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# PARENTAL NUTRITION AND CHICK PRODUCTION IN CAPTIVE WILLOW PTARMIGAN (LAGOPUS L. LAGOPUS)

By

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HANSSEN, I., J. NESS and J. B. STEEN: Parental nutrition and chick production in captive willow ptarmigan (Lagopus l. lagopus). Acta vet. scand. 1982, 23, 528—538. — The breeding performance of captive willow ptarmigan on different diets has been studied. The nutritional factors tested were protein concentration, natural feed supplement and grass meal and flavonoid admixture, and effects on egg numbers, fertility, hatchability, chick weights at hatching and 0—14 days mortality have been recorded. The breeding performance of ptarmigan hen in captivity showed great individual variations. Egg numbers were not statistically different in groups fed the different diets. Hens fed a 15 % crude protein died tended to produce smaller chicks with significantly lower viabi-

The breeding performance of ptarmigan hen in captivity showed great individual variations. Egg numbers were not statistically different in groups fed the different diets. Hens fed a 15 % crude protein died tended to produce smaller chicks with significantly lower viability than chicks from hens fed a 20 % crude protein diet. Supplement of natural feed tended to increase the number of chicks hatched through a combination of tendency to higher egg numbers and improved fertility. These tendencies were, however, statistically nonsignificant. Inclusion of 34 % grass meal to the diet also tended (nonsignificantly) to improve fertility and hatchability, while inclusion of flavonoids had no positive effect on reproduction. Eggs from captive hens showed significantly lower fertility, and a tendency to lower hatchability than eggs from wild hens. The former

Eggs from captive hens showed significantly lower fertility, and a tendency to lower hatchability than eggs from wild hens. The former difference was probably caused by the close cage confinements for the captive ptarmigan, while the latter condition probably was due to different start of incubation, most of the eggs from wild hens being started naturally.

willow ptarmigan; nutrition; chich production.

Rearing of willow ptarmigan was started at the University of Tromsø in 1972. During the first 2 years it became apparent that fertility and hatchability of eggs taken from wild hens were significantly higher than for eggs layed by our captives.

Moss et al. (1971) found that feeding a dietary supplement of growing heather (Calluna vulgaris) stimulated captive red grouse to lay more eggs than birds given a supplement of dormant winter heather. There was, however, no difference in fertility, hatchability and survival of chicks between these groups. Savory (1975) showed that captive red grouse hens started to increase their feed intake 5 weeks before laying, and suggested that both fertility and hatchability might be improved if protein rich breeding diets were given from that time on. Raa et al. (1976) found an unidentified substance in wild willow ptarmigan eggs that was not present in eggs from captive birds. Hanssen et al. (1979) demonstrated that chicks from eggs of wild ptarmigan performed better on a vitamin C-deficient diet than did chicks from eggs of captive hens, although vitamin C is not present in ptarmigan eggs.

On this background some feeding experiments were made with captive, breeding willow ptarmigan. The aim has been to understand why eggs from wild birds perform better than those from captives.

#### MATERIAL AND METHODS

### Ptarmigan and management

The ptarmigan were derived from eggs of both wild and captive ptarmigan hens. All eggs were incubated and hatched in machines, and the chicks were reared as described by *Hanssen & Ness* (1982) until they were  $1\frac{1}{2}$ —2 months old. At that age they were transferred to cages, singly, in pairs of in small groups. The cages were cubes of about 90 cm<sup>3</sup> with wire floors ( $3/_8 \times 1''$ ). Birds were kept both outdoors and indoors. The indoor birds were kept on a light regime adjusted to the outdoor daylength. The hens used were from 1 to 3 years old.

During the winter all birds were fed ptarmigan maintenance feed (Moss & Hanssen 1980) and water and grit ad libitum. Six weeks before expected start of laying the birds were divided into groups. Care was taken that the groups were composed of birds with similar body weight, age and location (outdoors/indoors).

The pairing of hens and cocks started 2—3 weeks later. This work was done by a trained person well aware of the fact that optimal fertility can only be achieved if the pair is synchronous in moult, similar in aggression and otherwise enjoy each other (Gjesdahl 1977). Each cock was paired to 2 hens so that the hen lived with its cock every other day. The 2 hens which shared a

cock were kept on the same diet in neighbouring cages with a sliding partition between. Thus disturbance by catching was avoided.

# Experiments

The experiments were performed during 3 years. They were designed to elucidate the effect of protein contents, supplement of natural ptarmigan feed, inclusion of grass meal and admixture of flavonoids on breeding performance. The experimental diets were given from 6 weeks before expected start of laying.

Experiment I was performed with 4 groups of birds. One group got maintenance feed only (15% protein), another group got breeder feed only (20% protein). The third and fourth group got these diets, supplemented with willow twigs (Salix spp.) and crowberries (Empetrum spp.) ad libitum. Blueberry plants (Vaccinium myrtillus) were given as soon as they became available, usually in the middle of May which coincided with the time when hens started to lay. The composition and analysis of the artificial diets are given in Table 1.

Experiment II was performed with 2 groups of birds testing the effect of grass meal admixture. The background for this experiment was the fact that grass meal admixture seemed to improve the general health of willow ptarmigan (*Hanssen* 1982).

Experiment III was designed to test the effect of the flavonoids rutin and quercetin on reproductive performance. These compounds are shown to enhance the biological potency of ascorbic acid (*Harper et al.* 1969), and since chicks from eggs of wild birds perform better on vitamin C deficient diet compared to those from captives (*Hanssen et al.* 1979), flavonoids might be the unidentified substances demonstrated in wild ptarmigan eggs (*Raa et al.* 1976).

# Eggs

Eggs were collected once a day. They were weighed and individually marked before they were stored (never more than a week) in paper trays at 10-13°C, blunt end up. The trays were inclined at about 30° to horizontal, and turned once daily. Once a week eggs were put into a rotating incubator (Victoria model

Ingredients	Maintenance	Breeder	Breeder with grass meal
Herring meal	2.0	6.0	5.0
Soya bean meal (extracted)	2.0	14.0	10.0
Corn grain	15.0	5.0	5.0
Barley grain	10.0	6.0	6.0
Oats grain	10.0	6.0	6.0
Wheat grain	10.0	6.0	6.0
Oat husks	29.0	29.0	
Grass meal			34.0
Wheat bran	12.6	18.6	18.6
Brewers yeast	1.0	1.0	1.0
Soya oil	2.0	2.0	2.0
Kelp meal	1.5	1.5	1.5
Limestone	1.5	1.5	1.5
Calcium phosphate	2.0	2.0	2.0
Trace mineral premix <sup>a</sup>	0.4	0.4	0.4
Vitamin premix <sup>b</sup>	1.0	1.0	1.0
Analy <b>s</b> is			
Dry matter %	89.6	89.5	90.0
Crude protein %			
(Total N · 6.25)	14.6	20.3	17.1
Crude fat %	6.8	5.7	5.5
Crude fibres %	7.4	7.9	9.5
Ca g/kg	17.4	15.3	14.5
P g/kg	12.0	10.7	9.0
Mg g/kg	2.1	2.1	2.0
K g/kg	14.5	15.8	13.8
Na g/kg	2.6	1.9	1.8
Cl g/kg	4.1		3.6

Table 1. Composition (%) and analysis (% of dry weight) of experimental diets.

<sup>a</sup> Supplies per kg: 7500 I.U. vit. A, 1480 I.U. vit. D<sub>3</sub>, 250 mg vit. E, 25 mg vit. B<sub>1</sub>, 150 mg vit. B<sub>2</sub>, 45 mg vit. B<sub>6</sub>, 55 mg Ca-D-pantothenate, 550 mg Niacin, 10 mg Folic acid, 3525 mg Choline chlorid, 0.45 mg Biotin, 0.01 mg B<sub>12</sub>, 10 mg vit. K<sub>3</sub>, 550 mg Inositol, 25 mg para-aminobenzoic acid, 265 mg Ascorbic acid, 75 mg Etoxiquin.

<sup>h</sup> Supplies per kg: 172 mg Fe, 228 mg Mn, 200 mg Zn, 57.2 mg Cu, 4.4 mg Co, 8 mq I.

654). Incubation temperature was 37.6°C and degrees of humidity was 27.2—27.7°C in Experiment I, 26.7—27.2°C in Experiment II and 27.7—28.2°C in Experiment III.

After 19 days of incubation the eggs were transferred from the incubator to the hatcher (Victoria 991) where the temperature

was  $37.6^{\circ}$ C and degree of humidity was  $31.1^{\circ}$ C. Each egg was hatched in its own wire-mesh container, so that the origin of every chick was known. The chicks were kept in the hatcher for 12-24 h, before they were taken out, weighed and banded.

The eggs were gassed with formalin for 20 min at the moment they were put into the incubator. This was repeated on the 7th and 14th day of incubation. Eggs that did not hatch were examined concerning fertility and embryo development in all experiments. Contents from 5 to 10 eggs from each of the 4 groups in Experiment I was submitted to chemical analysis.

# Chemical analysis of egg contents

Selen was determined by a fluorometric method (*Ihnat* 1974) after wet digestion (*Rygge et al.* 1977), while the concentrations of the other elements were monitored using a Perkin Elmer 370 AAS in conjunction with a suprapur  $HNO_3/HClO_3$  acid digestion of the samples (*Julshamn & Braekkan* 1975).

Vitamin A and  $\alpha$ -tocopherol was determined spectrophotometrically after chromatographical separation of the unsaponified part of the specimens. The methods are described in US *Pharmacopoea* (16th ed. and later) and *Lambertsen & Brækkan* (1959). Thiamin, riboflavin and pantothenic acid were determined microbiologically using Lactobacillus viridescens (ATCC 12706) (*Deibel et al.* 1957), Leuconostoc mesenteroides (ATCC 10100) (*Barton Wright* 1963) and Lactobacillus plantarum (ATCC 8014) (*Horwitz* 1980), respectively as test organisms. Growth was measured turbidimetrically at 660 nm after incubation under fixed conditions.

Bacteriological examinations of contents from 120 infertile eggs and dead embryos from Experiment I, was done. Specimens were streaked onto the surface of 5 % human blood agar and bromthymol blue lactose agar plates (*Nordic Commitee on Food Analysis* 1969). The plates were incubated aerobically at  $37^{\circ}$ C and read after 24 and 48 h. Shell thickness was measured by means of a micrometer screw in the blunt end of all incubated eggs in Experiment III.

# Rearing of chicks

The chicks were put into brooder houses and reared as described by *Hanssen & Ness* (1982).

Experi- ment (and year)	Group	Total num- ber of eggs per hen, $\overline{x} \pm s$	Percent fertile eggs	Percent of fer- tile eggs hatched	Number of chicks hatched per hen $\overline{x} \pm s$	Chick weights (g) at hatching $\bar{x} \pm s$	Percent of chicks survived 0—14 days
	15 % protein $(n=8)^{1}$	$21 \pm 9$	892	70	$11.1^3 \pm 5.7$	$13.7\pm1.6$	60*
	20 % protein (n=9)	$23 \pm 10$	76	80	$11.4 \pm 5.5$	$14.7 \pm 1.1$	74
I (1975)	15 % protein and natural food supple-	$24 \pm 7$	86	81	$14.4 \pm 5.8$	14.1 ± 1.1	78
	ment $(n=9)$ 20 % protein and natural food supple- ment $(n=7)$	26 ± 8	87	78	$14.6 \pm 2.8$	14.2 ± 1.1	78
	20 % protein	$21 \pm 7$	76	62	$7.4 \pm 5.5$	$13.1 \pm 1.4$	66
(1976)	(n=10) 17 % protein and 34 % grass meal (n=10)	20 ± 8	86	76	$10.9 \pm 6.2$	13.7 ± 1.2	72
	17 % protein and 34 % grass	18 ± 5	86	86	11.4 ± 4.7	$15.1 \pm 1.0$	39
(1980)	ineal $(n=9)$ 17 % protein, 34 % grass meal and 0.15 % rutin and quercetin (n=9)	$23 \pm 6$	68	83	$10.2 \pm 5.2$	14.4 ± 1.2	37
	Wild hens 1975	11 ± 1	91	80	$9.6 \pm 1.4$	$14.2 \pm 1.3$	83
	(n=14) Wild hens 1976 (n=15)	11 ± 1	98	89	10.0 ±1.6	13.6 ± 1.1	71

T a ble 2. Egg numbers, percent fertile and hatched eggs, chick numbers, weights, and percent survival at day 14 after hatching. Reproduction results for eggs from wild hens, artificially incubated, hatched and reared, are included for comparison.

<sup>1</sup> Hens that did not lay eggs, 1-2 in each group, were excluded.

<sup>2</sup> Very small eggs, eggs with rough shell surface or without shell, alltogether 5-10 percent of total number, are not considered.

<sup>3</sup> Corrected for eggs cracked by accidents or used in chemical analyses.

\* Significantly different from the other groups in Exp. I, P < 0.025.

# Statistics

Student's t-test was used to compare mean values, and chisquare test to compare mortality frequencies. Significance was set at the 2.5 % level.

### RESULTS

In Experiment I (Table 2) the number of eggs, per cent fertility and hatchability were statistically similar in all groups. There was, however, tendency (non-significant) that hens fed a natural feed supplement provided a higher number of chicks than hens fed the artificial diets only. And hens fed a low protein diet only, provided chicks that showed a significantly higher mortality than chicks from the other groups.

In Experiment II hens fed a grass meal containing diet tended to produce more fertile and hatchable eggs, and chicks with stronger survival fitness than hens fed the diet without grass meal. The differences were, however, non-significant. Both numbers of chicks hatched and chicks weights at hatching were lower in this experiment compared to Experiment I.

In Experiment III no positive effects of flavonoid admixture on reproduction could be demonstrated. The reason for the extremely low chick survival this year was that our ordinary way of chick rearing did not function, due to lack of suitable blueberry plants. They were too dry and fibrous for small chicks (Hanssen & Ness 1982).

In Experiments I and II embryo mortality showed peaks at day 2—5 and day 21. In Experiment III embryo mortality during the first week of incubation was low.

No significant difference in weight at hatching could be demonstrated between chicks that survived and chicks that died during the first 14 days after hatching. Shell thickness and chemical analysis of contents from eggs layed in Experiment I are shown in Table 3. There were no differences between the groups, and therefore the total material is combined in the table. Analysis of wild eggs is included for comparison. For 1 captive hen the shell was significantly thicker in fertile unhatched compared to hatched eggs. For another hen the opposite was true. Among the other 18 hens no difference was demonstrated in shell thickness of hatched and unhatched eggs.

			Mine	erals in egg c	ontents. Rar	iges in μg/g	dry matt	er	
Group	Shell thicknes ranges (mm)	Mg	Ca	Сп	Fe		nz	Mn	Se <sup>2</sup>
Eggs from captive wil- low ptarmigar (n=26)	1.8—2.4	279—365	1610-2500	3.66.	6 80	120 53	72	3.5—5.1	0.16-0.28*
Eggs from wild willow ptarmigan (Hanssen et a 1981)	1.6—2.5	322433	2148—5017	3.9-1	0.1 75	110 52	-63	4.04.6	0.070.15
		T	able 3 (co	ontinued).					
		Vitam	tins in egg conte itamin A, and μ	ents <sup>1</sup> . Ranges 1g/g dry matt	in I.U/dry r er for the o	natter for thers			
	Group	Vit. A	α-toco- phenol	Thiamin	Ribo- flavin	Pantothe- nic acid			
	Eggs from captive wil- low ptarmigan (n=26)	15—23	565	17 <sup>3</sup>	19—25	10226	*		
	Eggs from wild willow ptarmigan (Hanssen et al. 1981)	11	2083	410	1626	4457			

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<sup>3</sup> Only one egg analysed. • The difference is significant, P < 0.025.

The bacteriological examinations of egg contents showed a frequency of 2/120 infected eggs and embryos combined. The actual microorganism was E. coli.

#### DISCUSSION

It is evident that there are great variations between individual hens in ability to reproduce in captivity (Table 2). This makes it very difficult to test effects of different diets on reproduction, because a high number of hens in each group is needed. The breeding routines were slightly changed from year to year. This makes it difficult to compare results from different years.

One important reason for the lower chick production on 20 % protein diet only, observed in Experiment II (1976) compared to Experiment I (1975), probably was that the humidity in the incubator was slightly lower in Experiment II. The lowering of incubator humidity resulted in increased weight loss from eggs during incubation (67 against 63.5%), and on an average smaller chicks at hatching (Table 2). In Experiment III the humidity in the incubator was slightly increased compared to the preceding experiments, and a marked improvement of hatchability and hatching weights were recorded.

The reason for the great variation in fertility of captive hens was probably of behavioral character. In close cage confinements the natural display of mating, preparing the ptarmigan for copulation and conception, may easily be disturbed. An obvious sign for this was the heavy pecking marks on the head of some hens. Embryos in eggs from wild hens survived the first days of incubation better than embryos in eggs from captive hens. The fact that embryo death in eggs from captive hens was reduced when humidity in the incubator was increased, indicates that a natural start of the incubation, which most of the eggs from wild hens were submitted to, may be the reason why eggs from wild hens showed a tendency (non-significant) to hatch better than those from the captive. From this it may be concluded that the grass meal containing breeder diet, richly supplemented with natural feed, is a good nutrition for breeding ptarmigan. Except for the first week, the incubation regime is also fairly good. A further optimalization of the reproductive performance of captive willow ptarmigan may be achieved by improvements of the breeding cages to obtain an increased number of normal, fertile eggs from the more nervous ptarmigan hens.

#### REFERENCES

- Barton Wright, E. C.: Practical methods for the Microbiological Assay of the Vitamin B-complex and Amino Acids. United Trade Press Ltd., London 1963.
- Deibel, R. H., J. B. Evans & C. F. Niven jr.: Microbiological assay for thiamin using Lactobacillus viridescens. J. Bact. 1957, 14, 818— 821.
- Gjesdahl, A.: Social rank, mating and egg fertilization in willow ptarmigan (Lagopus lagopus lagopus). Poult. Sci. 1977, 56, 41-44.
- Hanssen, I., H. J. Grav, J. B. Steen & H. Lysnes: Vitamin C deficiency in growing willow ptarmigan. J. Nutr. 1979, 109, 2260-2276.
- Hanssen, I.: Nephritis and uric acid diathesis in captive willow ptarmigan (Lagopus l. lagopus). Effect of feed protein concentration and grass meal admixture. Acta vet. scand. 1982, 23, 446-455.
- Hanssen, I. & J. Ness: Chick nutrition and mortality in willow ptarmigan (Lagopus l. lagopus). Acta vet. scand. 1982, 23, 456-465.
- Harper, K. A., A. D. Morton & E. J. Rolfe: The phenolic compounds of black-currant juice and their protective effect on ascorbic acid. III The mechanism of ascorbic acid oxidation and its inhibition by flavonoids. Food Technol. 1969, 4, 255-267.
- Horwitz, W.: Official Methods of Analysis of the Association of Official Analytical Chemists. 13th ed. Published by the Ass. Off. Analyt. Chem., Washington 1980.
- Ihnat, M.: Fluorometric determination of selenium in foods. J. Ass. Off. Anal. Chem. 1974, 56, 368-372.
- Julshamn, K. & O. R. Brækkan: Determination of trace elements in fish tissues by standard addition method. At. Absorb. Newsl. 1975, 14, 49-52.
- Lambertsen, G. & O. R. Brækkan: The spectrophotometric determination of α-tocopherol. Analyst. 1959, 84, 706-711.
- Moss, R., A. Watson, R. Parr & W. Glennie: Effects of dietary supplement of newly growing heather on the breeding of captive red grouse. J. Anim. Ecol. 1971, 25, 135-143.
- Moss, R. & I. Hanssen: Grouse nutrition. Nutr. Abst. Rev. Ser. B. 1980, 50, 555-567.

- Raa, J., P. Moen & J. B. Steen: A nutrition dependent antimicrobial principle in tissue of willow ptarmigan (Lagopus lagopus). J. Sci. Food Agric. 1976, 27, 773-776.
- Rygge, J., G. Norheim & A. Frøslie: Fluorometrisk bestemmelse av selen i biologisk materiale. (Fluorometric determination of selenium in biological material). Poster 16th Nord. Chemist Meeting, Bergen 1977.
- Savory, C. J.: Seasonal variations in the food intake of captive red grouse. Brit. Poult. Sci. 1975, 16, 471-479.

#### SAMMENDRAG

# Foreldreernæring og kyllingproduksjon hos liryper (Lagopus l. lagopus) i fangenskap.

Reproduksjonsevnen hos liryper i fangenskap på forskjellig ernæring ble undersøkt. Næringsfaktorene som ble testet var henholdsvis proteinkonsentrasjon, tilskudd av naturlig føde og tilblanding av grassmel og flavonoider. Det ble registrert effekt på antall lagte egg, befruktningsprosent, klekkeprosent, klekkevekter og mortalitet hos kyllinger 0—14 dager etter klekking.

Det var store individuelle variasjoner i reproduksjonsevnen hos lirypehønene. Antall lagte egg var ikke statistisk forskjellig i de forskjellige fóringsgruppene, men høner som hadde fått en diett som inneholdt 15 % råprotein, viste tendens til å klekke lettere kyllinger med signifikant lavere levedyktighet enn høner som hadde fått en diett som inneholdt 20 % råprotein. Tilskudd av naturlig føde syntes å bevirke en økning i antall klekte kyllinger gjennom en tendens til høyere eggtall og bedre fruktbarhet. Denne tendensen var imidlertid ikke statistisk signifikant.

En tilblanding av 34 % grasmel til dietten ga også tendens til bedring av befruktnings- og klekkeprosenten, mens tilblanding av flavonoider ikke ga antydning til positiv effekt på reproduksjonen.

Sammenlignet med egg fra ville liryper viste eggene fra våre ryper i fangenskap en signifikant lavere befruktningsprosent og tendens til lavere klekkeprosent. Den førstnevnte forskjellen skyldes antagelig de trange burene som rypene ble holdt i, mens det sistnevnte forholdet sannsynligvis skyldes at de ville eggene var ruget naturlig i begynnelsen av rugeperioden.

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