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APPENDIX ONE

Developing a standard diet for potoroine marsupials

Introduction

AN intrinsic component of many nutritional studies is the precise measurement of food intake and hence the consumption of individual nutrients and other dietary constituents.

There are both practical and theoretical considerations for feeding a standard diet in preference to conducting research using natural foods. The first is that measurements of ingestion in free-living animals are difficult. The problem is exacerbated in potoroines because little is known about their natural diets (Chapter 3). A complete study of the diets of potoroine marsupials would require constant observation, at night, of feeding by animals of different ages and different reproductive states. Also, observation would need to continue through several seasons in order to identify all the foods eaten. A study of this kind was beyond the scope of the present work. It follows that, if nutritional studies are based on natural food items, only a subsample of the vast array of foods available could be offered. It would be impossible to incorporate the temporal variation found in natural diets. Also, this approach would exaggerate the risk of imposing nutritional deficiencies, which free-living organisms largely avoid by selective feeding.

In view of these limitations, it is preferable to develop a standard diet, thereby minimising variation in nutrient composition during long study periods.

The adoption of a standard diet should not suggest that the effect of variation in food quality on digestion and assimilation of nutrients does not deserve serious attention. Instead, a resolution of this issue is central to bridging the gap between the degree of control possible in the laboratory and in the complex reality of life in the field.

The following Appendix describes the development of the basal ration and of the experimental rations used throughout the study.

Materials and methods and Results

Introduction

Several factors must be considered before formulating artificial diets. These include the form of the diet, the potential ingredients and the method of evaluating the diet.

Potoroine marsupials manipulate feed with their forepaws. This imposes a physical constraint upon the artificial diet: animals must be able to handle it. A pelleted diet solves the problem. Calet (1965) reviewed the advantages and disadvantages of pelleted rations. Apart from enabling animals to pick up their food, an important advantage of pelleting in the current research is in minimizing selection, and thus enabling manipulation of nutrient intake.

In Chapter 3 it was concluded that potoroine marsupials probably select concentrated diets — those containing relatively high levels of available nutrients per unit dry matter. Thus, convenient ingredients for an artificial diet are cereal grains such as wheat and maize, and oilseed meals such as soybean meal.

How do we evaluate an artificial diet? The essential criterion is that a standard diet must allow animals to grow and reproduce. Ideally, the diet would maintain the body's metabolites at levels similar to those in free-living animals. Because there is a lack of data on tissue and blood parameters in wild potoroines, the present study used growth, reproduction and faecal characteristics as indices of dietary adequacy.

The approach

At the start of the current project, the small captive colony of *A. rufescens* was housed in outdoor enclosures and fed a diet of mainly sweet potato and commercial dog-food pellets (Allied Checkerboard dog food) and occasionally a few thistle roots. The dog food, which was always freely available to the *Aepyprymnus*, was formulated from cereals, oil-seed meals and animal byproducts. Each animal's intake of sweet potato was limited to about 100 $g.d^{-1}$. *Aepyprymnus* grew and reproduced on this mixed diet, which satisfied the main requirements of a basal ration. However, for several reasons, the diet was considered unsuitable for a general nutritional study of potoroines. First, the dog food contains much nitrogen (3.5%). Based on the mean maintenance nitrogen requirement of marsupials (300 $mg.kg^{-0.75}.d^{-1}$; Hume 1982), and assuming that they obtain sufficient energy but no additional nitrogen from other foods, *Aepyprymnus* would need to eat only about 9 $g.kg^{-0.75}.d^{-1}$ of the manufactured dog food to remain in nitrogen balance. Thus, dog-food diets were unsuitable for some of the planned experiments — for example, the determination of nitrogen requirements and the mechanisms for conserving nitrogen — because these require that the experimental animals stay at about zero nitrogen balance. Second, the only way to change the concentration of dietary-plant-cell-wall constituents in dog food is by grinding the dog food, incorporating plant cell-wall constituents and repelleting. This takes much time. Third, the dog food contains 1% sodium. It was found by Wright (1983) that a potoroine's water intake is strongly correlated with its intake of dog food. Finally, the manufacturer states that different ingredients may be used in each batch of dog food. This is undesirable in the present study. In conclusion, a sweet potato and dog-food diet lacks the flexibility needed for a broad nutritional study of potoroine marsupials. However, the nutrient intake by potoroines fed dog food and sweet potato was a useful index for the formulation of alternative diets.

Three *A. rufescens* (1 adult male, 1 juvenile male and 1 adult female) were placed in metabolism cages and treated as described in Chapter 4. The animals were offered, for 10 days, dog food *ad libitum* and $250 g.d^{-1}$ (10% more than their *ad libitum* intake) of fresh sweet potato. Feed intake and body mass were measured; results for the last five days are shown in Table A1.1.

Table A1.1 Intake and body mass measurements of A. rufescens fed fresh sweet potato[1] *and dog food*[1].

Animal	Intake ($g.kg^{-0.75}.d^{-1}$) dog food	sweet potato	N intake ($mg.kg^{-0.75}.d^{-1}$)	Body mass (g) start	finish
A	18	92	0.88	2750	2700
B	20	106	0.98	2650	2700
C	24	131	1.19	2100	2200

1 - intake of air-dry matter; the dog food contained 97% dry-matter and 3.55% nitrogen on a dry matter basis; the sweet potato contained 27% dry matter and 0.99% nitrogen on a dry-matter basis.

The *Aepyprymnus* ate most of the sweet potato offered, but usually left the skin. The animals maintained their body mass. Based on Wright's (1983) results and on the estimates of maintenance nitrogen requirements determined in later experiments (Chapter 5), all animals were probably in positive nitrogen balance. One problem with feeding so much sweet potato was that the animals produced very wet faeces. This violates one of the criteria for a standard diet — that the faeces should resemble those from free-living animals. Therefore, the study was repeated with the same three animals and a recently captured female. However, this time the animals were offered less sweet potato (150 $g.d^{-1}$). The results of the 10-day measurement period are shown in Table A1.2.

Table A1.2 Intake and body mass measurements of ***A. rufescens*** *fed fresh sweet potato*[1] *and dog food*[1].

Animal	Intake ($g.kg^{-0.75}.d^{-1}$) dog food	sweet potato	N intake ($mg.kg^{-0.75}.d^{-1}$)	Body mass (g) start	finish
A	28	66	1.15	2700	2700
B	27	71	1.13	2700	2700
C	25	81	1.09	2200	2300
D	15	59	0.68	3100	3000

1 - The dog food and sweet potato were of similar composition to those described in Table A1.1

Apart from small amounts of skin, the four *Aepyprymnus* again ate all of the sweet-potato offered. The three original *Aepyprymnus* ate similar quantities of dog food (25-28 $g.kg^{-0.75}.d^{-1}$). Also, animals tended to compensate for their lower intakes of sweet potato by eating more dog food than during the first measurement period. The fourth *Aepyprymnus* — the recently captured female — ate less dog food than the other animals. As a result it lost about 3% of its body mass. This loss was almost certainly due to factors other than a nitrogen deficiency, because the animal's nitogen intake was about three times the maintenance nitrogen requirement determined in Chapter 5. Reducing the amount of sweet potato caused animals to produce faeces that closely resembled those from wild animals in shape, consistency and water content.

The higher intakes of dog food in the second measurement period and the fact that about 60% of the animal's dry-matter intake was dog food suggested that it might be possible to maintain *Aepyprymnus* on a pelleted diet only. Thus, a diet was formulated (Table A1.3) so that animals eating about 30 $g.kg^{-0.75}.d^{-1}$ would have similar intakes of energy and nitrogen as those fed dog food supplemented with 150 $g.d^{-1}$ of sweet potato.

Table A1.3 Composition ($g.kg^{-1}$ air-dry matter) of the pelleted diet[1] fed to A. rufescens in Measurement Periods three and four

Ingredient	Level of inclusion
maize	600
wheat	200
dog food[2]	150
HCl-casein	49
mineral/vitamin premix[3]	1

[1] - The diet contained 2.5% nitrogen on a dry matter basis.
[2] - Allied Checkerboard dog food.
[3] - Fielder's pig grower premix (Table A1.6).

In Measurement Periods Three and Four the pelleted diet (Table A1.3) was fed to the four animals studied in Period Two and also to a juvenile male captured on the same night as Animal D. In Period Three, the animals were allowed free access to the pellets and were also given 90 $g.d^{-1}$ of fresh sweet potato. Measurements were made for ten days. Only pellets were fed in Period Four, in which a seven-day collection period followed seven days of adaptation. On three occasions Animal C spilt its food. Thus results for this animal are not included in Table A1.5.

Table A1.4 Intake and body-mass measurements of A. rufescens fed a pelleted concentrate diet (Table A1.3) and fresh sweet potato[1]

Animal	Intake ($g.kg^{-0.75}.d^{-1}$) pellets	sweet potato	N intake ($mg.kg^{-0.75}.d^{-1}$)	Body mass (g) start	finish
A	32	38	0.90	2700	2800
B	27	39	0.95	2700	2700
C	31	43	0.89	2300	2400
D	27	44	0.78	3100	3200
E	36	62	1.07	1500	1800

[1] - The sweet potato was of similar composition to that described in Table A1.1

Table A1.5 Intake, digestibility and body mass measurements from **A. rufescens** *fed a pelleted concentrate diet (Table A1.3)*

Animal	Food intake (g ADM.$kg^{-0.75}.d^{-1}$)	N intake (mg.$kg^{-0.75}.d^{-1}$)	DMD %	Body mass (g)[1] start	finish
A	30	0.75	70	2800	2800
B	33	0.83	70	2600	2550
D	27	0.68	73	3300	3300
E	40	1.00	72	1900	2500

1 - measured over 14 days

The *Aepyprymnus* ate similar quantities of pellets in both measurement periods. This was surprising because in Period 3 they ate between 10 and 16 g DM.$kg^{-0.75}.d^{-1}$ from sweet potato also. Nevertheless, even without the sweet potato in Period 4, the animals maintained their body mass.

Aepyprymnus always found sweet potato an attractive food. Indeed, they would often eat the sweet potato as soon as it was offered, regardless of the time of day. However, for the rest of the study, sweet potato was used only occasionally to provide some variety in the diet of breeding females.

As mentioned above, dog food was regarded as an unsuitable food for potoroines in the present study, mainly because it contains much nitrogen. Therefore, the dog food was removed from the basal diet as was the casein, which is both high in nitrogen and expensive. The nitrogen removed from the diet in these two ingredients was replaced by soybean meal. The dog food was also an important source of minerals. Thus, a mineral mix was formulated to provide similar levels of macro-elements as those contained in the diets fed to *M. r. erubescens* by Brown and Main (1967). This mineral formulation was used in all basal rations mixed after the removal of the dog food, and also in all experimental rations.

Table A1.6 Composition ($g.kg^{-1}$) of the mineral mix and mineral/vitamin premix used in the basal and experimental rations

	Ingredient	Level of inclusion (per kg feed)
Mineral mix		29
This provided:	NaCl (g)	9.1
	$CaCO_3$ (g)	5.2
	$CaHPO_4.2H_2O$ (g)	9.1
	$MgCl_2.6H_2O$ (g)	3.3
	KCl (g)	2.3
Mineral/vitamin premix[1]		1.0
This provided:	Co (µg)	750
	Cu (mg)	35
	Fe (mg)	80
	I (µg)	750
	Mn (mg)	64
	Se (µg)	230
	Zn (mg)	230
	Vitamin A (iu)	12500
	Vitamin D (iu)	2650
	Vitamin E (iu)	25
	Vitamin K (µg)	600
	Thiamine (mg)	1
	Riboflavin (mg)	7
	Pyridoxine (mg)	2.3
	Cyanocobalamin (µg)	24
	Biotin (µg)	335
	Folic acid (µg)	400
	Nicotinic acid (mg)	30
	Pantothenic acid (mg)	6

1 - Fielder's pig grower premix. The premix was always kept separate from the rest of the mineral mix.

Table A1.7 The composition ($g.kg^{-1}$ ADM) of the pelleted basal diet[1] *after removal of the dog food*

Ingredient	Level of inclusion
maize	615
wheat	200
soybean meal	155
mineral mix (Table A1.6)	29
mineral/vitamin premix (TableA1.6)	1

1 - The diet contained 2.5% nitrogen on a dry matter basis

By this stage the animals used in Measurement Periods 1-4 were restless after several weeks in metabolism cages. Therefore, the new basal diet was tested in the outdoor enclosures with the five *A. rufescens* used in Measurement Periods 3 and 4. Because the animals fed the basal diet maintained or gained body mass over a three-week period, the diet was judged adequate.

The development of potoroine diets now turned to the formulation of three experimental diets containing about 1, 1.5 and 2% nitrogen (on a dry-matter basis) for the preliminary study of maintenance nitrogen requirements. Because maize — the basal ingredient with the least nitrogen — contains at least 1.3% nitrogen and because the basal diet contained 2.5% nitrogen, it was clear that the basal ration had to be markedly changed to obtain a diet with only 1% nitrogen. The soybean meal, which contains about 8% nitrogen, was removed. The remaining cereals were diluted with cornflour (wheat starch: Fielders, which is essentially wheat devoid of cell-wall constituents and nitrogen) to give the dietary-nitrogen levels required. Thus, the energy content of the three diets was similar, but the nitrogen concentration varied. The composition of these experimental diets and the results and discussion of the balance study, in which they were fed to nine *Aepyprymnus*, are provided in Chapter 5. However, it is important to note here that eight animals ate the diet they were offered first; the remaining animal refused to eat the low-nitrogen ration and was transferred to the medium-nitrogen diet.

The basal ration (Table A1.7) was fed in all non-experimental periods and to all animals not being used in experiments until the end of the major experiment on nitrogen requirements of *Aepyprymnus* (Chapter 5). In this experiment it was shown that animals could maintain positive nitrogen balance on diets containing as little as 1% nitrogen. Furthermore, the faeces showed even closer resemblance to those of wild animals when the diet contained about 6% acid detergent fibre — as in the "low-fibre diets" described in Chapter 5. These observations were used in further development of the basal ration. The concentrations of soybean and maize in the basal ration were both reduced, and oaten straw was incorporated to provide about 10% acid detergent fibre in the diet (Table A1.8).

Table A1.8 Composition (g.kg^{-1} ADM) and typical chemical composition (g.kg^{-1} ODM) of the final basal diet

Ingredient	Level of inclusion
maize	555
wheat	200
soybean meal	140
oaten straw	75
mineral mix (Table A1.6)	29
mineral/vitamin premix (Table A1.6)	1
Analysis	
dry matter	910
organic matter	947
ash	53
nitrogen	22
acid detergent fibre	110
neutral detergent fibre	223
cellulose	95
hemicellulose	113
lignin	15

This basal diet was fed, for the remainder of the study, to all *A. rufescens*, *P. tridactylus* and *B. penicillata* that were not being used in experiments. The same formulation was used also in the calorimetric measurements of fed animals described in Chapter 11. Some typical intake, digestibility and balance data from animals fed the basal diet in Chapter 11 are shown in that chapter.

Summary

Because there is limited knowledge of the natural diets of potoroine marsupials, it was decided to develop a standard diet for use in the present studies. There is a lack also of data on tissue and blood parameters in wild potoroines. Accordingly, the present study used growth, reproduction and faecal characteristics as indices of dietary adequacy. The resulting standard diet — formulated from cereals and straw or oat-hulls — proved suitable for the maintenance of captive potoroine marsupials. Furthermore, the standard ration was modified to provide the experimental diets used in most of the present studies.

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APPENDIX TWO

The digestibility of plant-cell walls by potoroine marsupials

The low and variable digestibility of plant-cell walls by potoroine marsupials was first reported in Chapter 5. Similar findings in Chapters 6 and 7 (Table A2.1) prompted the studies reported in Chapter 8.

Table A2.1 The digestibility of plant-cell wall constituents in various experiments with potoroine marsupials.

		Digestibility (%)		sed	
Experiment	**Species**	**ADF**	**NDF**	**ADF**	**NDF**
Experiment 6.1[1]	*A. rufescens*	5	9	3.7	3.8
	P. tridactylus	13	16	3.7	3.8
Experiment 6.1[2]	*A. rufescens*	7	16	3.5	3.6
	P. tridactylus	9	16	3.5	3.6
	B. penicillata	8	15	3.5	3.6
Experiment 6.2	*A. rufescens*	12	23	8.7	7.7
	P. tridactylus	16	26	8.7	7.7
Experiment 7.2	*A. rufescens*	10	23	8.1	6.1
	P. tridactylus	19	28	8.1	6.1
	B. penicillata	19	26	8.1	6.1
Experiment 7.3	*A. rufescens*	12	16	4.7	3.7
	P. tridactylus	14	16	4.7	3.7
Experiment 9	*A. rufescens*	6	11	3.1	4.4
	P. tridactylus	6	11.4	3.1	4.4

1 - Collection periods 1 and 2 analysed as a cross-over design
2 - Collection period 1 analysed as a completely random design

Because there were no significant differences between treatments or collection periods for cell-wall digestibilities, data were pooled.

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APPENDIX THREE

Data from the calorimetric and balance studies described in Section 11.2

Animal	Mass	PY mass	DMI	RQ	Heat production		ME intake[3]		Energy balance		FHP[4]	Nitrogen intake		Nitrogen balance	
	(g)[1]	(g)	(g/d)		(kJ/d)	(kJ.MW/d)[2]	(kJ/d)	(kJ/MW/d)	(kJ/d)	(kJ/MW/d)	(kJ/d)	(g/d)	(g/MW/d)	(g/d)	(g/MW/d)
Ar009	2809	0	79	1.07	760	350	1006	464	246	113	568	2.02	0.93	0.61	0.28
Ar010	2837	0	49	0.98	802	367	591	270	-211	-96	584	1.29	0.63	-0.06	-0.03
Ar014	2385	0	40	0.95	671	350	493	257	-178	-93	586	1.04	0.54	-0.07	-0.04
Ar203	2581	0	56	1.05	840	412	702	345	-138	-68	562	1.43	0.45	0.16	0.08
	2550	27	65	1.05	757	375	858	425	101	50		1.66	0.45	0.41	0.20
	2860	151	75	1.10	847	385	467	179	179	81		1.95	0.68	0.68	0.30
	3190	526	62	1.04	1001	419	852	357	-149	-62		1.47	0.43	0.33	0.14
Ar112	2780	0	39	0.91	687	319	490	228	-197	-91	535	1.00	0.29	-0.25	-0.12
	2807	16	53	1.04	667	308	730	337	63	29		1.36	0.48	0.16	0.08
	2830	52	60	1.08	700	320	847	388	147	67		1.40	0.50	0.25	0.11
	2865	111	52	1.08	693	315	694	315	1	0		1.37	0.46	0.01	0.01
	2898	206	55	1.04	747	336	725	326	-22	-9		1.50	0.52	0.36	0.16
Ar012	2580	0	67	1.07	722	354	883	433	161	79	582	1.52	0.52	0.29	0.14
	2803	ca 4	85	1.13	822	379	1167	539	345	159		2.20	0.77	0.93	0.43
	2825	19	84	1.19	865	397	1171	537	306	140		1.98	0.66	0.48	0.21
	2850	46	69	1.06	758	346	956	436	199	91		1.82	0.65	0.57	0.26
	2959	82	75	1.11	793	351	1028	456	235	104		2.03	0.68	0.83	0.37
	3102	140	76	1.10	854	365	1014	434	159	68		1.94	0.63	0.51	0.22
	3157	265	84	1.08	981	414	1181	499	200	84		2.36	0.77	0.96	0.41
	3370	365	79	1.08	990	398	1061	427	70	28		2.03	0.61	0.65	0.26
	3454	476	77	1.05	1008	398	1066	421	59	23		2.00	0.59	0.58	0.23

Data from the calorimetric and balance studies described in Section 11.2 (continued)

Ar206	3186	0									612				
	3220	34	69	1.00	776	323	1012	421	235	98		1.82	0.61	0.61	0.25
	3212	61	68	0.99	812	338	980	408	168	70		1.84	0.61	0.81	0.24
	3207	96	71	1.09	813	339	986	411	174	72		1.82	0.59	0.53	0.22
	3487	190	70	1.06	910	357	991	388	81	32		1.97	0.59	0.64	0.25
	3485	355	72	1.03	1026	402	997	391	-29	-11		1.89	0.55	0.36	0.14
Ar135	2992	0													
	2950	85	28	0.9	729	324	334	148	-395	-180	598	0.70	0.21	-0.17	-0.07
	2917	150	41	0.96	739	331	513	230	-226	-101		1.05	0.37	0.15	0.07
	2935	275	63	1.05	877	391	843	376	-34	-15		1.76	0.57	0.59	0.26
	3037	371	62	1.05	879	382	841	366	-38	-17		1.60	0.50	0.53	0.23
	3179	480	69	1.04	958	402	978	411	20	8		1.81	0.61	0.46	0.19
	3264	590	73	1.03	980	404	968	399	-12	-5		1.86	0.58	0.64	0.26
Ar011	3153	0	99	1.10	873	369	1274	538	402	170	628	2.21	0.66	0.64	0.27
	3595	33	90	1.14	937	359	1263	484	327	125		2.27	0.68	0.64	0.25
	3700	140	86	1.09	943	353	1196	448	253	95		2.27	0.65	0.75	0.28
	3955	222	92	1.07	1017	363	1259	449	242	86		2.65	0.72	0.94	0.33
	4212	390	88	1.00	1161	395	1224	416	63	21		2.24	0.59	0.83	0.28
	4303	618	76	1.03	1141	382	1022	342	-119	-40		1.94	0.46	0.08	0.03
Ar013	2590	0	59	0.96	764	374	781	383	17	8	585	1.33	0.48	0.07	0.03
	2818	ca 17	63	1.06	795	365	849	390	35	16		1.58	0.54	0.02	0.01
	2689	53	56	1.05	773	368	724	345	-40	-19		1.47	0.49	0.22	0.11

1 - combined mass of the female and pouch young
2 - MW = $kg^{-0.75}$
3 - ME = metabolisable energy
4 - FHP = fasting heat production

APPENDIX FOUR

Blood parameters in free-living *A. rufescens*

In the course of the field studies reported in Section 11.3 many blood samples were collected for analysis of ^3HOH and $H^{18}OH$. Within one hour of bleeding, packed-cell volumes (Chapter 10) were measured and the remainder of the blood was centrifuged. The plasma was separated and stored frozen (-4°C). It was later analysed for several compounds.

Table A5.1 The concentrations of various compounds in the plasma of free-living A. rufescens. "Background" refers to a blood sample taken soon after the animal's capture at night; the "Equilibrium" sample was taken four hours after the background; "Daytime" refers to samples from later recaptures of the animals during the day. Values are means ± standard errors of the mean.

Parameter	Background	Equilibrium	Daytime
number	17	9	31
Glucose ($mg.dl^{-1}$)	179 ± 17.7	145 ± 17.2	180 ± 7.6
Lactate ($mg.dl^{-1}$)	261 ± 21.0	127 ± 16.3	330 ± 8.2
Urea-N ($mg.dl^{-1}$)	30.5 ± 1.56	33.0 ± 1.63	26.4 ± 1.34
Creatinine ($mg.dl^{-1}$)	1.87 ± 0.173	1.44 ± 0.069	1.62 ± 0.076
Protein ($g.dl^{-1}$)	6.9 ± 0.13	6.0 ± 0.22	6.9 ± 0.14
Albumen ($g.dl^{-1}$)	3.5 ± 0.06	3.2 ± 0.11	3.25 ± 0.097