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Nutritional significance of coprophagy in the rat-like hamster *Tscherskia triton*

Abstract: Coprophagy is widespread among rodent species and has nutritional significance in providing microbial protein to animals via feces. However, studies of coprophagy in rodents have focused mainly on species that are cecal fermenters. In this study using rat-like hamsters (*Tscherskia triton*), which have a large forestomach and cecum, we investigated the contribution of coprophagy to protein nutrition in pregastric and cecal fermenters and also examined whether or not the cecum is involved in protein nutrition enhanced by coprophagy. With or without a forestomach, coprophagy may affect protein digestion in *T. triton*, and coprophagy cannot provide beneficial effects without a cecal contribution. Prevention of coprophagy increased the fecal concentration of crude protein in animals with an intact cecum. Therefore, we conclude that coprophagy is closely related to the cecum in terms of protein nutrition, even in the pregastric and cecal fermenter *T. triton*.

Keywords: cecum; coprophagy; forestomach; protein.

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Introduction

Most omnivorous and herbivorous small rodents are monogastric and commonly possess a well-developed cecum as a fermentation chamber in which they harbor symbiotic microorganisms. In terms of symbiotic nutrition, the cecum is thought to provide microbial protein to the host animals through coprophagy (Torrallardona et al. 1996, Sakaguchi 2003). Thus, studies of coprophagy in rodents have focused mainly on species that are cecal fermenters (Barnes et al. 1957, Hintz 1969, Takahashi and Sakaguchi 1998, Hirakawa 2001). Hamsters and voles have stomachs with multiple chambers and can synthesize protein from urea via microbes in their forestomachs (Sakaguchi et al. 1978). However, the nutritional significance of coprophagy and the related role of the cecum and forestomach have not been well documented in these animals.

In this study, using the rat-like hamster *Tscherskia triton*, which has a large forestomach and cecum, we investigated the role of coprophagy and the function of the forestomach and cecum in nutrition by pregastric and cecal fermenters, and also examined whether or not the cecum is involved in protein nutrition enhanced by coprophagy.

Material and methods

Experimental procedures in this study were examined and approved by the Animal Experimentation Committee at the University of Miyazaki (Permission No. 2005-054-6).

Animals and feeding

Forty adult rat-like hamsters were used for the experiments. The animals were divided into four groups and subjected to sham operation (SHAM), cecum resection (CX), forestomach resection (FX) or resection of the forestomach and cecum (FCX) (Sakaguchi et al. 1981). After the operation, all animals were given 7–8 days to recover. Subsequently, they were divided into two groups in which coprophagy was either allowed or prevented. Thus, there were five individuals in each of eight groups, depending upon the treatment received and whether or not they had been able to engage in coprophagy. Animals capable of coprophagy (coprophagic animals) were placed in ordinary metabolic cages, whereas animals incapable of coprophagy (non-coprophagic animals)

Table 1 Composition of the diet (%).

Moisture	9.2
Crude protein	18.8
Crude fat	3.9
Crude fiber	6.6
Crude ash	6.9

were housed in anti-coprophagy cages (modified after Torrallardona et al. 1996). These cages were constructed of galvanized wire mesh (mesh size, 13 mm), shaped as a tube and suspended from a frame. The hamster's feces fell easily through the 13-mm mesh floor. The diameter of the cages was adjusted so that the hamsters could move about comfortably, but could not turn round, so they were prevented from taking feces directly from the anus. The experiment consisted of an acclimation period of 4–5 days and a subsequent experimental period of 8 days. A powdered commercial diet (Labo MR Stock, Nippon Nosan Corporation, Yokohama, Japan) and distilled water were available *ad libitum* throughout the experiment. The composition of the diet is shown in Table 1. During the experimental period, the animals were weighed every other day, and their food consumption was recorded every day.

Sample preparation and analysis

Feces were collected daily, frozen and stored for analysis. The fecal samples were oven dried at 60°C and ground. Feces and food were analyzed for dry matter (DM) and chemical composition according to the standard method of AOAC (1990). The apparent digestibility of DM and crude protein (CP) (nitrogen×6.25) was calculated by dividing the difference between dietary intake and fecal excretion by dietary intake for each item.

Data analysis

Initial and final body weights during the experimental period were compared statistically using a paired t-test. The effects of forestomach, cecum and coprophagy (independent variables) on DM digestibility, CP digestibility and fecal CP concentrations (dependent variables) were analyzed using a three-way analysis of variance (ANOVA), followed by Tukey's HSD test. The values obtained using the ANOVA are shown in Table 2. A p-value <0.05 was considered significant.

Results

During the experimental period, all animals maintained their body weights, as shown in Table 3. Apparent DM digestibility is shown in Figure 1. CX reduced DM digestibility significantly ($p<0.05$), whereas prevention of coprophagy had no significant effect on DM digestibility in animals with a cecum (SHAM and FX animals). In CX animals, prevention of coprophagy elevated the DM

Table 2 ANOVA for the effects of forestomach, cecum and coprophagy.

Dependent variables			**Main effects**	
	F	**C**	**Cop**	
Apparent digestibility				
Dry matter	0.3814	<0.0001	0.1091	
Crude protein	0.1274	<0.0001	0.0004	
Fecal concentrations				
Crude protein	0.1018	<0.0001	<0.0001	
Dependent variables				**Interactions**
	F×C	**F×Cop**	**C×Cop**	**F×C×Cop**
Apparent digestibility				
Dry matter	0.9297	0.2215	0.0018	0.4385
Crude protein	0.7464	0.6121	<0.0001	0.6503
Fecal concentrations				
Crude protein	0.4801	0.9638	<0.0001	0.8855

Each number shows a p-value. A p-value of <0.05 was taken as significant.
F, forestomach; C, cecum; Cop, coprophagy.

Table 3 Initial and final body weights during the experimental period (g).

	Treatment	Initial body weight	Final body weight	p-Value
Coprophagic	SHAM	135.2±7.4	135.5±5.4	0.9470
	CX	110.5±16.1	112.8±18.0	0.8365
	FX	138.9±8.6	138.6±10.1	0.9598
	FCX	122.1±18.4	122.3±19.8	0.9872
Non-coprophagic	SHAM	134.3±6.9	133.1±7.0	0.7920
	CX	111.2±16.8	110.1±17.8	0.9210
	FX	135.1±11.8	136.0±8.8	0.9019
	FCX	122.2±19.1	124.4±19.6	0.8605

Values are means±SD. SHAM, sham-operated animals; FX, forestomach-resected animals; CX, cecum-resected animals; FCX, forestomach/cecum-resected animals.

digestibility significantly. Meanwhile, FX had no significant effect on DM digestibility.

Apparent CP digestibility is shown in Figure 2. The prevention of coprophagy reduced CP digestibility markedly in SHAM and FX animals but did not affect it in animals without a cecum (CX and FCX animals). FX did not affect CP digestibility significantly.

Fecal CP concentrations are shown in Figure 3. The prevention of coprophagy increased fecal concentrations of CP in SHAM and FX animals, whereas it did not affect fecal CP concentrations in animals without a cecum (CX and FCX animals). The CP concentration in feces was unaffected by FX.

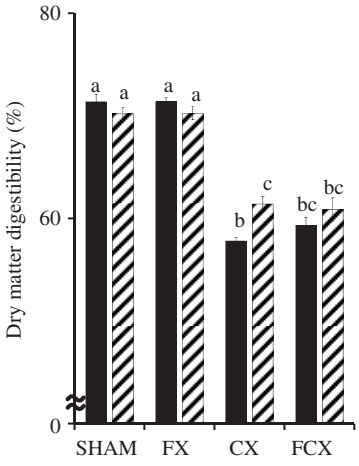

Figure 1 Apparent dry matter digestibility.
■, coprophagic; ▨, non-coprophagic; SHAM, sham-operated animals; FX, forestomach-resected animals; CX, cecum-resected animals; FCX, forestomach-/cecum-resected animals. a, b, c: different letters indicate significant difference ($p<0.05$).

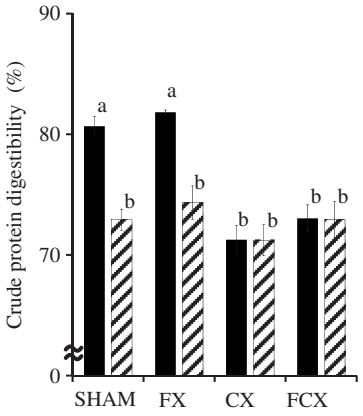

Figure 2 Apparent crude protein digestibility.
■, coprophagic; ▨, non-coprophagic; SHAM, sham-operated animals; FX, forestomach-resected animals; CX, cecum-resected animals; FCX, forestomach-/cecum-resected animals. a,b: different letters indicate significant difference ($p<0.05$).

Discussion

There was no significant change in body weights (Table 3) before and after the experiment, which suggests that stressors from surgery and housing in anti-coprophagy cages may be negligible. The forestomach was not involved in apparent DM digestibility (Figure 1). Thus, the forestomach may not play an important role in the digestion and absorption of food. Similar results have been reported in the golden hamster, *Mesocricetus auratus*, which is a closely related species (Musser and Carleton 1993). However, an *in vitro* study (Banta

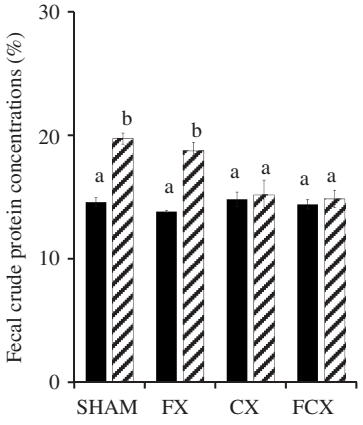

Figure 3 Fecal crude protein concentrations.
■, coprophagic; ▨, non-coprophagic; SHAM, sham-operated animals; FX, forestomach-resected animals; CX, cecum-resected animals; FCX, forestomach-/cecum-resected animals. a,b: different letters indicate significant difference ($p<0.05$).

et al. 1975) comparing inocula from the forestomach and cecum of the golden hamster with that from a bovine rumen revealed that plant cell wall digestibility was identical for the three inocula when alfalfa was used as the substrate. With more-fibrous, lower-quality substrates, such as straw, digestibility values were lower for the forestomach and cecal inocula than those for the bovine inocula (Banta et al. 1975). From these findings, it is likely that the forestomach of the hamster contains microorganisms capable of digesting forage cell walls to some extent. One reason that hamsters do not use the ability of their forestomachs to digest fibrous food is likely to be a shorter stomach retention time compared with ruminant digesta. The retention time of food in the hamster forestomach was reported to be approximately 1 h (Sakaguchi 1991), which may be too short for cell wall digestion by microbial fermentation. In the Norway rat, *Rattus norvegicus*, the retention time of the digesta in the whole stomach (including the forestomach) was reported to be approximately 2 h (Sakaguchi 1991), but under restricted feeding conditions, the ingested food remains much longer in the whole stomach (Robinson and Stephenson 1990). Compared to rats, hamsters have a much bigger and more clearly partitioned forestomach, which has the potential to hold more digesta for a longer time than in rats. If retention time in the forestomach were to be extended, microbial fermentation could contribute more to food digestibility. However, at least under an *ad libitum* feeding regimen, the forestomach does not affect food digestibility significantly in *Tscherskia triton*. CX reduced DM digestibility significantly, irrespective of coprophagy. Unlike the forestomach, the cecum therefore plays an important role in the digestion and absorption of food, even if foods are provided *ad libitum*. In golden hamsters, Banta et al. (1975) showed that fermentation activity in the cecum exceeded that of the forestomach, whereas the retention time of digesta in the cecum was approximately 3–4 h. This retention time was longer than that in the forestomach. Therefore, to some extent, microbial fermentation in the cecum may degrade the non-digestible fraction of DM that is passed through the small intestine into the large bowel, resulting in significantly better digestibility of food in rat-like hamsters. Reduced DM digestibility in coprophagic CX animals is presumably due to low digestibility of the reingested feces. As previously noted, the feces for reingestion produced by a colonic separation mechanism is rich in protein and with high digestibility. CX animals unable to produce such feces in the cecum were forced to reingest feces with a high non-digestible fraction. Therefore, the increased intake of a non-digestible fraction leads to a reduction in the apparent digestibility of food and DM (Zhao et al. 1995, Pei et al. 2001). Non-significant differences between coprophagic and non-coprophagic animals subjected to SHAM and FX suggest that metabolic fecal products derived from the cecum, which could be utilized as protein to some extent via coprophagy, might not be reflected by changes in DM digestibility.

In SHAM and FX animals with cecal function intact, apparent CP digestibility (Figure 2) was lowered and fecal CP concentrations (Figure 3) were elevated by prevention of coprophagy. As the increase in fecal CP concentrations reflects endogenous CP not utilized by reingestion, the reduction in apparent CP digestibility by the non-coprophagic animals could be due to the increased CP excretion derived from endogenous products, including intestinal microbial protein. However, in (CX and FCX) animals with the cecum removed, there was no difference in CP digestibility or fecal CP concentration between coprophagic and non-coprophagic animals. These results are interpreted as meaning that the main fermentation chamber in the large bowel was deprived by CX and that intestinal microbial CP production may have been depleted. Another explanation is that CX may cause a drastic reduction in coprophagic frequency so that animals reingest ordinary feces, which are not rich in protein. As a result, the effects due to coprophagy may disappear. Consequently, there is no benefit to CP nutrition if animals lack either cecum or coprophagy.

CX reduced protein digestibility in coprophagic animals. Small herbivores, such as rabbits, could operate retrograde transport of fine particle digesta and bacteria with the fluid phase, which are linsed from the larger particle phase through proximal colon function. Such a function is known as a colonic separation mechanism (Sperber 1985, Björnhag 1987). Although inferior to the rabbit, a similar function was reported in small rodents. Rodents have folds in the proximal colon that create furrows between them. Bacteria and only a few food particles are trapped in the mucus in the furrow and transported from the proximal colon to the cecum through the furrow by antiperistaltic movement of this part of the proximal colon (Sperber et al. 1983). This type of colonic separation mechanism is called a 'mucus-trap' type (Cork et al. 1999) and results in selective retention mainly of bacteria in the cecum. Retained bacteria are excreted as soft feces that contain a lot of bacteria and are rich in protein. The habit of producing and eating distinctive soft feces has a beneficial effect on protein uptake. However, CX deprives animals of the ability to produce special feces rich in protein. Consequently, beneficial effects on CP nutrition are not gained if animals lack either a cecum

or coprophagy. Our experiment suggests that rat-like hamsters may have a colonic separation mechanism and reingest soft feces. As no relationship between coprophagy and the forestomach was found, we conclude that, similar to cecal fermenters, coprophagy affects protein alimentation of the rat-like hamster and that, without cecal function, coprophagy does not provide any nutritive benefit.

However, this does not imply that all cecal function is involved in coprophagy.

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References

AOAC International. 1990. Official methods of analysis of AOAC international, 15th ed., AOAC International, Gaithersburg, MD.

Banta, C.A., R.G. Warner and J.B. Robertson. 1975. Protein nutrition of the golden hamster. J. Nutr. 105: 38–45.

Barnes, R.H., E. Kwong and G. Fiala. 1957. Effects of the prevention of coprophagy in the rat III. Digestibility of protein and fat. J. Nutr. 65: 251–258.

Björnhag, G. 1987. Comparative aspects of digestion in the hindgut of mammals. The colonic separator mechanism (CSM). DTW Dtsch. Tierarztl. Wochenschr. 94: 33–36.

Cork, S.J., I.D. Hume and G.J. Faichney. 1999. Digestive strategies of non-ruminant herbivores. The role of the hindgut. In: (H.J.G. Jung and G.C. Fahey Jr., eds.) Nutritional ecology of herbivores. American Society of Animal Science, Savoy. pp.210–260.

Hintz, M.F. 1969. Effect of coprophagy on digestion and mineral excretion in guinea pig. J. Nutr. 99: 375–378.

Hirakawa, H. 2001. Coprophagy in leporids and other mammalian herbivores. Mammal Rev. 31: 61–80.

Musser, G.G. and M.D. Carleton. 1993. Family Muridae. In: (D.E. Wilson and D.M. Reeder, eds.) Mammal species of the world. 2nd ed. Smithsonian Institute Press, Washington and London. pp. 501–755.

Pei, Y.X., D.H. Wang and I.D. Hume. 2001. Selective digesta retention and coprophagy in Brandt's vole (*Microtus brandti*). J. Comp. Physiol. [B] 171: 457–464.

Robinson, P.H. and J.S. Stephenson. 1990. Dietary restriction delays gastric emptying in rats. Appetite 14: 193–201.

Sakaguchi, E., M. Horiguchi and T. Matsumoto. 1978. Effect of genistin on the utilization of ^{15}N-urea in the lactating hamster. Jpn. J. Zootech. Sci. 49: 653–658.

Sakaguchi, E., J. Itoh, H. Shinohara and T. Matsumoto. 1981. Effects of removal of the forestomach and caecum on the utilization of dietary urea in golden hamsters (*Mesocricetus auratus*) given two diets. Br. J. Nutr. 46: 503–512.

Sakaguchi, E. 1991. Comparative aspects of fiber digestion and digesta transit in cecum fermenters. In: (T. Sakata and R.L. Snipes, eds.) Hindgut 91. Senshu Univ. Press, Tokyo. pp. 49–69.

Sakaguchi, E. 2003. Digestive strategies of small hindgut fermenters. Anim. Sci. J. 74: 327–337.

Sperber, I., G. Björnhag and Y. Ridderstrale. 1983. Function of proximal colon in lemming and rat. Swed. J. Agr. Res. 13: 243–256.

Sperber, I. 1985. Colonic separation mechanism (CSM). Acta Physiol. Scand. 124: 87.

Takahashi, T. and E. Sakaguchi. 1998. Behaviors and nutritional importance of coprophagy in captive adult and young nutrias (*Myocastor coypus*). J. Comp. Physiol. [B] 168: 281–288.

Torrallardona, D., C.I. Harris and M.F. Fuller, 1996. Microbial amino acid synthesis and utilization in rats: the role of coprophagy. Br. J. Nutr. 76: 701–709.

Zhao, X., H. Jorgensen and B.O. Eggum. 1995. The influence of dietary fibre on body composition, visceral organ weight, digestibility and energy balance in rats housed in different thermal environments. Br. J. Nutr. 73: 687–699.