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A COMPARISON OF THE MICROBIOLOGICAL
CONDITIONS IN THE SMALL INTESTINE
AND CAECA OF WILD AND CAPTIVE
WILLOW GROUSE
(LAGOPUS LAGOPUS LAGOPUS)*

By

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HANSEN, INGOLF: *A comparison of the microbiological conditions in the small intestine and caeca of wild and captive willow grouse (Lagopus lagopus lagopus)*. Acta vet. scand. 1979, 20, 365—371. — The study compares the microbiological conditions in the small intestine and caeca of captive and wild willow grouse. The small intestine of wild willow grouse scarcely contained bacteria, while the caeca, without exception, contained high numbers of microorganisms including spirochetes, small gram-negative anaerobe rods, flagellates and amoebae. In 50 % of the birds a low number of *E. coli* was found in the caeca. The types, numbers and distribution of intestinal microorganisms of captive willow grouse were very similar to that of the domestic fowl and thus quite unlike that of the wild grouse.

These results help to explain why captive grouse digest natural food less efficiently than wild birds. Hence captive grouse should not be used in experiments which aim to clarify digestive capacity and functions in the wild grouse.

bacteria; spirochetes; flagellates; amoebae; gut;
willow grouse.

Digestion and fermentation by microbes have been demonstrated in the caeca of wild willow grouse and ptarmigan (*Suomalainen & Arhimo 1945, McBee & West 1969, Gasaway 1976a, b*). A reduced gut size in captive red grouse, willow grouse and rock ptarmigan compared to wild birds has been demonstrated

* The project was financially supported by the Agricultural Research Council of Norway.

(Moss 1972, Gasaway *et al.* 1976, Gasaway 1976a, Hanssen 1979). This reduction in size is associated with reduced digestion and fermentation of dry matter in the caecum of captive ptarmigan (Gasaway 1976a, Gasaway *et al.*) and red grouse (Moss 1977). Changes in gut microflora and fauna accompanying morphological and functional changes in the gut of grouse and ptarmigan held in captivity have not previously been investigated. It is recently reported (Hanssen) that the caecal epithelium of the wild willow grouse contains large numbers of spiral-shaped microorganisms and amoebae which are absent in captive birds. This finding indicates differences in gut microbiology between captive and wild willow grouse which may be responsible for changes in caecal function, thus limiting the use of captive birds for the development of digestive models for wild grouse. The present study describes microbiological differences in the small intestine and caeca in wild and captive willow grouse.

MATERIAL AND METHODS

The material consisted of the same three groups of adult willow grouse described by Hanssen (1979). Hereafter they will be referred to as captive, summer or winter birds. The captive birds consisted of one wild-caught adult (♀) kept five months in captivity on concentrates before being killed, and five (2♀/3♂) hatched and raised in captivity. These birds were killed by a blow on the head. The summer and winter birds were wild grouse. The winter birds included eight (3♀/5♂) shot in the winter (March/April) on Karlsøy and Ringvassøy islands, latitude about 70°N, and the summer birds consisted of nine (2♀/7♂) shot at the same locations in the summer (July).

The captive birds were eviscerated and examined immediately after being killed. The winter birds were brought to the laboratory unopened to avoid freezing, while the summer birds were aseptically eviscerated in the field immediately after shooting. All wild birds were brought to the laboratory within 10–18 h after being shot.

The small intestines were opened from the duodenum to the ileo-caecal-colic (I-C-C)-junction. The caeca were opened from the proximal ends. Specimens for qualitative bacteriological investigation were taken from the gut contents in the duodenum (5 cm posterior to the gizzard-duodenal junction), in the ileum

(5 cm anterior to the I-C-C-junction) and in the middle caecum. The specimens were taken using an inoculating loop to streak out the material on 5 % ox-blood agar plates and bromthymolbluelactose agar plates (*Nordic Committee on Food Analysis* 1969). Parallels of blood agar plates were incubated aerobically for 24 h at 37°C, and anaerobically (Gas Pak System) for five-seven days before reading. The bromthymolbluelactose agar plates were incubated aerobically at 37°C and read after 24 h. For cultivation of the microorganisms in the caecal contents of the wild willow grouse, the medium described by *Bryant & Robinson* (1961) augmented with 0.1 % salicin (*McBee & West* 1969) was used. Trypticase soy agar with 5 % ox-blood (*Kinyon & Harris* 1974) was used for cultivating the spiral-shaped microorganisms in the caeca of the wild willow grouse. The latter two media were incubated anaerobically for five-seven days. The isolated bacteria were classified into families, genera and species on the basis of cultural, morphological and biochemical properties (*Bergey's manual of determinative bacteriology*, 1974).

Quantitative bacteriological investigations were performed by taking 1.0 g samples of gut contents from the first and second half of the small intestine and from the caeca. These samples were diluted 10^{-2} , 10^{-4} , 10^{-6} and 10^{-8} in 0.9 % saline, and 0.1 ml of the dilutions were transferred onto the surface of blood agar plates and incubated for 24 h aerobically and five-seven days anaerobically at 37°C before being read. For some of the wild birds direct microscopic counts were made on gram-stained dried films, prepared by spreading 10 μ l of the 10^{-2} or 10^{-4} dilution over an area of 1 cm².

RESULTS

The quantitative bacteriological data are summarized in Table 1. In the captive willow grouse bacteria were not demonstrated in the contents of the anterior small intestine, but bacteria were constantly present in the posterior small intestine. All the captive birds had a large number of bacteria in the caeca. Both in the caeca and posterior small intestine the microflora was mainly coliforms, enterococci and *Bacterioides* spp. with some contribution from *Lactobacillus* spp., *Bacillus* spp., yeasts and *Clostridium* perfringens.

In the wild willow grouse very few bacteria were noted in

Table 1. Mean number of bacteria in contents from the anterior (A) and posterior (P) small intestine and the caeca (C) of willow grouse (ranges in parentheses, N means none).

Groups	Number of birds	Log ₁₀ viable counts per g			Log ₁₀ microscopic counts, cells per g		
		A	P	C	A	P	C
Captive birds	6	N	4.8 (3.7—6.3)	9.8 (8.5—11.0)	Not counted		
Wild summer birds	8	N	N	3.6 (N—6.4)	N	N	8.7**
Wild winter birds	9	N	N—3.9*	(N—6.2)	N—5.1*	N	9.3 (8.7—9.7)

* Bacteria recognized in only one of the birds.

** Microscopic count made only for one bird in the group.

the small intestine. Viable counts from all caecal content specimens were low, but microscopic examinations revealed, without exception, a high number of microorganisms here. These microorganisms were spiral-shaped (most of them 20—40 μ , some up to 80 μ long) (Fig. 1), small gram-negative rods, singly and

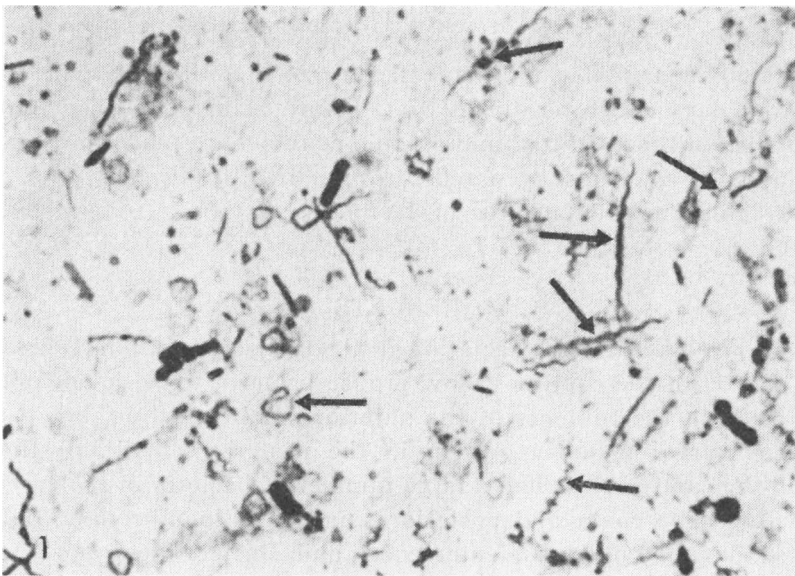


Figure 1. Gram-stained preparation from caecum of wild willow grouse showing spiral-shaped microorganisms (arrows) of different morphological types. 1350 \times .

in chains, and flagellates and amoebae. In 50 % of the wild birds *E. coli* was demonstrated in low numbers. The small gram-negative rods grew as small pin-point colonies on 5 % ox-blood agar, trypticase soy agar with 5 % ox-blood (*Kinyon & Harris* 1974), and Bryant & Robinson's medium supplied with 0.1 % salicin (*McBee & West* 1969) after anaerobic incubation for five-seven days. Growth of spiral-shaped microorganisms was never demonstrated. Due to the methods employed seasonal variation in gut microflora could not be investigated.

DISCUSSION

The wild willow grouse feed on a low-quality, fiber-rich diet (*Moss & Hanssen*, in press) and there is reason to believe that it relies upon a specialized microbial digestion. The present study revealed a complex society of microorganisms in the caeca of the wild willow grouse. These microorganisms were spirochetes, which have been previously recognized in the caeca contents of red grouse (*Fantham* 1910) and Norwegian willow grouse (*Brinkmann* 1922), and flagellates and amoebae previously found in the caecal contents of red grouse (*Fantham*). The mucous filaments observed in the caecal epithelium of several wild browsing gallinaceous species (*Schumacher* 1922, 1925) were recently shown to be spirochetes (*Hanssen* 1979). Hence there is reason to believe that the spirochetes, flagellates and amoebae are autochthonous microbes of browsing gallinaceous birds.

The captive grouse were fed a diet very similar to commercial chicken food (*Hanssen*) and this has generated a gut microflora very similar to that of the domestic fowl, both with regard to types, numbers and distribution (*Barnes* 1972). The main gut and caecal microorganisms were *Bacteroides* spp. and facultative anaerobic microbes — coliforms and enterococci, while spirochetes, flagellates and amoebae were never demonstrated. This finding, combined with the extensive differences in caecal morphology of captive and wild willow grouse (*Hanssen*) explains why captive grouse digest natural food, and especially cellulose and lignin, only half as efficiently as wild ones, and cannot survive on it (*Moss* 1977, *Moss & Hanssen*). Experiments aimed at understanding digestive capacity and functions of wild grouse should thus be carried out on wild birds. These

birds should preferably be kept gnotobiotically on natural food, or be used very soon after capture as gut microbiology and morphology (Hanssen) probably begin to change rapidly. Within four-five months gut morphology and microbiology will be similar to that of birds kept their entire lives in captivity.

ACKNOWLEDGEMENT

I thank Dr. O. Sandvik for his encouraging support when I started this project, and Drs. J. B. Steen, K. Fossum, H. J. Grav and H. Parker for their valuable criticism of the manuscript.

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SAMMENDRAG

*Komparative studier av de mikrobiologiske forholdene i tynntarmen og blindtarmene hos ville liryper (*Lagopus lagopus lagopus*) og hos liryper holdt i fangenskap.*

Det er foretatt en sammenligning av de mikrobiologiske forholdene i tynntarmen og blindtarmene hos ville liryper og liryper som har vært holdt i fangenskap. I tynntarmen hos ville ryper ble det som regel ikke påvist mikroorganismer, mens det i blindtarmene alltid fantes et stort antall. Disse mikrobene var spirocheter, små gram-negative, anaerobe staver, flagellater, amøber og hos enkelte ryper et lavt antal *E. coli*. Hos liryper holdt i fangenskap fant en at tarmfloraen var svært lik den som er beskrevet for kylling både med hensyn til typer, antall og utbredelse. Ville ryper og ryper som har vært holdt i fangenskap har således helt forskjellig tarmflora. Dette forholdet, sammen med forskjellene i tarm-morfologi, er sannsynligvis årsaken til at ryper i fangenskap ikke fordøyer naturlig rypemat så godt som de ville rypene. Ryper som er oppdrettet eller har vært holdt i fangenskap over lengere tid bør derfor ikke brukes i eksperimenter som har som siktemål å avsløre fordøyelsesfunksjoner og -kapasiteter hos ville ryper.

(Received December 21, 1978).

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