelgrootte in de pellets had verder geen effect op de water- en voederopname, of op de schijnbare verteerbaarheid van de macronutriënten. Aangezien het vochtgehalte in de mest gelijk was bij beide behandelingen, werd het effect op de mestconsistentie veroorzaakt door een verschil in waterbindend vermogen. Mogelijks was dit een gevolg van een verschil in de beschikbaarheid van niet-zetmeel polysacchariden.

Als conclusie kan worden gesteld dat de beschikbaarheid van vers fruit in granivore papegaaien de spontane energieopname kan reduceren, zonder een beduidend negatief effect uit te oefenen op de voorziening in essentiële nutriënten. Hierdoor kan overgewicht bij papegaaien, zelfs indien de vogels in groep worden gehuisvest, op een veilige manier worden bestreden. Het energiegehalte in vers fruit kan echter hoger zijn in vergelijking tot sterk verdunde nectar. Hierdoor leidt het vertrekken van vers fruit bij nectarivore papegaaien niet altijd tot een lagere energieopname. In lories is het effect van zowel fruit als een hogere verdunningsgraad van nectar op de dagelijkse energieopname species afhankelijk.

Supplementatie van zadenmengelingen met kleine supplementen is omwille van segregatie- en onvoldoende opname geen efficiënte methode om deficiënties inherent aan zaden te voorkomen. Een gepelleteerd voeder laat beter toe om de vogels te voorzien in een uitgebalanceerd rantsoen. De mestconsistentie bij gepelleteerde voeders kan worden verhoogd door een grotere partikelgrootte in de pellets. Hierdoor kan de perceptie van papegaaieneigenaars tegenover dergelijke mono-component voeders worden verhoogd.



# ACKNOWLEDGEMENTS



Aagje, ‘not last, but not least’, maar ‘first and most significant’... Zonder jouw steun, hulp, late-night catering services en begrip voor mijn soms onconventioneel werkschema was het schrijven van dit boekje veel minder interessant en aangenaam geweest. Laat staan dat het ooit op tijd was klaar geraakt. Daarom, en voor alles wat je doet, merci!

Geert, bedankt om mijn promotor te willen zijn, voor de kansen en het gestelde vertrouwen, waarbij ik in alle vrijheid ‘mijn ding’ mocht doen. Veel toffe collega’s zijn gekomen en gegaan, maar graag had ik een speciaal woord van dank gericht aan: Adronie, Donna, Enass, Hannelore, Herman, Jenny, Kristel, Lien, Marleen, Myriam, Rebekka, Sarah, Steven, Tim en Veronique. Aan An, Jo-Ann en Stephanie, merci voor de olijke intermezzo’s en de steun ‘whenever needed’. Christel, ons collegaschap was maar tijdelijk, maar onze vriendschap is dit zeker niet. Bedankt om er voor te zorgen dat ik mij de afgelopen 6 jaar zal herinneren als jaren van collegialiteit en vriendschap.

Ik had ook graag mijn respect geuit voor de vogeltjes uit Loro Parque en van Patrick Ghysels. Deze prachtige, lieve en intelligente vogels zijn de belangrijkste figuranten in dit onderzoek.

David, Mattias and Marcellus, I would like to thank you for kindly sharing your extensive knowledge and experience on working with parrots, and for the use of your facilities and birds at the Loro Parque Fundación. Marjolein and Bjorn, thank you for all the precise and thorough work you performed at the La Vera breeding centre. Guy and Patrick, to you as well, thank you for your efforts to formulate all test diets, for sharing information and for lending us the African greys. Petra, thank you for your valuable input, for giving me confidence in myself and in the value and relevance of my work on parrots.

I would also like to thank the members of my guidance and examination committee, for their constructive contribution to this PhD thesis. Thank you An Martel, Andrea Fidgett, Christel Moons, Guy Werquin, Joeke Nijboer, Marcus Clauss and Petra Wolf.

Tot slot een woord van dank aan mijn familie en vrienden. In de eerste plaatste aan mijn ouders, broer, Julien, nonkel Pierre en tante Rita, dankjewel voor alles wat jullie doen en voor mij betekenen. Elke, bedankt om jouw linguïstische blik te werpen op mijn thesis. Aan alle vrienden in het oog of in het hart, bedankt om er te zijn.

Aagje, jij bent uiteraard het begin en het einde, bedankt om mijn bolleke te zijn,

*Nakupenda Mbuni,*

*Isabelle*





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**SUBMITTED BOOK CHAPTERS**

**Kalmar ID.** Calcium metabolism in rabbits. In *Small Mammal Nutrition*. Versele-Laga Ltd., Deinze, BE. (submitted)

**Kalmar ID.** Nutrition of the ferret. In *Small Mammal Nutrition*. Versele-Laga Ltd., Deinze, BE. (submitted)

**Kalmar ID.** Nutrition of the prairie dog. In *Small Mammal Nutrition*. Versele-Laga Ltd., Deinze, BE. (submitted)

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**Kalmar ID** and CPH Moons. Nutrition of degus. In *Small Mammal Nutrition*. Versele-Laga Ltd., Deinze, BE. (submitted)

**Kalmar ID** and CPH Moons. Dietary specialisation. In *Small Mammal Nutrition*. Versele-Laga Ltd., Deinze, BE. (submitted)

Moons CPH and **ID Kalmar**. Nutrition of the Mongolian gerbil. In *Small Mammal Nutrition*. Versele-Laga Ltd., Deinze, BE. (submitted)



# CURRICULUM VITAE

Isabelle Dominique Kalmar werd geboren op 9 december 1977 te Gent, België. In 1995 behaalde ze het diploma van het hoger secundair onderwijs (Wiskunde-Wetenschappen) aan het Sint-Bavo Humaniora te Gent. Datzelfde jaar begon zij haar studies aan de Universiteit Gent en behaalde in 1998 het diploma Bachelor in de Geneeskunde. Vervolgens vatte ze de studie Diergeneeskunde aan en behaalde in 2004 met grote onderscheiding de academische graad van Dierenarts. Haar afstudeerrichting was Onderzoek & Industrie, met de klinische optie: vogels, bijzondere diersoorten en laboratoriumdieren. Tijdens haar studies liep ze gedurende twee maanden een klinische stage in de dierenkliniek van Mangai Zoo en Jurong Bird Park in Singapore. Voor haar afstudeerthesis verrichtte ze in samenwerking met het Laboratorium voor Dierenvoeding, Genetica en Ethologie van de Faculteit Diergeneeskunde en Versele-Laga Ltd. gedurende drie maanden voedingsonderzoek op papegaaien in het onderzoekscentrum van de Loro Parque Fundación te Tenerife. Voor deze scriptie behaalde ze een award voor 'Excellent Research in Parrot Nutrition'.

Aansluitend op haar afstuderen trad ze in dienst aan bovenvermeld laboratorium. Haar onderzoek betrof enerzijds voedingsonderzoek op papegaaien, welke deels beschreven is in dit proefschrift. Anderzijds, deed ze in samenwerking met Taminco NV onderzoek naar nieuwe veevoederadditieven, waarop ze op 17 maart 2011 hoopt te doctoreren aan het Wageningen Institute of Animal Sciences. Verder was zij aangesteld als studiomonitor voor de registratieproeven van Taminco NV. Andere activiteiten waren onder andere het geven van practica and gastlessen, het begeleiden van masterproeven van studenten Diergeneeskunde, dienstverlening en projectcoördinator van Project Pathway. Dit laatste bestond enerzijds uit de ontwikkeling van een flowcytometrie model voor het *in vitro* testen van de antimicrobiële activiteit van nieuwe voederadditieven en anderzijds uit het onderzoek van effecten van gemethyleerde voederadditieven op het niveau van genexpressie aan de hand van microarray en PCR methoden. In 2006 behaalde ze het certificaat van proefleider (FELASA categorie C). Vervolgens behaalde ze in 2007 met grootste onderscheiding het diploma Master in Laboratory Animal Science (FELASA categorie D). In 2008 behaalde ze het post academisch certificaat Praktijkgerichte Statistiek aan het Instituut voor Permanente Vorming.

## CURRICULUM VITAE

Isabelle Dominique Kalmar was born on December 9<sup>th</sup>, 1977 in Ghent, Belgium. In 1995 she finished secondary grammar school (Mathematics and Science) at Sint-Bavo Humaniora in Ghent. That year, she started her education at Ghent University and graduated as Bachelor in Medicine in 1998. Subsequently, she took up Veterinary Medicine studies and graduated magna cum laude in 2004. Her graduation option was Research & Industry, with the clinical option: birds, special animals and laboratory animals. During her studies she did a two-month clinical internship at the Animal Clinic of Mangai Zoo and Jurong Bird Park, Singapore; and a three-month research internship at the Research Centre of the Loro Parque Fundación in Tenerife. Her master thesis, which was performed in cooperation with the Laboratory of Animal Nutrition, Genetics and Ethology and Versele-Laga Ltd., is entitled “Nutrient Requirements and Digestibility in Parrots”. For this research, she was granted an award for “Excellent Research in Parrot Nutrition”.

Immediately after graduation, she got employed at the above mentioned laboratory where she conducted research activities on parrot nutrition, which is partly described in this PhD thesis. Meanwhile, she investigated new feed additives in production animals in cooperation with Taminco NV, on which she will defend a PhD in Animal Sciences at the Wageningen Institute of Animal Sciences on March 17<sup>th</sup> 2011. Other activities included teaching, supervision of master’s projects in Veterinary Medicine, consultancy, and project coordinator of ‘Project Pathway’. The latter included on the one hand development of a flowcytometry model for *in vitro*-testing of antimicrobial potential of new feed additives, and on the other hand investigation of effects of methylated feed additives at the level of gene expression using microarray and PCR methods. She was also appointed as study monitor of the registration trials of Taminco NV. In 2006, she earned the FELASA category C certificate and in 2007 she acquired the degree of Master in Laboratory Animal Science (FELASA category D) summa cum laude. In 2008, she earned the post academic certificate in Practice-Based Statistics.





