

ORIGINAL ARTICLE

Water intake in domestic rabbits (*Oryctolagus cuniculus*) from open dishes and nipple drinkers under different water and feeding regimes

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welfare, drinking system, urolithiasis, calcium, water balance, water requirement, water restriction, husbandry

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Summary

Rabbits (*Oryctolagus cuniculus*) are often presented suffering from urolithiasis. A high water intake is important in the prophylaxis of uroliths. We investigated the influence factors for water intake using 12 rabbits subjected to different feed and water regimes with practical relevance: Hay, fresh parsley, a seed mix and two different pelleted feed were offered in diverse combinations. Water was provided either by open dish or nipple drinker. Water was accessible *ad libitum* except for four treatments with 6 h or 12 h water access. Under the different feeding regimes, the drinker had no influence on water intake, but faecal dry matter content was significantly higher with nipple drinkers [60.0 ± 2.1 vs. $57.2 \pm 2.1\%$ of wet weight (mean \pm 95% confidence interval), $p = 0.003$]. Dry food led to a higher drinking water intake but total water intake was still lower than with addition of 'fresh' food. With restricted water access, rabbits exhibited a significantly higher water intake with open dishes compared with nipple drinkers (54.9 ± 9.8 vs. 48.1 ± 8.2 g/kg^{0.75}/day (mean \pm 95% confidence interval), $p = 0.04$). High proportions of fresh parsley or hay in the diet enhanced total water intake and urine output, and led to lower urinary dry matter content and lower urinary calcium concentrations. Restricted access to drinkers led to a decreased total daily water intake and increased dry matter content of urine and faeces. For optimal water provision and urolith prophylaxis, we recommend a diet with a high 'fresh food' proportion as well as additionally hay *ad libitum* with free water access, offered in an open bowl.

Introduction

Rabbits (*Oryctolagus cuniculus*) are frequently seen in small animal veterinary practice. Most of their disease conditions are due to inappropriate husbandry and feeding (Morgenegg, 2003). This is also valid for urolithiasis, which belongs to the 10 most important conditions diagnosed in pet rabbits at the Clinic for Wildlife, Zoo Animals and Exotic Pets in Zurich

(Langenecker et al., 2009). Pathogenesis of urolithiasis in rabbits is considered multifactorial, but particularly associated with an excessive dietary calcium (Ca) intake (Kamphues, 1991, 2001). However, in a study in rabbits with a comparatively high Ca intake, neither uroliths nor macroscopically detectable nephrocalcinosis could be triggered (Burger, 2009). In that study, water intake was allowed *ad libitum* from open drinkers, which led to the assumption

that water intake might have prevented urolith formation.

Water intake is an important component of animal nutrition as it is a crucial constituent of body metabolism and temperature control (Pond et al., 2005). Rabbits exhibit a comparatively high water intake (Cizek, 1961). Water intake in rabbits occurs in numerous small portions (Prud'hon et al., 1972) mainly during night (Denton et al., 1985) and often in parallel to food intake (Wayner, 1974). Factors that influence water intake are age (Cizek, 1961), breed (Zumbrock, 2002), environmental temperature (Marai et al., 2005), lactation (Scheelje et al., 1975), husbandry conditions (Potter and Borkowski, 1998), water restriction (Prud'hon et al., 1975) and diseases (Ewringmann, 2005). Of special importance are dry matter intake (DMI) and food composition, in particular the presence of 'fresh' food (Buchner, 1994; Schwabe, 1995; Wenger, 1997; Wolf et al., 1999). Water intake correlates linearly with DMI (Cizek, 1961).

A frequently-asked question in veterinary consultancy is whether rabbits do need drinking water when being fed 'fresh' food. Experimental results are equivocal; sometimes, rabbits may meet their water requirements through 'fresh' food if large amounts are available (Schwabe, 1995), but they may also use drinking water even if 'fresh' food is available *ad libitum* (Wenger, 1997). Therefore, in general, water access is recommended even if 'fresh' food is offered (Schall, 2008; Wolf et al., 2008).

Another controversial topic in rabbit husbandry is the drinking system. In pet rabbits, mostly open dishes and nipple drinkers are used (Winkelmann, 2006; Tetens, 2007). Open dishes have the advantages of allowing an easy and natural way of drinking and of rapid and easy cleaning – but are easily contaminated by bedding, food, urine and faeces, and water may be spilled from them. Nipple drinkers in contrast save space and prevent spilling but drinking occurs in an unnatural, strenuous way; thorough cleaning of nipple drinkers is difficult (Drescher and Hanisch, 1995; Morgenegg, 2003; Quesenberry and Carpenter, 2004).

We investigated water intake and excretion of adult rabbits under different feeding and watering regimes, comparing nipple drinkers and open dishes, to identify conditions that lead to a high water throughput and are therefore particularly suitable for urolithiasis prophylaxis, especially as in human medicine high water intake was shown to constitute a prophylactic factor in idiopathic

calcium nephrolithiasis recurrence (Borghini et al., 1996).

Animals, materials and methods

Animals

Twelve adult dwarf rabbits of different sex (3.9), age (0.6 to 5.6 years old) and breed (four dwarf blue tan rabbits, four dwarf lops and four dwarf mixed breed) were used. Prior to the study all animals underwent a clinical examination, blood samples were taken and ultrasound of the urinary tract was performed in all animals to exclude urinary tract diseases. All animals were considered clinically healthy. All the animals survived and were returned to their owner at the end of the trial. The rabbits used in this experiment were also part of another study in which the preference for a drinking system had been investigated by a choice trial, and in which behavioural aspects of drinking (drinking speed, drinking frequency) had been documented by videotaping (A. Tschudin, M. Clauss, D. Codron, M. J. Hatt, unpublished).

Experimental design

Rabbits were divided into two groups of six animals. Each animal underwent 14 different treatments; each treatment lasted 15 days (Table 1). The order of the treatments was randomized to exclude environmental influences. A total of 10 different feeding regimes were used, of which each was given to six animals during two treatments: once with nipple drinker and once with open dish as the drinker system. Two of the treatments were additionally investigated for the influence of restricted water access (6 h and 12 h water access; water supply started at around 8:00 clock in the morning).

Measurements and sampling regime

Animals were checked daily for general condition, urination and defaecation throughout the whole experimental period. Body mass (BM) was recorded three times per treatment. After 2 days of acclimatization to a new diet, water and food intake were measured during a 9-day adaptation period. Water and food intake were measured as the weight difference after 24 h (scale: Missil ML0301, Bengt EK, EK Inter AG, Rothenburg, Switzerland, 1 g, max. 3 kg), taking into account evaporation losses (measured by blind probe for water and fresh parsley). After the adaptation period, rabbits were placed in metabolism cages, where water intake, food intake, urinary and

Table 1 Different treatments used in the study for group A (rabbits 1–6, upper case letters) and group B (rabbits 7–12, lower case letters) and the corresponding results

Treatment					Results						
Code	Diet	Diet abbreviation	Drinking system	Water access (h)	Total water intake (g/kg ^{0.75})	Water intake from food (g/kg ^{0.75})	Dry matter intake (g/kg ^{0.75})	Faeces (g/kg ^{0.75})	Faecal dry matter content (%)	Urine (g/kg ^{0.75})	Urinary dry matter content (%)
					Mean (per day) ± SD						
A	hay <i>ad libitum</i>	Hay100	OD	24	144 ± 57	9 ± 2	54 ± 10	57 ± 20	51 ± 6	58 ± 39	7.6 ± 3.4
B			ND	24	147 ± 38	9 ± 1	52 ± 8	51 ± 11	55 ± 4	52 ± 28	7.4 ± 3.8
C	90% Healthy	HRP90	OD	24	144 ± 29	11 ± 1	73 ± 5	72 ± 15	52 ± 7	41 ± 30	10.6 ± 5.3
D	Rabbit Pro		ND	24	128 ± 30	10 ± 1	67 ± 4	62 ± 14	59 ± 5	34 ± 20	12.7 ± 4.2
E	90% fresh parsley	Parsley90	OD	24	212 ± 38	195 ± 32	51 ± 10	33 ± 15	52 ± 6	104 ± 25	5.1 ± 0.9
F			ND	24	212 ± 25	193 ± 32	51 ± 6	34 ± 13	55 ± 8	102 ± 23	5.6 ± 1.5
G	50% fresh parsley	Parsley50	OD	24	176 ± 24	121 ± 8	56 ± 5	44 ± 12	53 ± 8	76 ± 24	6.6 ± 1.3
H			ND	24	167 ± 19	120 ± 7	52 ± 9	39 ± 16	54 ± 3	56 ± 11	7.7 ± 2.1
I	33% seed mix,	SeedParsley33	OD	24	122 ± 25	73 ± 11	46 ± 3	29 ± 11	59 ± 10	45 ± 27	8.9 ± 3.3
J	33% fresh parsley		ND	24	122 ± 21	75 ± 8	46 ± 5	26 ± 9	60 ± 8	43 ± 26	9.4 ± 3.0
K	33% seed mix,	SeedParsley33	OD	12	112 ± 21	78 ± 4	44 ± 4	24 ± 8	65 ± 7	36 ± 24	12.1 ± 4.5
L	33% fresh parsley		ND	12	108 ± 14	77 ± 5	42 ± 3	22 ± 5	67 ± 8	34 ± 23	12.4 ± 4.0
M	33% seed mix,	SeedParsley33	OD	6	110 ± 17	82 ± 4	52 ± 3	32 ± 8	61 ± 7	36 ± 20	10.7 ± 3.6
N	33% fresh parsley		ND	6	98 ± 10	81 ± 8	47 ± 4	24 ± 7	65 ± 9	31 ± 19	12.3 ± 4.6
a	90% laboratory	Pellet90	OD	24	86 ± 19	6 ± 1	47 ± 6	29 ± 10	63 ± 6	32 ± 14	9.4 ± 4.2
b	pellets		ND	24	86 ± 18	6 ± 1	46 ± 5	28 ± 9	65 ± 10	32 ± 12	10.1 ± 4.4
c	90% seed mix	Seed90	OD	24	56 ± 31	6 ± 3	43 ± 18	25 ± 16	58 ± 7	19 ± 17	11.5 ± 6.3
d			ND	24	72 ± 18	6 ± 2	43 ± 14	23 ± 14	63 ± 9	22 ± 15	10.0 ± 5.2
e	70% seed mix,	Seed70Parsley20	OD	24	69 ± 40	47 ± 3	40 ± 13	22 ± 12	60 ± 9	22 ± 25	11.0 ± 4.7
f	20% fresh parsley		ND	24	87 ± 19	47 ± 2	43 ± 10	21 ± 8	63 ± 11	26 ± 11	11.0 ± 4.5
g	45% seed mix,	SeedParsley45	OD	24	92 ± 47	97 ± 9	38 ± 7	18 ± 8	60 ± 8	39 ± 24	7.4 ± 2.7
h	45% fresh parsley		ND	24	114 ± 14	96 ± 9	37 ± 6	17 ± 6	64 ± 5	43 ± 21	7.7 ± 2.7
i	50% seed mix	Seed50	OD	24	79 ± 27	5 ± 2	35 ± 8	19 ± 9	63 ± 6	29 ± 13	7.7 ± 5.5
j			ND	24	74 ± 16	5 ± 1	33 ± 5	20 ± 7	63 ± 8	26 ± 11	7.9 ± 4.9
k	50% seed mix	Seed50	OD	12	77 ± 31	5 ± 1	34 ± 5	19 ± 5	70 ± 7	27 ± 15	9.1 ± 6.8
l			ND	12	66 ± 19	5 ± 2	33 ± 9	18 ± 6	70 ± 8	25 ± 13	8.9 ± 5.6
m	50% seed mix	Seed50	OD	6	73 ± 22	6 ± 2	41 ± 9	27 ± 9	62 ± 6	27 ± 13	9.9 ± 5.4
n			ND	6	66 ± 12	5 ± 2	37 ± 11	21 ± 9	66 ± 3	26 ± 16	9.6 ± 5.1

OD, open dishes; ND, nipple drinkers.

faecal output were measured for four consecutive days. Excrements were collected quantitatively and stored at −21 °C until further analysis.

Housing

During the adaptation period, the rabbits were housed in a room with natural lighting by windows, and kept individually in wooden boxes (97 cm × 130 cm). Bedding consisted of wood shavings, and each animal had a wooden shelter. Holes in the walls (diameter of 5 cm) allowed contact among the rabbits. Food and water were offered on an elevated area and hay in a hay rack. Metabolism cages (53 cm × 43 cm × 40 cm) either had a wire-mesh ($n = 3$) or perforated metal floor ($n = 9$); here, food and water were offered on ground level and

hay in a hayrack. Air temperature and humidity were measured daily.

Food

Hay, fresh parsley, a commercial seed mix, a premium pellet food [Healthy Rabbit Pro (HRP)] and a laboratory pellet food were used (Table 2) in 10 different combinations (Table 1). The animals were divided into two groups (A and B). Each animal received five of the combinations twice for 15 days. The treatments were calculated for the actual BM with respect to the daily digestible energy (DE) requirement (440 kJ DE kg/BM^{0.75}; Kamphues *et al.*, 2009). After the first four treatments the calculation was adapted to the target BM, because most of the animals gained weight. All animals were offered hay

Table 2 Assumed energy content and assumed and effective composition of the different feeds used in the trial with manufacturer's details

Parameter	Unit	Hay		Fresh parsley		Healthy Rabbit Pro		Laboratory pellet food		Seed mix	
		Own analysis	Kamphues et al., 2009	Own analysis	Kamphues et al., 2009	Own analysis	Manufacturer	Own analysis	Manufacturer	Own analysis	Manufacturer
Assumed energy content	MJ DE/kg	n.s.	5.42	n.s.	1.88	n.s.	9*	n.s.	9*	n.s.	10.2
Dry matter	%	85.20	86.00	11.38	18.10	88.40	n.s.	89.95	89.00	89.40	87.50
Crude ash	% DM	10.09	n.s.	15.26	n.s.	8.72	8.40	7.25	8.31	7.22	n.s.
Crude protein	% DM	12.00	13.72	18.42	24.31	11.63	12.00	15.89	15.17	11.25	11.43
Crude fat	%DM	2.00	2.67	2.06	1.66	2.41	2.00	3.36	3.93	4.08	4.57
Crude fibre	%DM	25.66	31.05	12.53	23.76	27.29	28.00	16.95	18.65	10.51	11.43
N-free extracts	%DM	50.25	n.s.	51.73	n.s.	49.95	n.s.	56.55	53.93	66.94	n.s.
NDF	%DM	59.67	n.s.	23.10	n.s.	55.93	n.s.	38.14	n.s.	35.30	n.s.
ADF	%DM	29.96	n.s.	20.23	n.s.	32.67	n.s.	22.86	n.s.	14.43	n.s.
ADL	%DM	3.28	n.s.	2.89	n.s.	6.03	n.s.	6.46	n.s.	3.78	n.s.
Na	% DM	0.03	0.07	0.33	0.18	0.30	n.s.	0.44	0.35	0.30	n.s.
K	%DM	2.27	n.s.	5.04	n.s.	1.59	n.s.	0.88	1.01	0.78	n.s.
Ca	%DM	0.73	0.74	1.00	1.35	0.50	0.60	0.56	0.90	1.04	n.s.
P	%DM	0.20	0.27	0.28	0.71	0.21	0.40	0.34	0.61	0.41	n.s.
Ca:P		3.65	2.78	3.57	1.91	2.38	1.50	1.65	1.48	2.54	n.s.
Mg	%DM	0.10	n.s.	0.09	n.s.	0.08	n.s.	0.08	0.20	0.06	n.s.
Manufacturer's details						Healthy Rabbit Pro, Oxbow Enterprises, Murdock, USA		Alleinfuttermittel für Kaninchen und Meerschweinchen, Provimi Kliba SA, Kaiseraugst, Switzerland		Hobby Corn Mix, Landi Schweiz AG, Dotzingen, Switzerland	

DE, digestible energy; DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; n.s., not specified.

*Kamphues et al. (2009)

ad libitum and expected to consume it to meet their overall requirements.

Water

Fresh water and food were offered daily in the morning. Water was given in commercially available two-parted open dishes (Rössler Porzellan AG, Ersigen, Switzerland; 23 cm × 8.5 cm × 4.5 cm, content: 7.8 dl, filled with 4.5 to 6 dl water) or nipple drinkers (Classic, large bunny, Pet products, Caldex Ltd., Halifax, UK; height: 18 cm, diameter: 6.5 cm, maximum content: 6.2 dl, diameter of the metal nipple: 7 mm) and was exchanged daily. Spillage and contamination (no, little, much) were additionally recorded.

Analysis

Feed and faecal samples were dried at 60 °C (Vulkan Trocknungsschrank, Elektroapparate Fabrik AG, Rorschach, Switzerland), and ground (1 mm, Retsch Mühle, Retsch GmbH, Haan, Germany). Feed samples were investigated by Weender analysis and Van

Soest fibre analysis; faeces, urine and a water sample were analysed for dry matter (DM) and crude ash (AOAC 1997). All samples were additionally tested for sodium, potassium, calcium, phosphorus and magnesium, using the crude ash residue as base material. Drinking water was analysed for mineral content as well using the same methods. Drinking water contained 65 mg Ca/kg water and 21 mg Mg/kg water. The calcium and magnesium intake from water were included in the calculations of total calcium and magnesium intake.

Statistics

General linear models with repeated measures ANOVA were applied for statistic analysis using PASW 18.0 (SPSS, Chicago, IL, USA) and STATISTICA 7.1 (StatSoft Europe GmbH, Hamburg, Germany). For the evaluation of temperature and ration composition effects, the samples were treated as independent with non-normal distribution and were analysed with a Spearman correlation. To further analyse this data, General linear models were used with testing for

normal distribution of residuals and equal variance. Different measures of water metabolism were dependent variables, the individual animal was set as a random factor, and temperature, DMI, DM content of the diet and proportion of hay in the diet were covariates. The significance level was set at $p \leq 0.05$.

Results

Different diets with *ad libitum* water intake

Food intake

Dry matter intake varied significantly among diets (Table 3). Dry matter intake was especially high with HRP90 and was lowest with Seed50 (Table 1). No influence of the drinking system on DMI was found. Dry matter intake was negatively correlated to air temperature (Spearman correlation: $\rho = -0.323$, $p < 0.001$, $n = 120$). Daily Ca intake was highest with Parsley90 and Parsley50 and lowest with Seed50 (Fig. 1). Calcium intake was notably not related to the Ca content of the diet (Spearman correlation: $\rho = -0.129$, $p = 0.159$, $n = 120$).

Water intake

Both drinking water intake and total water intake (TWI; water intake from drinker and from food combined) differed significantly among diets (Table 3). Drinking water intake was high on Hay100 and HRP90, and it was lowest with Parsley90 (Table 1). Still, water intake from the drinker was never zero, even when the ratio of parsley in the diet was high. Total water intake was positively correlated to DMI (Spearman correlation: $\rho = 0.552$, $p < 0.001$, $n = 117$). Total water intake was highest with Parsley90 in the diet (Fig. 2). With the exception of the 'high fibre diets' (hay, HRP) TWI basically increased

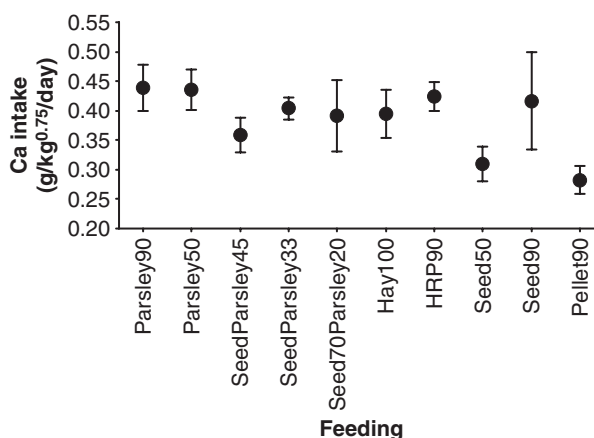


Fig. 1 Mean calcium intake per kg metabolic body mass in 12 rabbits (*Oryctolagus cuniculus*), each being fed five different diets with *ad libitum* water, during 26 days each. Mean \pm 95% confidence interval.

with diets of decreasing DM content (Fig. 3a). The lowest TWI was measured with diets with high proportions of seed mix. Diets with a higher DM content led to a higher *drinking* water intake, but this did not result in a higher *total* water intake (Fig. 3). Not only DM content but also hay ratio in the diet influenced TWI – a high proportion of hay favoured a high TWI (Table 4). Water intake was not dependent on temperature (Table 4) or drinking system (Table 3).

Faeces

The amount of faeces (on fresh matter basis) depended on DMI (Spearman correlation: $\rho = 0.886$, $p < 0.001$, $n = 120$), but the drinking system also had an influence (Table 3): with open drinkers more faeces were excreted than with nipple drinkers.

Table 3 Results of a repeated measures ANOVA of the influence of drinking system and diet on different parameters in 12 rabbits (*Oryctolagus cuniculus*) split into two groups with a total of 10 different diets and *ad libitum* water access

Dependent variable	Within-subjects effects					
	Between-subjects effect Group		Drinking system		Diet	
	F	p	F	p	F	p
Dry matter intake (g/kg ^{0.75} /day)	16.538	0.002*	0.786	0.396	13.932	<0.001*
Total water intake (g/kg ^{0.75} /day)	36.206	<0.001*	0.185	0.678	16.606	<0.001*
Drinking water intake (g/kg ^{0.75} /day)	3.518	0.093	0.181	0.680	75.989	<0.001*
Food water intake (g/kg ^{0.75} /day)	236.960	<0.001*	0.031	0.864	335.924	<0.001*
Water:dry matter intake (g/g)	8.145	0.019*	0.479	0.506	50.941	<0.001*
Faecal output (g/kg ^{0.75} /day)	18.858	0.001*	5.028	0.049*	22.972	<0.001*
Faecal dry matter content (%)	5.786	0.037*	6.400	0.030*	1.767	0.155
Urinary output (g/kg ^{0.75} /day)	7.976	0.020*	3.002	0.117	16.633	<0.001*
Urinary dry matter content (%)	0.749	0.409	1.560	0.243	6.922	<0.001*

*Significant results.

The italics are the results which were not significant but showed a tendency ($p < 0.10$).

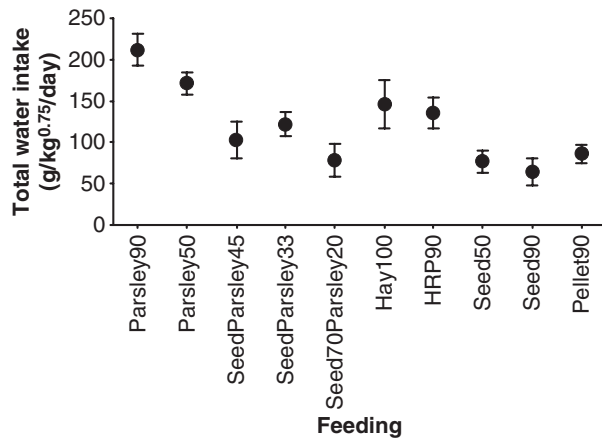


Fig. 2 Mean total water intake per kg metabolic body mass in 12 rabbits (*Oryctolagus cuniculus*), each being fed five different diets with *ad libitum* water, during 26 days each. Mean \pm 95% confidence interval. Note that evidently, drinking water intake did not compensate for low water intake via food.

DM content of faeces was not influenced by diet but again by the drinking system (Table 3): with nipple drinkers, faeces were drier than with open dishes [dry matter 60.0 ± 2.1 vs. $57.2 \pm 2.1\%$ of wet weight (mean \pm 95% confidence interval)].

Urine

The amount of urine excreted and the DM content of urine differed among diets (Table 3). In each group the treatment with the highest parsley proportion led to most, and most diluted, urine (Table 1). A higher DM content in the diet led to less urinary output (Spearman correlation: $\rho = -0.528$, $p < 0.001$, $n = 117$) and to higher DM content of the urine (Spearman correlation: $\rho = 0.219$, $p = 0.018$, $n = 117$). The Ca concentration in urine depended on

both, total water intake and Ca intake (univariate ANOVA: random factor: individual: $F = 5.016$, $p < 0.001$; covariate TWI: $F = 9.200$, $p = 0.003$; covariate Ca intake: $F = 11.114$, $p = 0.001$); the higher TWI, the lower urinary calcium concentration. Urinary Ca concentration was lowest with Parsley90 and was highest with Seed90, HRP90 and Seed70Parsley20 (Fig. 5).

Water restriction treatments

Food intake

Dry matter intake varied significantly among the different water access times (Table 5), with highest DMI in 6 h water access and lowest in 12 h (Table 1). With nipple drinkers, DMI was lower compared with open dishes. Dry matter intake also differed between feeding regimes: group A being fed 33% parsley in the ration exhibited a higher DMI than group B without parsley (Table 5).

Water intake

The more hours the rabbits had access to water, the higher were water intake from drinker and TWI. The presence of parsley in the diet led to a 10 times higher food water intake in group A than group B (without parsley). Group B, in contrast, exhibited a higher water intake from the drinker, but a lower TWI than group A. The drinker itself also influenced water intake: water intake from the drinker (Fig. 4a) and TWI were lower with nipple drinkers than with open dishes (Table 5).

Faeces

Both the amount and the DM content of faeces differed significantly with water access (Table 5). With *ad libitum* access, rabbits excreted the highest

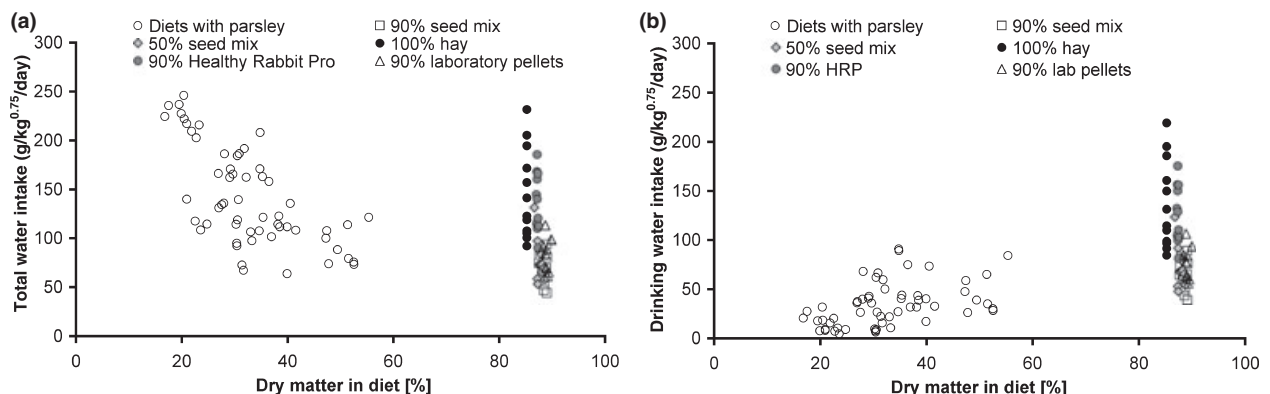


Fig. 3 Total water intake (a) and drinking water intake (b) in relation to the DM content of the diet in 12 rabbits (*Oryctolagus cuniculus*), each being fed five different diets with *ad libitum* water, during 26 days each.

Table 4 Results of a general linear model in a univariate anova of the influence of individual, temperature, dry matter intake, dry matter and hay content of the diet on different parameters in 12 rabbits (*Oryctolagus cuniculus*) split into two groups with a total of 10 different diets and *ad libitum* water access

Dependent variable	Random factor		Covariates							
	Individual		Temperature (°C)		Dry matter intake (g/kg ^{0.75} /day)		Dry matter content of diet (%)		Hay in diet (%dry matter intake)	
	F	p	F	p	F	p	F	p	F	p
Total water intake (g/kg ^{0.75} /day)	8.35	<0.001*	2.26	0.136	20.20	<0.001*	71.55	<0.001*	9.74	0.002*
Drinking water intake (g/kg ^{0.75} /day)	30.95	<0.001*	3.82	0.053	40.48	<0.001*	254.76	<0.001*	35.00	<0.001*
Food water intake (g/kg ^{0.75} /day)	0.87	0.574	7.10	0.009*	0.02	0.887	323.71	<0.001*	0.66	0.418
Water:dry matter intake (g/g)	10.60	<0.001*	2.68	0.105	9.91	0.002*	79.79	<0.001*	5.05	0.027*
Faecal dry matter content (%)	5.98	<0.001*	10.10	0.002*	1.92	0.169	0.83	0.365	6.52	0.012*
Urinary output (g/kg ^{0.75} /day)	8.42	<0.001*	0.49	0.486	3.71	0.057	41.82	<0.001*	5.16	0.025*
Urinary dry matter content (%)	14.93	<0.001*	0.26	0.611	0.78	0.380	14.41	<0.001*	14.48	<0.001*

*Significant results.

amount of faeces with the lowest DM content compared with 12 h and 6 h water access. Neither drinking system nor group influenced these faecal parameters (Table 5).

Urine

The amount and DM content of urine also depended on water access: with 24 h water access more, and more diluted, urine was excreted than during water restriction (Table 5) because of the higher TWI with *ad libitum* water access. The drinking system tended to influence urinary output in the way that the amount of urine was lower with nipple drinkers than with open dishes (Table 5).

Water contamination and spillage

In the wooden boxes where feeding and drinking equipment was offered on the elevated area, spillage and contamination of open dishes did not turn out to be of importance (Table 6). When open dishes

were placed on the floor in the small metabolism cages, contamination was more frequent. Nipple drinkers were macroscopically clean, but calcareous accretions were visible after few weeks of use.

Discussion

This study evaluated different feeding and drinking regimes in rabbits with respect to water intake and hence urolith prophylaxis in rabbits. Large individual differences in food and water intake as well as in urinary and faecal output were observed – the individual animal as a random factor was highly significant in most analyses (Table 4). These can be explained by different effects of age and breed (Cizek, 1961; Marai et al., 2005) as well as by unknown individual factors.

Drinking system

With *ad libitum* water access, the drinking system did not have an influence on water intake. However,

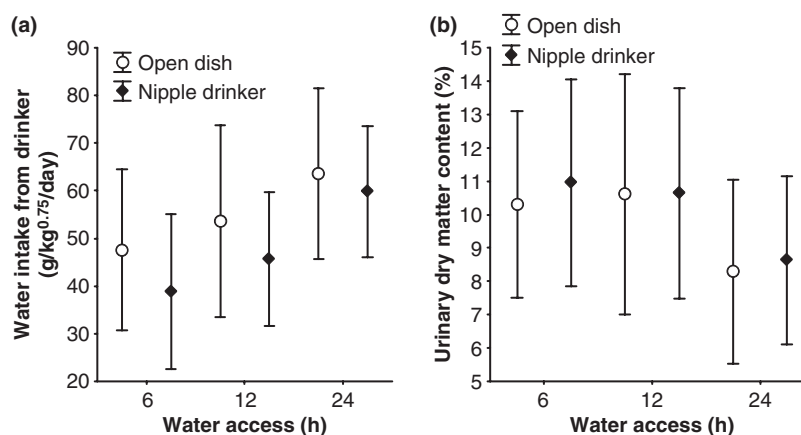


Fig. 4 Mean water intake from the drinker per kg metabolic body mass (a) and mean dry matter content of urine (b) with open dish and nipple drinker by 12 rabbits (*Oryctolagus cuniculus*) during water restriction (12 and 18 h) and *ad libitum* water access. Mean \pm 95% confidence interval.

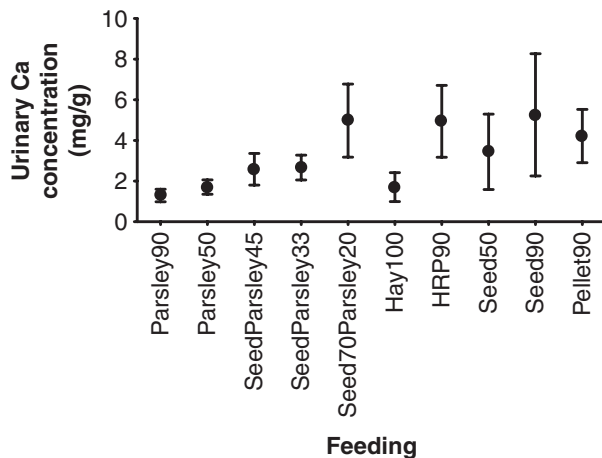


Fig. 5 Mean calcium content per g urine in 12 rabbits (*Oryctolagus cuniculus*), each being fed five different diets with *ad libitum* water, during 26 days each. Mean \pm 95% confidence interval.

faecal output was less and dry matter content of faeces was higher with nipple drinkers compared with open dishes, indicating a physiological response in the sense of a water-saving mechanism. Under water restriction, the nipple drinker had a negative effect on water and food intake, and tended to affect urinary and faecal output. Both nipple drinkers and open dishes were well tolerated by the rabbits in this trial. Still, some rabbits displayed a drinking behaviour with signs of an additional endeavour at nipple drinkers which could be explained by the prolonged drinking time which is unavoidable with nipple drinkers (A. Tschudin, M. Clauss, D. Codron, M. J. Hatt, unpublished). In combination with the finding that adult rabbits obviously prefer open dishes over nipple drinkers (A. Tschudin, M. Clauss, D. Codron, M. J. Hatt, unpublished) we

therefore recommend using open dishes. Open dishes are often criticized because of their possible contamination and spillage (Drescher and Hanisch, 1995; Kamphues and Schulz, 2002; Quesenberry and Carpenter, 2004; Schall, 2008). In our trial, we found that both of these undesirable conditions were only of concern if the open dish was offered on the floor in the small metabolism cages. We therefore suggest to place the open dish on a elevated area, according to Morgenegg (2003). Other options would be to use heavy dishes with high rims, or to weigh open dishes down with a stone (Morgenegg, 2003; Quesenberry and Carpenter, 2004).

Diet

The selected diets were chosen for practical reasons. Hay should be the basis of rabbit nutrition (Lowe, 1998) and was therefore included in all diets *ad libitum*. Seed mixes are not recommended in rabbit feeding because of their high starch and energy content (Irlbeck, 2001), because they allow selective feeding, and because of negative effects on dental health (Harcourt-Brown, 1996); nevertheless, such feeds are still often used in pet rabbit husbandry (Mullan and Main, 2006; Schepers et al., 2009; Tschudin et al., 2010). The laboratory pellets were chosen to represent a conventional pelleted feed as sold for laboratory rabbits. The other pelleted feed, HRP, has a much higher fibre content than the conventional laboratory pellet and might therefore be particularly suitable for rabbits. Fresh parsley was used as a source of fresh food with a relatively high Ca and water content.

As the aetiology of urolithiasis does not seem to depend on Ca content of the diet alone (Burger,

Dependent variable	Between-subjects effect Group		Within-subjects effects			
			Drinking system		Water access	
	F	p	F	p	F	p
Dry matter intake (g/kg ^{0.75} /day)	14.318	0.004*	5.845	0.036*	19.757	<0.001*
Drinking water intake (g/kg ^{0.75} /day)	11.399	0.007*	5.508	0.041*	12.909	<0.001*
Food water intake (g/kg ^{0.75} /day)	1128.360	<0.001*	0.040	0.846	2.276	0.129
Total water intake (g/kg ^{0.75} /day)	14.805	0.003*	4.936	0.051	10.453	0.001*
Water:dry matter intake (g/g)	1.899	0.198	0.033	0.860	33.195	<0.001*
Faecal output (g/kg ^{0.75} /day)	2.189	0.170	3.794	0.080	13.016	<0.001*
Faecal dry matter content (%)	0.791	0.395	1.970	0.191	16.173	<0.001*
Urinary output (g/kg ^{0.75} /day)	1.086	0.322	4.116	0.070	10.367	0.001*
Urinary dry matter content (%)	0.619	0.450	0.843	0.380	12.618	<0.001*

*Significant results.

The italics are the results which were not significant but showed a tendency ($p < 0.10$).

Table 5 Results of a repeated measures ANOVA of the influence of drinking system and water access on different parameters in 12 rabbits (*Oryctolagus cuniculus*) with 6 h, 12 h and 24 h water access. Rabbits were divided into a group with fresh food in the diet and one without fresh food

Table 6 Contamination and spillage of open dishes in 12 rabbits (*Oryctolagus cuniculus*) during a total of 104 measure days per animal (72 days per animal during adaptation period, 32 days per animal during measure period)

	Adaptation period (9 days)		Measure period (4 days)	
	Contaminated	Spilled	Contaminated	Spilled
Degree	%	%	%	%
No	84	96	58	93
Little	15	3	31	3
Much	1	1	11	4

2009), other prophylactic options than lowering Ca content should be considered. To prevent urolith formation a high TWI is generally advisable (Fritz, 2009) as also shown in human medicine where the recurrence of idiopathic calcium nephrolithiasis could be lowered by increased water intake (Borghini et al., 1996). In our study, TWI was highest with diets consisting of parsley and hay. Other studies in which rabbits were fed fresh food (e.g. grass, carrots) also found an increasing water intake with its addition (Bucher, 1994; Schwabe, 1995; Wenger, 1997; Zumbrock, 2002; Wolf et al., 2008). 'Fresh food' therefore not does not necessarily have to be parsley as in our study. Dry diets stimulated water intake from the drinker but led basically to smaller TWI than diets with a high water content, such as Parsley90. A high food water intake seemed to be more important to achieve a high TWI than only a high water intake from the drinker itself. This is also valid in other species, such as dogs or cats where TWI can be enhanced by increasing the moisture content of the diet (Kane et al., 1981; Stevenson et al., 2003).

Not only water content of the diet influences TWI, there are also marked differences between different dry feeds (Fig. 2). With hay-only TWI was higher than with seed-based diets. Part of this can be explained by the higher DMI with hay-only but there is still a considerable disparity as Hay100 led to much higher TWI:DMI ratios of around 2.7 compared with around 1.5 for Seed90. For dry feeds such as seed mixes and pelleted feeds TWI:DMI of about 2 are reported in rabbits (Wolf et al., 1999); with hay-only diets, TWI:DMI ratios of up to 9.9 can be reached (Zumbrock, 2002). Compared with the other dry rations Hay100 and HRP90 led to high TWI – both of these diets are high in fibre content. Generally diets high in fibre seem to enhance water intake in rabbits (Harkness and Wagner, 1995). Furthermore, the longer chewing time which is spent

for diets high in fibre (Wenger, 1997; Zumbrock, 2002) could lead to more salivation. Another possible reason could be the special separation mechanism in the colon (Björnhag and Snipes, 1999): the chymus in the colon is flushed retrogradely with actively secreted fluid to bring bacteria and small particles back into the caecum for caecotrophe formation. Because of the higher usage of caecotrophes with diets high in fibre (Fekete and Bokori, 1985), more fluid could be required on these diets. Based on the comparison of water intake and urinary Ca levels in hay-only and seed mix-dominated diets (Figs 2 and 5), we must conclude that a hay-only diet, in itself, has a prophylactic effect against urolithiasis.

As parsley and hay increased water intake they also enhanced water excretion, while seed mix and pelleted feed lowered urine excretion and/or led to higher DM contents. Healthy rabbit pro 90 for example, which led to a similar TWI as Hay100, caused much higher urinary DM content than the hay-only diet. This could be explained by the difference in DMI which was notably higher with HRP90 than with Hay100 and thus also leading to a lower TWI:DMI ratio. Hay100 and HRP90 only markedly differed in their physical structure and in their sodium content (Table 2). Interestingly, the 10-fold higher sodium content of HRP90 did not lead to a higher water intake than with Hay100, potentially indicating that the physical structure of the diet is a more important factor for water intake, even if sodium is known as a dipsogenic stimulus in various species (Antunes-Rodrigues et al., 2004). In rabbits, however, sodium chloride only acts dipsogenically under certain circumstances (Denton et al., 1985).

As in other studies, an increasing Ca intake led to higher urinary Ca concentrations (Burger, 2009). Nevertheless, the Ca intake alone must not be considered as the sole decisive factor for urinary Ca levels. For example, the total Ca intake was particularly high in this study in diets with high proportions of fresh parsley, and also on the hay-only diet (Fig. 1), yet the Ca content of the urine was low on these diets due to the high urine volume (Fig. 5). Parsley90 even led to the highest Ca intake but to the lowest urinary Ca concentration, supporting the findings of Wolf et al. (2008). They found that Ca intake with forage (450 mg Ca/day) was higher than with a mixed feed of native components (250 mg Ca/day) but urinary Ca concentration was still lower with forage (0.87 mg Ca/ml urine) than with the mixed feed (1.98 mg Ca/ml urine). In this study, Ca content of urine was not positively

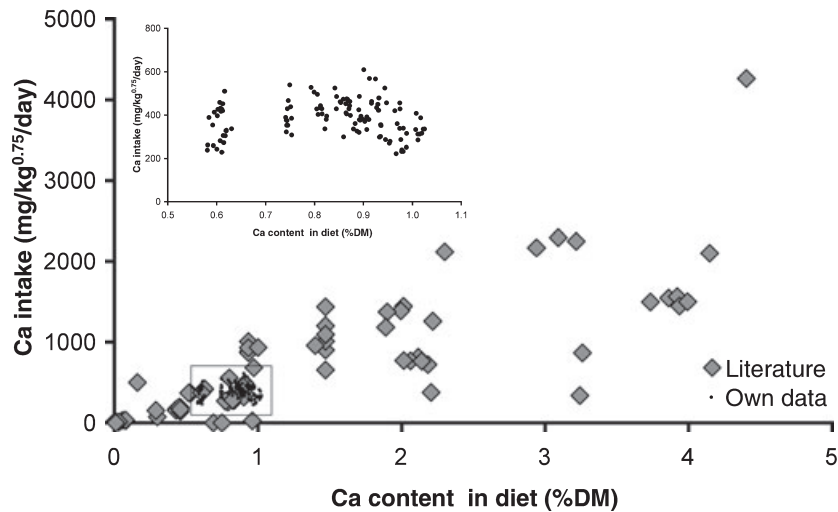


Fig. 6 Calcium intake in rabbits (*Oryctolagus cuniculus*) in relation to the calcium content in the diet. Own data (insert) added to literature data (Bourne and Campbell, 1932; Buss and Bourdeau, 1984; Carstensen, 1984; Bourdeau et al., 1986; Eddy et al., 1986; Kamphues et al., 1986; Barr et al., 1991; Ritskes-Hoitinga et al., 2004; Burger, 2009). Multiple regression: $R = 0.811$, $p < 0.001$, $n = 202$.

correlated to Ca content in the diet, because of the lack of correlation between Ca content of the diet and total Ca intake: Ca intake was higher on low-Ca diets because on these diets, rabbits had a generally higher food intake. However, if our results are added to literature data, the expected pattern of increasing urinary Ca content with increasing dietary Ca levels is nevertheless evident (Fig. 6). Our study differs from many other studies insofar that our diets did not consist of a pelleted diet with varying concentrations of a mineral premix, but were composed of different heterogeneous components as fed in practice. In pet rabbit husbandry, urolith prophylaxis (in terms of preventing high urinary Ca levels) is not a matter of considering dietary Ca levels alone, but ingested amounts of Ca and, particularly, of the overall water intake.

Water restriction

The water restrictions of 12 h and 18 h in this study were chosen to simulate situations in rabbit husbandry where an involuntary water deprivation happens. This condition may possibly occur if water freezes in outdoor husbandry, if the water container is empty and not immediately refilled or if a less dominant animal is prevented from approaching the drinker (Kamphues et al., 2009). Similar to numerous other studies with different types and degrees of water restriction (e.g. Carles and Prud'hon, 1979; Schwabe, 1995; Verdelhan et al., 2004), water intake was also lower in our study when water access was restricted. Water restriction does not only lower water intake, but also interrupts the natural circadian drinking pattern (A. Tschudin, M. Clauss, D.

Codron, M. J. Hatt, unpublished). Restricted water access of 6 h and 12 h did not lead to clinical signs of dehydration in this study, but still compensatory physiological mechanisms were evident: urinary output was lowered and DM content of urine and faeces were increased with water restriction. In contrast, in a study where rabbits had no drinking water access but fresh food *ad libitum*, no significant differences could be found in faeces and urine composition (Schwabe, 1995). In our study, group A did, and group B did not receive fresh food during water restriction trials. Group A exhibited a higher TWI and tended to show more urinary output than group B; nevertheless TWI was reduced in both groups compared with *ad libitum* water access. Even if fresh food enhances TWI, we agree in the recommendation of other authors (e.g. Kamphues and Schulz, 2002; Fritz, 2009) that drinking water should always be accessible *ad libitum* in rabbit husbandry and not be temporarily restricted.

Conclusion

Drinking systems influenced water intake only under conditions of water restriction, but still with nipple drinkers faecal output and DM content pointed into a direction of water conservation. Because of the negative influence of nipple drinkers during water restriction and due to findings of preference trials (A. Tschudin, M. Clauss, D. Codron, M. J. Hatt, unpublished) we recommend offering drinking water in open dishes in rabbit husbandry, best offered on elevated areas. For the purpose of urolith prophylaxis diets consisting of hay and large proportions of 'fresh food' proved to be advisable, and seed

mixes should be avoided. Under water restriction, compensatory mechanisms in kidneys and gut were active and water intake was decreased. For animal welfare as well as for physiological reasons we reject limited water access.

Acknowledgements

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