CECAL FERMENTATION IN THE WILLOW PTARMIGAN

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Gallinaceous birds have two relatively large ceca arising from the junction of the small and the large intestines. The size of these organs has led to speculation regarding their role in digestion of cellulose and other resistant materials (Farner 1960). Suomalainen and Arhimo (1945) showed by the use of enrichment cultures that cellulose fermentation could be induced by inoculation with cecal content from several grouse species, including the Willow Ptarmigan, *Lagopus lagopus*. The responsible bacteria were not isolated.

The Willow Ptarmigan in northern Alaska subsist on a diet composed almost exclusively of willow (*Salix*) buds and twigs from October until May (West and Meng 1966). It was thought that this limited diet should select for a specialized microbial population in the ceca and that the apparently low concentration of soluble nutrients might make such birds dependent upon absorption of cecal fermentation products for part of their maintenance energy.

PROCEDURE

COLLECTION

Ptarmigan were collected by shooting in the vicinity of Umiat, Alaska. Collecting dates were from 20 to 23 March 1965; chance dictated the time of day, but it varied from 09:30 to 17:20. All birds were mature males since the females and most of the juvenile males migrate south of the Brooks Range before winter (Irving et al. 1967). Field work was done in a tracked vehicle enclosed in order to minimize the effect of cooling upon the microbial population and the fermentation rates.

FERMENTATION RATES

A modification of a procedure developed for the study of the elk rumen fermentation was used. The ceca were removed immediately following the death of the bird. The tip one-third of one cecum was cut off, placed in a plastic bag, and quickly cooled in snow to stop the fermentation. The remainder was placed in another plastic bag and incubated in a vacuum bottle containing water at about 40°C. At two successive intervals, varying from 10 to 30 minutes according to circumstances, the remaining two-thirds of that cecum were removed and cooled in snow. The times of death of the bird and of the three sample collections were recorded. Upon return to the laboratory building the contents from each section were squeezed into a weighed tube containing 9 ml of 0.1N H_2SO_4. The amount of the sample was determined by reweighing. This preserved material was examined for volatile fatty acids, lactic and succinic acids, and ethanol after returning to Bozeman. The total volatile acids were determined by titration following steam distillation. The amounts of the individual volatile acids were determined by gas chromatography (Johnson and McBee 1967). Examination for succinic acid was by thin-layer chromatography. Lactic acid was determined by the method of Barker and Summerson (1941). Ethanol was measured by the diffusion method of Winnick (1942).

The concentrations of the fermentation products found in the three portions of each cecum were plotted against the time of incubation and the curves extrapolated back to the time of death in order to determine the rate of fermentation in the bird at the time it was killed. This zero-time method has been used effectively in studies of the rumen (Carroll and Hungate 1954) and rodent cecal fermentation (Johnson and McBee 1967). Narrow-range indicator paper was used to measure *p*H.

MICROBIAL POPULATION

A field laboratory set up in a bunk house was used for cultural and microscopic examination

TABLE 1. Representative average body weight and cecal size of Willow Ptarmigan on willow diet collected in the Brooks Range, Alaska.

Sex	Age	No. of birds	Avg. body wt. g	Avg. length each cecum cm	Avg. diameter cm	Avg. total wt. g	Avg. total vol. ml	% body wt.
M	Adult	6	625.2	55.2	0.7	28.4	20.1	4.54
M	Juvenile	4	585.6	55.0	0.7	29.2	21.1	4.99
F	Adult	5	523.4	53.0	0.7	26.2	20.4	5.01
F	Juvenile	5	524.4	55.0	0.7	31.0	21.1	5.91

of the digestive system for microorganisms. The culture procedure used the anaerobic roll tube method (Hungate 1950) with Bryant and Robinson's (1961) medium to which had been added 0.1 per cent salicin. The salicin was added because it was thought that the willow diet might be relatively rich in salicin and thus yield a microflora using salicin instead of some of the simple sugars. The initial dilution was made by placing a sample of the digestive system content in a previously weighed tube containing 9 ml of anaerobic diluting fluid. This was considered to be a 10-fold dilution but the tube was reweighed to determine the actual dilution. Serial dilutions were prepared and culture counts made in triplicate from the 10^6, 10^7, and 10^8 dilutions. A 5-ml portion of the initial dilution was mixed with an equal quantity of a 10 per cent aqueous solution of formaldehyde and saved for later direct microscopic counts. The cultures were incubated at about 42°C in a covered water bath while at Umiat and later in a 42°C incubator at the University of Alaska and at Bozeman. The cultures cooled to room and airplane temperatures during travel. Colonies were counted at 7 to 10 days of growth. A total of 150 colonies were picked at random from the 10^8 dilution cecal cultures of two birds and transferred to anaerobic slants of Bryant and Robinson's medium plus salicin. Colony descriptions, cell morphology, and several other characteristics were recorded for each culture.

Direct microscopic counts were made on dried films prepared by spreading 0.01 ml of a 10^2 or 10^3 dilution over an area of one square centimeter and mixing with a small drop of nigrosin.

RESULTS AND DISCUSSION

Examination of the crop showed that it was filled with willow buds and twig tips and was dry. There was no evidence of bacterial action there or in any section of the digestive tract other than the ceca. The small intestinal flora consisted exclusively of gram-positive cocci, less than 10^6 per gram. This concentration is too small to have any significant effect upon the small intestine digestion through enzyme production. Owing to the mechanical action of the gizzard, the contents of the small intestine were composed of a mixture of roughage and paste. The paste-like material was nearly all diverted into the ceca and the well-cleaned roughage all went into the large intestine. A comparable sorting of materials at the junction of the ceca with the small and large intestine was observed by Hungate (personal communication) when dried beet pulp was added to the diet of chickens. The beet pulp particles did not enter the ceca.

The gizzard action removes the bark and cambium layers from the willow twigs, leaving the central dowel-like wood core essentially unchanged. Therefore, it is assumed that the sources of energy for the bird are materials from the buds and from the bark and cambium of the twigs. The woody portion does not appear to be digested. Although birds were not collected at all hours, none of those examined had anything but a relatively dry collection of roughage in the large intestine.

Ptarmigan in captivity appear to empty the ceca only once a day. This conclusion is drawn from the observation that a single large dark dropping is excreted during the night. All other droppings consist of coarse or fibrous materials. Therefore, the ceca must fill only once a day. Data on ptarmigan ceca collected under similar conditions show them to have a total volume of about 20 ml (table 1). Whether this total volume of material is turned over daily or whether a significant portion remains for more than one day is not

TABLE 2. Concentrations of cecal fermentation products at time of death, in millimoles/100 g cecal contents.

Bird	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Ethanol
3	0.65	0.0	0.0	0.0	0.0
4	1.9	1.75	0.18	0.0	0.0
5	2.5	1.3	0.30	0.0	2.3
6	4.75	0.0	0.19	0.57	9.4
7	6.2	2.2	0.25	0.91	8.9
8	4.6	1.3	0.88	0.74	6.8
9	5.0	0.0	0.69	1.1	13.0
10	3.25	1.1	0.11	0.28	6.8

TABLE 3. Cecal fermentation rates, mmoles/100 g cecal contents per hour.

Bird	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Ethanol
3	3.85	2.6	0.93	0.59	6.1
4	0.9	1.1	0.30	0.72	3.7
5	2.5	–	0.17	0.44	0.5
6	0.0	0.0	0.61	0.34	0.0
7	0.0	0.0	0.20	0.0	0.0
8	0.0	0.4	0.0	0.0	0.0
9	0.0	1.1	0.84	0.0	0.0
10	2.25	0.0	0.04	0.0	0.0

known. Other than a gross analysis of willow buds, which showed them to be about 12 per cent protein, 8 per cent fat, 3 per cent ash and 77 per cent carbohydrate, little is known of the digestability of willow buds and twigs. It is assumed that the protein, fat, and soluble carbohydrates would be digested and absorbed from the small intestine, leaving only the difficultly digestible materials for bacterial action in the ceca. In rodents (Johnson and McBee 1967) no free amino acids or sugars passed from the small intestine into the cecum.

Measurable amounts of fermentation products and significant numbers of bacteria were found only in the ceca. The fermentation products found were ethanol and lactic, acetic, propionic, and butyric acids. No succinic acid was found. The concentrations of these products at the time of death (table 2) were calculated by extrapolating concentrations found after incubation for known times back to time zero (the time of death). In most instances this concentration did not change appreciably during the incubation period but instead remained nearly constant or had a very slight downward trend (table 3).

The bacterial counts of the cecal content averaged 1.4×10^{11} per gram microscopically whereas the culture counts were only about 1 per cent of this figure (1.6×10^{9}, table 4). Only 18 of the 150 colonies grew on subculture. These were mainly gram-negative nonmotile rods having characteristics of the genus *Bacteroides*, such as bipolar staining. None of them grew in glucose media. One culture was a mixture of gram-negative rods and gram-positive cocci in clusters; one was a gram positive coccus and one a small gram-positive rod. The 17 apparently pure cultures could be subcultured on the isolation medium, but only three grew in fluid carbohydrate media (table 5).

TABLE 4. Bacterial counts in cecal contents.

Bird	Colony counts/gram	Microscopic counts, cells/gram
1	4.5×10^{8}	4.9×10^{10}
2	1.5×10^{8}	5.4×10^{10}
3	2.4×10^{9}	2.0×10^{11}
4	8.7×10^{9}	2.4×10^{11}
5	3.5×10^{9}	2.0×10^{11}
6	7.1×10^{8}	—
7	1.1×10^{9}	1.5×10^{11}
8	2.0×10^{8}	1.5×10^{11}
9	5.1×10^{8}	1.4×10^{11}
10	4.2×10^{8}	1.1×10^{10}
11	6.2×10^{8}	1.8×10^{11}
12	1.0×10^{9}	1.3×10^{11}
Avg.	1.6×10^{9}	1.4×10^{11}

The majority of the bacteria cultured appeared to be bacteroides-like. These were generally the most numerous single type of cecal bacteria, but never constituted a majority of the bacteria in the cecum. Microscopic examination showed five major morphological groups: gram-positive, slender, straight rods; gram-positive, slender, curved rods; large, gram-negative, curved rods of the size of *Spirillum serpens* or a *Crustospira*; a bacteroides-like gram-negative rod; and cocci of both gram reactions. The relative proportions of these are given in table 6. Only the large spiral appeared to be a unique organism. The failure to culture the majority of the bacteria may have been due to the repeated heating and cooling between Umiat and Bozeman or may have been due to inadequate culture techniques. Dr. John Johnson had similarly poor results when attempting to cultivate the bacteria from the cecum of the brown lemming, *Lemmus trimucronatus*, (unpublished data) even though his technique was adequate for culturing bovine and elk rumen bacteria.

The fermentation rate studies yielded re-

TABLE 5. Characteristics of pure cultures isolated from ptarmigan cecum.

Culture type	Number of cultures	Growth in fluid carbohydrate media				
		Glucose	Xylose	Cellobiose	Starch	Salicin
Gram-neg oval rods	13	—	—	—	—	—
Gram-neg oval rods	1	—	—	+	—	—
Gram-neg oval rods	1	—	—	—	+	—
Gram-neg cocci	1	—	+	+	—	+
Gram-pos slender rod	1	—	—	—	—	—

TABLE 6. Abundance (in per cent) of morphological groups of cecal bacteria.

Bird	Gram + slender rods	Gram + curved rods	Gram — large spirals	Gram — oval rods[a]	Gram + and gram — cocci
1	24.7	30.7	22.3	19.9	2.4
2	16.4	18.6	50.0	12.5	5.5
4	26.3	20.7	7.4	34.2	11.4
5	22.3	23.0	5.9	34.8	14.0
7	24.4	16.0	2.3	42.0	15.3
8	23.8	11.1	14.3	38.1	12.7
9	15.4	11.5	15.4	37.5	20.2
10	28.1	12.4	6.7	42.7	10.1
11	19.1	13.9	8.7	30.4	27.9
12	15.3	13.6	9.3	44.9	16.9
Avg.	21.6	17.2	14.2	33.7	13.6

[a] Bacteroides-like.

sults that require some explanation. It was assumed on the basis of studies of the rodent cecum (Johnson and McBee 1967) that the contents of the cecum would be uniformly mixed. The dimensions of the ptarmigan ceca, however, may prevent this. They are each about 50 cm long and less than 1 cm in diameter (table 1). A relatively drier layer of material appeared to coat the inside wall of the cecum. The concentrations of fermentation products in all portions of each cecum may not have been the same at the time of death. The first sample was taken from the tip one-third, the second from the middle section and the third from the portion nearest the illeocecal valve. In birds 6 through 10 these three samples showed little if any difference in amounts of fermentation products even though two of them had been incubated. This could be interpreted as evidence that the cecal contents are not uniform in composition; i.e., fermentation product concentrations were initially lower in portions two and three and required incubation to reach the concentration found initially in the tip.

A second interpretation of the results could be that the contents were uniformly mixed but that no fermentation was occurring. Since the product concentrations were quite high this would also imply that product absorption was not occurring. This interpretation would lead to the conclusion that the fermentation was inhibited by the concentration of the products and thus arrested when blood levels of the fermentation products prevented further absorption. This would give a feed-back control of the fermentation rate. The rapid fermentation from birds 3, 4, and 5, however, produced product concentrations above those found in other birds, so product inhibition is probably not the explanation of the results found unless it occurs only following prolonged elevation of product concentration.

TABLE 7. Energy available from cecal fermentation products.

Bird	Calories/hr per 100 g cecal contents					
	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Ethanol	Total
3	805	955	504	192	2000	4456
4	188	404	163	235	1215	2205
5	522	–	99	143	164	928

Another possible explanation could be dependent upon the time of day of collecting and the cycle of filling and emptying of the ceca. Unfortunately little is known about this schedule except that it is a daily affair. It may be important that the three birds with high fermentation rates were all killed at about 17:00 whereas the other birds were killed between 09:30 and 14:05. This might support the theory of lack of uniform mixing during the cecal filling phase.

Whether any of these three theories has any support in fact will require more work. The rates could not be dependent upon the total bacterial numbers present since only minor differences were found between the microscopic counts of birds taken at different times. The culture counts of the birds having the highest fermentation rates were about 10 times those of the birds with no measurable fermentation. This may or may not be significant.

The fermentation rates in birds 3–5 could, if maintained for a significant period, provide a significant portion of the energy requirements of the bird (table 7). The average weight of cecal contents from adult male birds collected was 20 g. At the fermentation rates of birds 3, 4, and 5 a cecum of this size would then yield 891, 441, and 186 calories per hour, respectively.

A partial evaluation of the role of the cecal fermentation in the total energy budget of the Willow Ptarmigan can be made on the basis of known maintenance energy requirements of caged birds. It can be assumed from the consistency of night droppings that the bird fills and empties the ceca once each day and that the fermentation products are absorbed at approximately the same rate as produced, as was found in the rabbit cecum (Bailey and McBee 1964). Willow Ptarmigan living in small cages outdoors in Fairbanks, Alaska, maintained an average energy balance throughout the winter of 119 kcal/bird per 24 hrs, or 4.96 kcal/bird per hour (West et al.

1967). Birds tested at night during winter but at thermoneutral temperatures had a basal metabolic level of 2.97 kcal/bird per hour (West, unpublished data).

For birds 3, 4, and 5, the contribution of the cecal fermentation rate shown in table 3 would be about 30.0, 14.8, and 6.2 per cent of the hourly basal energy requirement at night, or 17.9, 8.8, and 3.7 per cent of the hourly maintenance energy level of birds in outdoor cages. With the increased energy requirement of free existence these contributions may well be reduced by 50 per cent.

A thorough evaluation of the cecal fermentation will require more complete knowledge of (1) the rate at which the ceca are filled, (2) the duration of significant cecal fermentation rates, and (3) the efficiency of absorption.

We still do not know the duration of significant fermentation rates or the rates of absorption. There is, however, some evidence that the absorption rate may influence the fermentation rate since in most instances where a high fermentation rate was found (table 3) the concentration of material found at death was low. If such a feedback mechanism for controlling fermentation rate were available, it would prevent an overloading of the liver with materials to be converted to reserve food materials. Blood levels of fermentation products determined at the time of death would help to establish whether such a control over fermentation rate were actually active. A complete evaluation of the energy value of the cecal fermentation requires further data.

Less can be said about the microbial flora since only 1 per cent of the microscopically visible flora grew in rolled tube cultures and only about 10 per cent of these grew on subculture. These, as might be expected, were bacteroides-like. Species were not identified. It is quite likely that organisms unique to the ptarmigan were not cultivated under the conditions used. It should be mentioned that cecal smears from White-tailed Ptarmigan collected during the winter in Colorado showed a microbial flora strikingly different from those collected in Alaska. Further cultures will be necessary to identify the important cecal bacteria of the ptarmigan and their roles in the cecal fermentation.

SUMMARY

The ceca of 12 Willow Ptarmigan collected at Umiat, Alaska, following several months on their winter diet of willow buds and twigs were examined for fermentation products, rate of fermentation, and bacterial types. The majority of the bacterial flora could not be cultivated. None could be identified to species. The principal fermentation products were ethanol and acetic, propionic, butyric and lactic acids in various concentrations. Active production of these materials was found only in birds killed late in the day, at about 17:00. In these birds, the rate found would provide from 6.0 to 30.0 per cent of the basal energy requirement of winter birds per hour of fermentation.

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