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From the Section of Physiology, Institute of Medical Biology, University of Tromsø, and the Department of Anatomy, Veterinary College of Norway, Oslo, and the National Veterinary Institute, Oslo, Norway.

MICROMORPHOLOGICAL STUDIES ON THE SMALL INTESTINE AND CAECA IN WILD AND CAPTIVE WILLOW GROUSE (LAGOPUS LAGOPUS LAGOPUS)*

By

Ingolf Hanssen

HANSSEN, INGOLF: *Micromorphological studies on the small intestine and caeca in wild and captive willow grouse (Lagopus lagopus lagopus)*. Acta vet. scand. 1979, *20*, 351—364. — The present study describes the micromorphology of the small intestine and caeca of wild and captive willow grouse. The micromorphology of the small intestine was similar in wild and captive birds, while typical differences were apparent in the caeca. Wild grouse had ciliated epithelium without goblet cells in the neck part of the caeca, captive birds had strongly atrophied cilia and a high number of goblet cells. The epithelium of the body part of caeca of wild birds lodged a great number of spiral-shaped microorganisms and amoebae, which were absent in captive birds. Both the caecal villi and the longitudinal folds were much larger in wild than captive grouse. In the captive grouse the caecal lamina propria was heavily infiltrated with mononucleated cells and very often also with polymorphonucleated heterophilic leucocytes.

Since only first generation captives were used in this study, the differences in gut morphology of captive and wild grouse must be due to different food and environmental conditions and not to genetic selection.

micromorphology; gut; willow grouse.

The gut morphology of the tetranoid species rock ptarmigan (Lagopus mutus), black grouse (Lyrurus tetrix), capercaillie (Tetrao urogallus) and hazelgrouse (Tetrastes bonasia), is rather similar (*Schumacher* 1921, 1922, 1925). Compared to the gut morphology of other birds (*Zisweiler & Farner* 1972) the most

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remarkable findings are zig-zag arrangement of the small intestinal villi, the long caeca, the ciliated epithelium in the neck part of the caeca and the secretion of filamentous mucus in the body part of the caeca.

Seasonal variations in the length of the caeca have been demonstrated in spruce grouse (Canachites canadenis) (*Pendergast & Boag* 1973) and rock ptarmigan (*Gasaway* 1976), the caeca being longest during winter. When red grouse (Lagopus lagopus scoticus) were reared in captivity on a concentrated food mixture, both the small intestine and caeca decreased in length (*Moss* 1972). The micromorphological aspects of these gross gut changes have not been investigated in any tetranoid species. The present work describes the gut micromorphology of thc willow grouse under natural and artificial nutrional and environmental conditions.

MATERIAL AND METHODS

The material consisted of three groups of healthy adult willow grouse. Hereafter they will be referred to as captive, summer or winter birds. The captive birds consisted of one adult, wild-caught bird (♀) kept five months in captive on concentrates before being killed, and five birds (2 ♀/3 ♂) hatched either from wild or first generation captive eggs and raised in captivity. The latter were fed concentrates (without antibiotics and coccidiostats) and blueberry plants until eight weeks of age, and later pellet concentrates ad libitum (Table 1). Grit and water were always available. The captive birds were never in contact with wild grouse or the reindeer, bantams and different tetranoid species reared at the same game farm. They were killed by a blow on the head.

The summer and winter birds consisted of wild birds. The summer birds included six (2 ♀/4 ♂) shot during July on Karlsøy and Ringvassøy islands, latitude 70° N, and the winter birds eight (3 ♀/5 ♂) shot on the same islands during March/April. Summer bird crops contained a variety of herbaceous and heather-like plants, while winter crops contained twigs and catkins of birch (Betula pubescens) and willow (Salix spp.). The mean body weights of the respective groups were 520, 572 and 609 g. Parasites were not present in the guts of captive and winter birds, while the small intestine of all summer birds were infested with roundworms (Ascaridia compar), tapeworms (Hy-

Table 1. Composition (% fresh wt) and analysis (% dry basis) of artificial grouse diet.

Ingredients	% fresh wt	Analysis	% dry basis
Herring meal	2.0	Dry matter	90.8
Soya meal (extracted)	2.0	Oil	5.6
Maize	15.0	Crude protein	11.8
Barley	10.0	Crude fibre	10.0
Oats	10.0	Ash	5.9
Wheat	10.0	Ca	1.9
Oat husks	29.0	P	0.8
Wheat bran	12,6		
Brewers yeast	1.0		
Soya oil	2.0		
Kelp meal	1.5		
Limestone	1.5		
Calciumphosphate	2.0		
Trace mineral premix[a]	0.4		
Vitamin premix[b]	1.0		

[a] Supplies per kg: Fe 172 mg; Mn 228 mg; Zn 200 mg; Cu 57.2 mg; Co 4.4 mg; I 8 mg.

[b] Supplies per kg: A 7500 i.u.; D_3 1480 i.u.; E 250 mg; B_1 25 mg; B_2 150 mg; B_6 45 mg; Nicotinic acid 550 mg; Ca-D-pantothenate 55 mg; Choline chloride 3525 mg; Folic acid 10 mg; K_3 10 mg; B_{12} 0.01 mg; Biotin 0.45 mg; Inositol 550 mg; Para-amino-benzoic acid 25 mg; Ascorbic acid 265 mg; Etoxyquin 75 mg.

menolepis microps and Raillietina urogalli), and low numbers of coccidia (Eimeria fanthami).

The birds were eviscerated immediately after being killed. The emptied gizzards were weighed, and the length of the small intestine and caeca measured to the nearest 0.5 cm. The small intestine and caeca were prepared for micromorphological examinations as follows: The mucosae were first flushed with 10 % formalin. Finally, the complete small intestine and caeca were immersed in 10 % formalin. Three birds from each group were selected for microscopic examination. The mucosa of the entire small intestine and one of the caeca were first examined by stereomicroscope. Nine gut tissue specimens from each bird were selected for histological examination; six from the small intestine and three from the caecum (Fig. 1). The six small intestine specimens were all taken opposite the mesentery: from

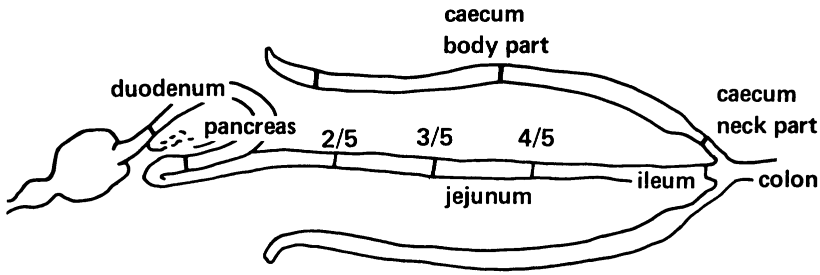


Figure 1. Schematic drawing of the grouse gut, showing the nine sampling sites.

the upper and lower duodenum, from the second, third and fourth fifths of the jejunum, and from the posterior ileum. The caecal specimens were taken from the neck part and the middle and distal body parts. Longitudinal and cross sections were prepared from each specimen and the sections were stained with haematoxylin-eosin. The spiral-shaped microorganisms in the caecal body mucosa of the wild willow grouse were examined after Warthin-Faulkner's method (*Culling* 1963). Electron microscopical examinations of these microorganisms were performed on caecal body mucosa specimens from three wild birds. These specimens were immersed in 3 % glutaraldehyde in Millonigs phosphate buffer in 3 % Macrodex, postfixed in 2 % OsO_4 in Millonigs phosphate buffer for about 2 h, and embedded in Araldite after acetone dehydration. Ultrathin sections were mounted on carbon coated grids and double stained with aqueous uranyl acetate and lead citrate (*Reynolds* 1963).

RESULTS

Gross examination

The gizzard weights and gut measurements showed that wild grouse had heavier gizzards and longer caeca during the winter than in summer, while the small intestine did not vary in length. Captive grouse had lighter gizzards and shorter small intestines and caeca than the wild summer birds (Fig. 2).

Small intestine

The microanatomy of the small intestine was very similar in the wild and captive willow grouse, and scarcely different from

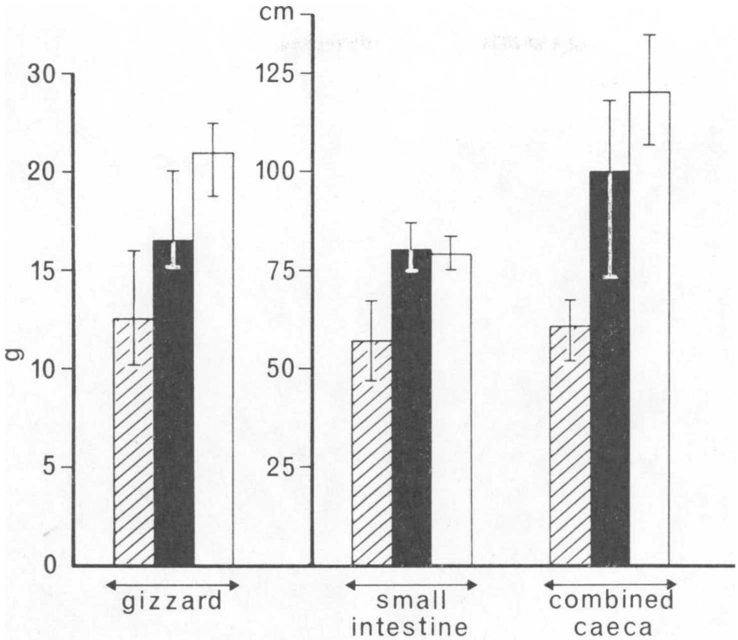


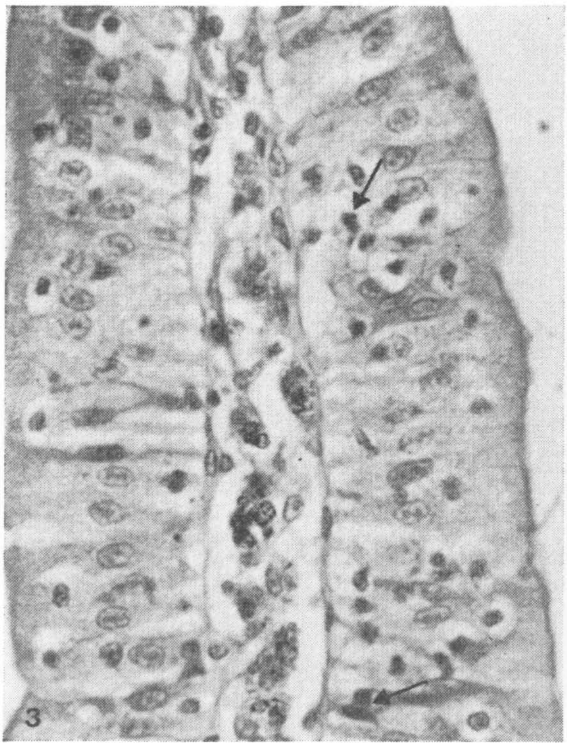
Figure 2. Mean gizzard weights (g) and gut lengths (cm) of captive ▨ and wild summer ■ and winter □ willow grouse. |——| indicates ranges.

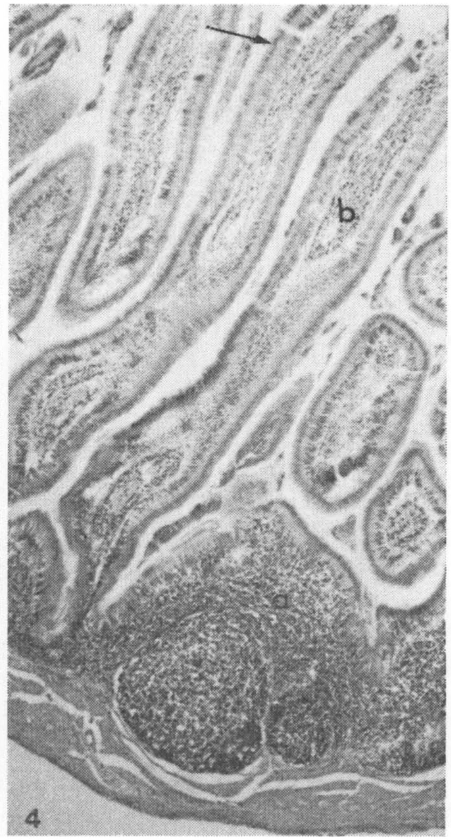
that in the wild rock ptarmigan (*Schumacher* 1921). Masses of goblet cells in the epithelium of the posterior ileum of both wild and captive birds were observed, and the epithelium on the tips of the duodenal villi of the captive willow grouse contained, in addition to ordinary chief and goblet cells, many degenerated epithelial cells (Fig. 3).

Caeca

The microanatomy of the caeca was similar in wild grouse collected during summer and winter. There were, however, several differences between wild and captive birds both in the neck and body parts of the caeca.

Neck part of caeca. The neck of the caeca had finger-shaped villi and an epithelium exclusively formed by columnar ciliated cells (Fig. 4). The longitudinal muscularis was very thick compared to that in the small intestine and body part of the caeca. The lamina propria was also well developed and consisted of loose connective tissue with considerable diffuse infiltration of

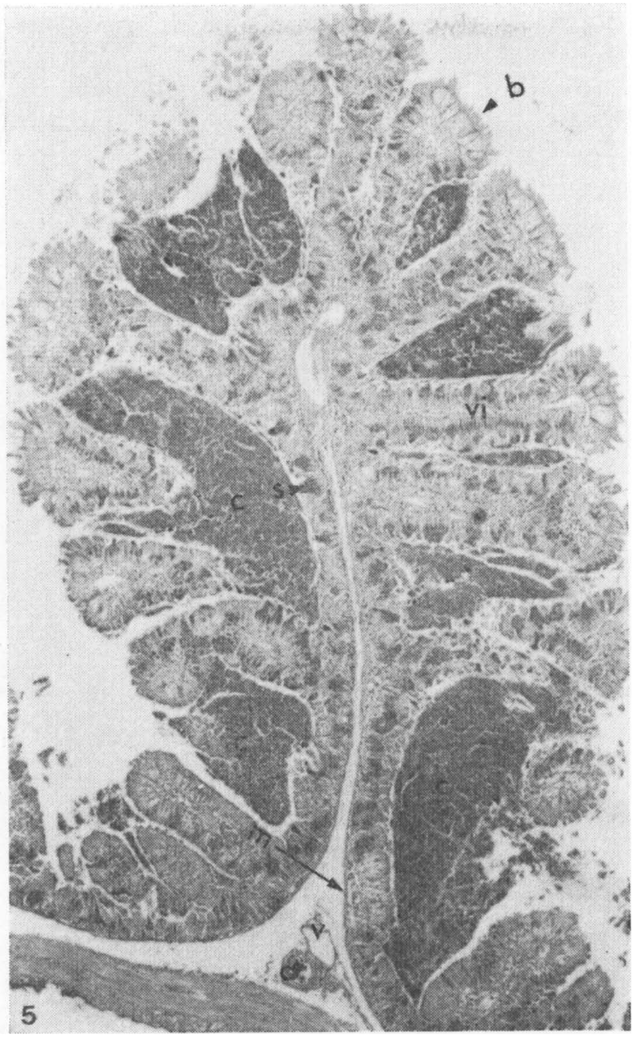




F i g u r e 3. Longitudinal section through a duodenal villi of a captive willow grouse. Arrows point to some of the degenerated epithelial cells. H.E. 540 ×.

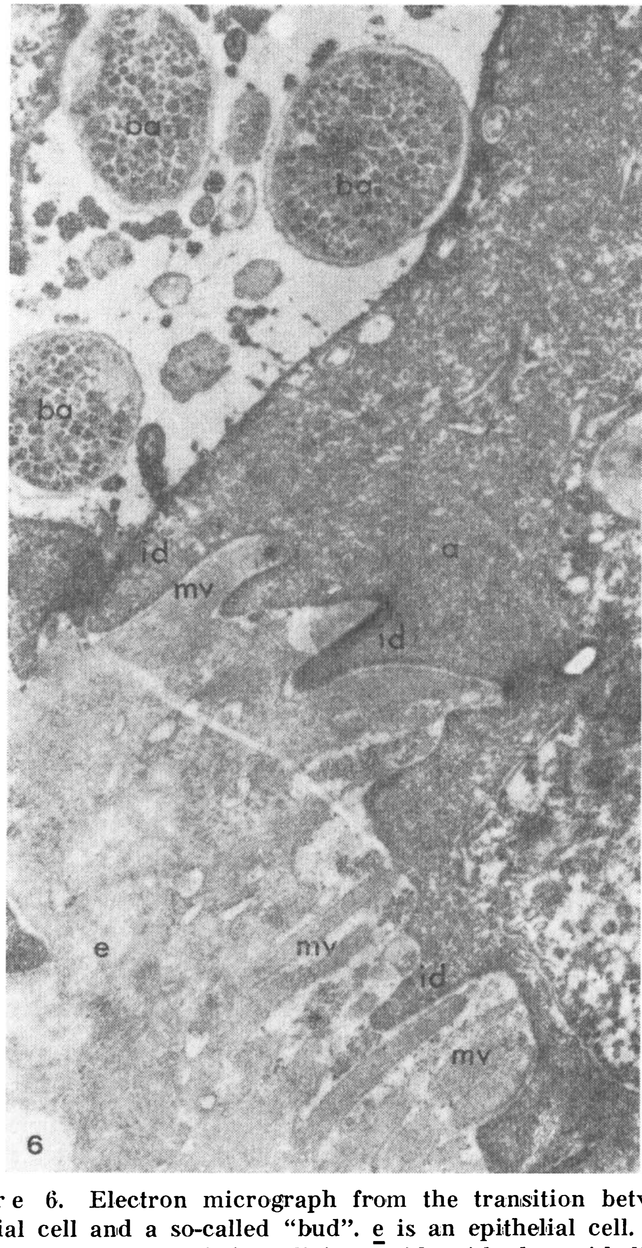
F i g u r e 4. Longitudinal section through the caecal neck part of a wild willow grouse. a is a lymph nodule, b is stroma in one of the villi which contains many mononuclear cells. The arrow points to the ciliated epithelium. H.E. 55 ×.

mononuclear cells, of which a great many were plasma cells. Mononuclear cells were also present in lymphoid nodules. A few rudimentary glands of Lieberkühn were recognized in the basal part of the lamina propria. The villi of captive birds were shorter and their stroma more heavily infiltrated by mononuclear cells than those of wild birds. Captive birds showed strong atrophy of the epithelial cilia and contrary to the wild ones, great numbers of goblet cells in this region of the gut.



F i g u r e 5. Cross section through a longitudinal fold in the caecal body of a wild willow grouse. a artery and v vein in the submucosal part of the longitudinal plica. m muscularis mucosae, vi villus, b eosinophilic "buds" on epithelial cells, s gatherings of spiral-shaped microorganisms attached to the epithelium. c shows caecal contents between the caecal villi. H.E. 55 ×.

Body part of the wild willow grouse's caeca. The epithelium in this gut region was formed by columnar cells. At the top of the villi many epithelial cells carried eosinophilic bud-like protuberances (Fig. 5), some of which showed a nucleus. Light



F i g u r e 6. Electron micrograph from the transition between an epithelial cell and a so-called "bud". e is an epithelial cell. a is the "bud", an amoeba which interdigitates, id, with the epithelial cell's microvilli mv. ba are bacteria on the surface of the amoeba. 30 000 ×.

Figure 7. Epithelial cells in the caecal body of a wild willow grouse. Basophilic filaments are shown to be spiral-shaped, partly intracellular microorganisms, s. Warthin-Faulkner. 1350 ×.

microscopical examination failed to reveal whether these buds were formations in continuity with the epithelial cell cytoplasm, or not. Electron microscopical examination, however, showed clearly that the buds were amoebae with pseudopods protruding between the microvilli of the epithelial cells and thus attaining an intimate contact with them (Fig. 6).

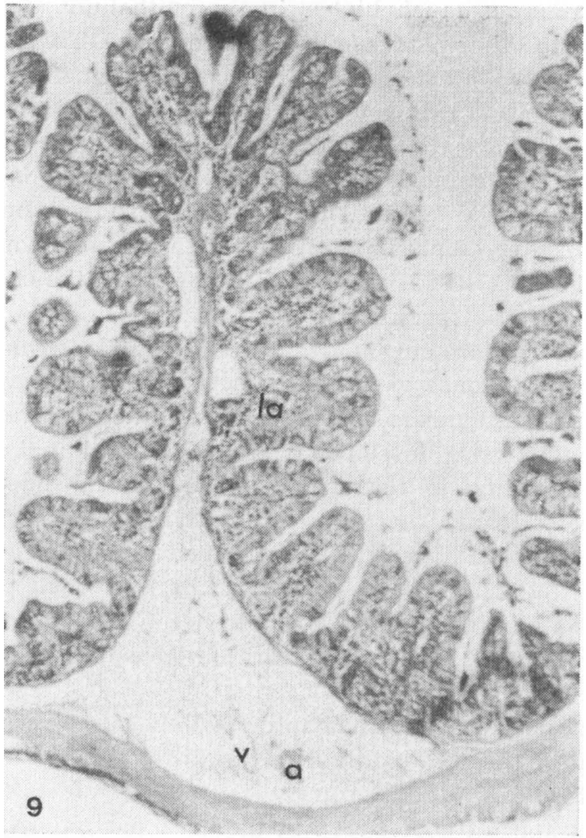
Between and along the lower half of the villi, the epithelial cells were basophilic from the nucleus and towards the lumen. This basophilic material seemed to be intracellular filaments, which extended into the caecal lumen. Warthin-Faulkner staining of caecal body sections revealed that these filaments were spirochetes (Fig. 7). This was also confirmed by electron microscopical investigation (Fig. 8).

The stroma of the villi consisted of loose connective tissue in which smooth muscle cells and small blood vessels could be seen. Diffuse infiltrations of mononuclear cells, among which plasma cells were prominent, occurred regularly. The lamina propria was narrow, but in some places distended by lymph nodules. The

F i g u r e 8. Electron micrograph of an epithelial cell in the caecal body of a wild willow grouse. The spiral-shaped microorganism, s, is cut in different directions and lies in invaginations which are formed in the epithelial cell. sp indicates the small space between microorganism and host cell, and c is the borderline between two epithelial cells. 14 700 ×.

present study could not demonstrate any differences in morphology and amoeba/spirochete fauna between wild birds collected in the winter and in the summer.

Body part of the captive willow grouse's caeca. The longitudinal caecal folds were lower and the villi shorter than in the wild birds (Fig. 9). The lamina propria was heavily infiltrated with mononucleated cells and in some instances by polymorphonucleated, heterophilic leucocytes. The epitelium consisted of regular, broad columnar cells, and neither spiral-shaped microorganisms nor amoebae could be recognized in connection with them. As was the case in wild birds, goblet cells were not present in the caecal body epithelium.



F i g u r e 9. Cross section through a longitudinal fold in the caecal body of a captive willow grouse. a artery and v vein embedded in the submucosal fat tissue. The lamina propria, la, contains many mononuclear cells and a few polymorphonucleated heterophilic leucocytes. Note the difference in size and morphology compared to the wild grouse (Fig. 5). H.E. 55 ×.

DISCUSSION

Gut measurements made during this study (Fig. 2) showed systematic variations which agree well with results of previous investigations on captive and wild red grouse (*Moss* 1972), wild spruce grouse (*Pendergast & Boag* 1973), and wild rock ptarmigan (*Gasaway* 1976).

The micromorphology of the small intestine and caeca of wild willow grouse agrees well with earlier descriptions given for wild rock ptarmigan (*Schumacher* 1921, 1922). The present study demonstrated that the number of goblet cells in both wild and captive birds was much higher in the epithelium of the lower ileum than in other parts of the small intestine. This indicates a high mucus production in this gut region. The function of gastrointestinal mucus is assumed to include that of lubrication of food and protection of epithelial surfaces against damage by ingested food, bacteria, chemicals and intestinal secretion (*Shora et al.* 1975). In the willow grouse this lower ileum mucus may act as a lubricant in the separation of the rough grouse chymus at the ileo-caecal-colic (I-C-C)-junction, and beyond in the caeca where goblet cells are not present.

The major micromorphological differences between wild and captive willow grouse were found in the caeca. The wild bird's caeca were distinguished from those of the captive by their greater length, well developed villi and longitudinal folds, the ciliated epithelium in the neck part, and spiral-shaped microorganisms and amoeba lodged in the epithelium of the body. There seems to be little doubt that the mucus filaments demonstrated in the caecal body epithelium of wild rock ptarmigan, capercaillie, hazelgrouse and black grouse (by *Schumacher* 1922, 1925) in reality must have been intracellular spiral-shaped microorganisms.

The caecal mucosa of the captive willow grouse is more similar to that of the seed-eating domestic fowl (*Hodges* 1974) than to that of wild browsing grouse. The atrophy of epithelial cilia and appearance of goblet cells in the neck part, and the intense cellular infiltrations in the lamina propria of both the body and neck part of the captive birds' caeca may all be tissue reactions against a caecal microflora (*Hanssen* 1979) to which the willow grouse is unaccustomed.

As the differences in gut morphology occurred in first generation captives and even in a wild bird after five months in captivity, they cannot be genetic, but must be caused by environmental, presumedly nutritional factors.

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SAMMENDRAG

Mikromorfologiske studier av tynntarmen og blindtarmene hos ville liryper (Lagopus lagopus lagopus) og hos liryper holdt i fangenskap.

Denne undersøkelsen beskriver de mikromorfologiske forholdene i tynntarmen og blindtarmen hos vill lirype og hos lirype holdt i fangenskap.

De mikromorfologiske forholdene i tynntarmen var svært like hos de ville lirypene og hos de som var holdt i fangenskap mens det i blindtarmene var typiske forskjeller. Epitelet i halsdelen av blindtarmene hos de ville rypene besto utelukkende av sylindriske, ciliekledte epitelceller. Hos rypene i fangenskap var disse ciliene sterkt

atrofierte, og epitelet inneholdt dessuten begerceller. I hoveddelen av blindtarmen hos de ville rypene ble det påvist store mengder spirocheter og amøber i tett kontakt med epitelet. Disse mikroorganismene fantes ikke hos rypene i fangenskap. Det ble videre registrert at både villi og de langsgående foldene i blindtarmens hoveddel var mye større hos de ville enn hos rypene i fangenskap.

Hos rypene i fangenskap var lamina propria i blindtarmene sterkt infiltrert av mononukleære celler og som regel også av en del polymorfkjernede, heterofile leukocyter.

Siden gruppen av ryper i fangenskap var første generasjon under slike forhold må forskjellene i tarm-morfologi skyldes forskjell i ernæring og miljømessig bakgrunn hos de to gruppene.

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Reprints may be requested from: I. Hanssen, University of Tromsø, Wildlife Research Station, Holtveien, N-9000 Tromsø, Norway.