ships in swine feeds. Canadian J. Anim. Sci. 40: 100–106.
Hill, F. W., and D. L. Anderson, 1958. Comparison of metabolizable energy determinations with growing chicks. J. Nutrition, 64: 587–604.
Jukes, T. H., 1955. Antibiotics in Nutrition. N. Y. Medical Encyclopedia, Inc., New York, N. Y.
Leong, K. C., L. S. Jensen and J. McGinnis, 1962. Effect of water treatment and enzyme supplementation on the metabolizable energy of barley. Poultry Sci. 41: 36–39.
Marshall, M. W., H. E. Hildebrand, J. L. Dupont and M. Womack, 1959. Effects of dietary fats and carbohydrates on digestibility of nitrogen and energy supply, and on growth, body composition and serum cholesterol of rats. J. Nutrition, 69: 371–382.
Nelson, F. E., 1962. Investigations of the mode of action of antibiotics in stimulating growth of chicks and turkey poults. Ph.hD. Thesis, Washington State University, Pullman.
Rice, E. E., W. O. Warner, P. E. More and C. F. Poling, 1957. Comparison of the metabolic energy contributions of foods by growth under conditions of energy restriction. J. Nutrition, 61: 253–266.
Sibbald, I. R., S. J. Slinger and G. C. Ashton, 1961. Factors affecting the metabolizable energy content of poultry feeds. 4. The influences of calcium, phosphorus, antibiotics and pantothenic acid. Poultry Sci. 40: 945–947.
Slinger, S. J., W. F. Pepper, I. Motzok and I. R. Sibbald, 1962. Studies on the calcium requirements of turkeys. 3. Influence of chlortetracycline and reserpine during the starting and growing periods. Poultry Sci. 41: 460–467.
Snedecor, G. W., 1956. Statistical Methods, Fifth Edition, Iowa State College Press, Ames, Iowa

The Effect of Seaweed Carotenoids on Egg Yolk Coloration

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CONTRADICTORY records are found in the literature on the effect upon egg yolk coloration of feeding seaweed meal to the laying hen. While Olsson (1941) and Black (1953) observed little or no effect, Høie and Sannan (1960) found a significant darkening of the egg yolk on addition of 5 percent of seaweed meal to the rations. A possible explanation for this discrepancy may be that some of the meals tested had lost their carotenoids prior to the trials. Neither Black nor Olsson gave any data on the carotenoid content of the meals they tested, and it is well known that commercial seaweed meals may be devoid of carotene and carotenoids, due to inferior production processes or prolonged storage of the product before use.

However, as it is feasible both technically and economically to produce a seaweed meal which contains notable amounts of β-carotene, violaxanthin (= zeaxanthin 5,6-5′,6′-diepoxide), and fucoxanthin (constitution unknown), it is of considerable interest to establish whether this type of seaweed meal may have any effect on egg yolk coloration when given to the laying hen. A series of feeding experiments with laying hens has therefore been carried out in cooperation with Professor Johs. Høie at the Institute of Poultry and Fur Animals of The Agricultural College of Norway. The influence of seaweed meal additives on feed consumption, egg production and live weight has been reported by Høie and Sannan

(1960), while the effect on the carotenoid content of the egg yolk is dealt with in the present report.

MATERIALS AND METHODS

The following three species of seaweeds were tested: *Ascophyllum nodosum* (L.) Le Jol., *Fucus vesiculosus* L. and *Fucus serratus* L. The algae were collected in the Trondheimsfjord and brought to the laboratory within 1 hour of harvesting. Drying was carried out at 40 C. in a compartment dryer with forced air circulation. After 48 hours the dry material was ground to pass a 1 mm. sieve in a Wiley laboratory mill, and the resulting meal was sent for testing at The Institute of Poultry and Fur Animals of The Agricultural College of Norway, Vollebekk. The experimental details of the feeding trials have been given by Høie and Sannan (1960). White Leghorn hens were used in all experiments.

TABLE 1.—*Composition of basal diets (Høie and Sannan, 1960)*

Component	Basal ration 1	Basal ration 2
Ground yellow corn	27.0%	—
Ground barley	27.0%	24.0%
Ground milo	—	31.0%
Ground oats	10.0%	9.0%
Wheat bran	10.0%	10.0%
Soybean oil meal, expeller	6.0%	6.0%
Peanut oil meal	3.0%	3.0%
Linseed oil meal	1.0%	1.0%
Herring meal	3.0%	4.0%
Feed yeast	1.5%	1.5%
Dry vitamin A, D and B_2 supplement	1.5%	1.5%
Mineral mixture	3.0%	—
Ground limestone	4.5%	5.0%
Dicalcium phosphate	2.5%	3.5%
Common salt (NaCl)	—	0.5%
Total	100.0%	100.0%
Content, calculated		
Scandinavian feed units/100 kg.	91.9	93.9
Crude protein %	15.4	16.6
Ca %	2.77	2.50
P %	1.10	1.04
Fibre %	4.6	4.6
Vitamin A I.U./kg.	16,400	15,200
Vitamin D I.U./kg.	1,500	1,500
Vitamin B_2 mg./kg.	8.2	8.3
Vitamin E mg./kg.	6.2	5.1

TABLE 2.—*Composition of the diets of the test groups*

Component	*A. nodosum* groups		*F. vesiculosus* group	*F. serratus* group
	1	2		
Basal diet 1	90%	85%	—	—
Basal diet 2	—	—	90%	85%
A. nodosum meal	10%	15%	—	—
F. vesiculosus meal	—	—	10%	—
F. serratus meal	—	—	—	15%

The meal of *Ascophyllum nodosum* was given at 10 and 15 percent levels to laying hens on a basal ration containing 27 percent of yellow corn. The control group received the basal ration only.

The rest of the experiments were carried out with ground milo instead of yellow corn in the basal ration. To this ration were added the seaweed meals at 10 or 15 percent levels in the test groups. The composition of the basal rations and of the different test rations are given in Tables 1 and 2.

Samples of the seaweed meals were analysed for carotenoid content by quantitiative paper chromatography (Jensen and Jensen, 1959). When the hens had been on the test diets for at least two weeks, eggs were collected for determination of the carotenoid content of the yolk.

The yolks of 10 eggs from each group were thoroughly mixed and 10 g. aliquots were extracted exhaustively with acetone. Considerable amounts of colorless lipids were removed from the extracts by storage over-night at −20° and decanting the colored solution. The latter was concentrated *in vacuo* at room temperature to an oil which was partitioned between petroleum ether (b.r. 60–80°) and 85 percent aqueous methanol. After drying over an-

TABLE 3.—*Carotenoid content of the seaweed meals tested (mg./kg. of dry matter)*

Meal	β-Carotene	Violaxanthin	Fucoxanthin
A. nodosum[1]	30	12	60
F. vesiculosus[3]	49	80[2]	270
F. serratus[3]	160	240	680

[1] Analyzed 2 months after the completion of the feeding experiment. (Freshly prepared meal of *A. nodosum* usually contains approximately 50, 40 and 300 mg. respectively of β-carotene, violaxanthin and fucoxanthin.)

[2] A mixture of zeaxanthin and furanoid carotenoids.

[3] Analyzed at the start of the feeding experiment.

hydrous sodium sulfate, the epiphase was concentrated to a small volume and the pigments separated on a neutral aluminium oxide column, activity grade III (Brockmann and Schodder, 1941), using petroleum ether—diethyl ether mixtures for the development. The hypophasic pigments were extracted from the methanolic solution into diethyl ether by the addition of water, and after drying, the ether solution was concentrated to a small volume, and petroleum ether was added. The hypophasic pigments were then separated on a calcium carbonate—Hyflo Supercel (1:1) column using petroleum ether containing 10 to 20 percent of acetone for the elution. The colored zones of the chromatograms were cut out from the columns after extrusion, the pigments extracted with acetone—methanol (1:1), and their amounts determined spectrophotometrically in the usual way (after having been transferred to petroleum ether). Aliquots of each fraction obtained from the columns were subjected to quantitative paper chromatography (Jensen and Jensen, 1959) if not homogenous; the proportion of the components being determined by extracting the separated pigmented zones with acetone and measuring the light absorption in the usual way. A more detailed description of the procedure will be published elsewhere.

RESULTS AND DISCUSSION

The carotenoid contents of the seaweed meals tested are given in Table 3.

In the feeding experiment with *A. nodosum* the yolks of the eggs from the control group appeared somewhat paler than those of normal eggs. A definite darkening of the color was observed for the egg yolks of the seaweed group animals. As seen from Table 4 this darkening was caused by a change in the carotenoid composition rather than by an increase in the pigment content. The fucoxanthin-like pigments appearing in the egg yolks of the seaweed groups exhibited broad absorption spectra in the visible region. This explains why relatively small amounts of these pigments led to a significant increase in total color of the egg yolks.

As the background effect of the carotenoids of the basal ration was rather high in the *Ascophyllum* trial, ground milo was used instead in the subsequent experiments. This gave very pale, almost colorless yolks in the control groups, while a marked increase in coloration was observed for all yolks of the test groups. In Table 5 the results of the analysis of the carotenoids in the yolks of the *F. vesiculosus* and the *F. serratus* groups are com-

TABLE 4.—*Carotenoid content of egg yolks in the* Ascophyllum nodosum *experiment, μg./100 g. of fresh yolk*

Carotenoid	Control	10% seaweed	15% seaweed
Carotene +cryptoxanthin	147	157	173
Lutein	648	683	520
Zeaxanthin	342	360	264
Others[1]	0	traces	194
Total	1,137	1,200	1,151

[1] Fucoxanthin-like carotenoids.

pared with those of their respective control groups. (A new control group had to be set up for the *F. serratus* trial since a new batch of basal ration had to be mixed.)

The addition of 10 percent of *F. vesiculosus* meal to the basal ration resulted in a tenfold increase in carotenoid content relative to the content of the control group. In the case of the *F. serratus* meal a 7.5 fold increase was observed.

In all the experiments carried out considerable variation between the hens was noticed, concerning their ability to convert the carotenoids of the diet into egg yolk pigments. Some animals gave constantly dark colored yolks, while others, on the same ration, laid eggs with light colored yolks only. In Table 6 the quantitative composition of different yolks from hens given 15 percent of the *A. nodosum* meal is summarized.

In some rare cases egg yolks from certain animals of the test groups which had received 15 percent seaweed meal showed a reddish color. However, in this and other experiments (Høie and Sannan, 1960) reddish yolks were only observed when the seaweed meal supplement exceeded 10 percent. In practical agriculture 3–7 percent seaweed meal of all mash in rations for laying hens is recommended.

TABLE 5.—*Carotenoid content of egg yolks in the* Fucus vesiculosus *and* Fucus serratus *trial, μg./100 g. of fresh yolk*

Carotenoids	Control	10% *F. vesiculosus*	Control	15% *F. serratus*
β-Carotene	0	+	0	61
Esterified lutein	20	+	50	47
Lutein, free	70	71	244	348
Zeaxanthin	0	370	0	40
Flavoxanthinoids	0	15	0	38
Auroxanthinoids	0	63	0	791
Fucoxanthinoids	0	340	0	866
Others	3	54	16	159
Total	93	913	310	2,350

TABLE 6.—*Composition of yellow and orange yolks of eggs from hens given 15% of* Ascophyllum nodosum, *μg./100 g. of fresh yolk*

Carotenoids	Yellow yolks	Orange yolks
Carotene+cryptoxanthin	156	208
Lutein	300	960
Zeaxanthin	139	515
Fucoxanthinoids	168	246
Total	763	1,929

It is interesting to note that the violaxanthin and the fucoxanthin of the seaweed meals were not transferred to the yolks as such, but were rearranged quite considerably, giving rise partly to furanoid compounds with light absorption properties similar to those of flavoxanthin and auroxanthin, and partly to compounds more closely related to fucoxanthin itself. Brockmann and Völker (1934) had previously found that violaxanthin disappeared completely in the intestinal tract of the hen, forming compounds with light absorption maxima at 426 mμ. (in benzene), and Strain (1938) claimed that violaxanthin is not transferred to the yolk. Strain did, however, find small amounts of flavoxanthin, a furanoid oxide, in the yolk. Karrer and Krause-Voith (1948) stated that epoxidic carotenoids in general are not carried to the egg yolk. Previous and present findings are thus in good agreement and show that in contrast to their furanoid isomers (such as the flavoxanthin- and auroxanthin-like carotenoids of Table 5), epoxidic carotenoids are not transferred to the yolk. The explanation for this may be that the epoxides are converted to their furanoid forms already in the intestinal tract of the hen, whereafter the latter pigments are taken up by the animal.

No record was found in the literature concerning the fate of fucoxanthin when given to the laying hen. It is not surprising, however, that this pigment, which is labile towards both acid and base, is radically rearranged in the intestinal tract of the hen.

SUMMARY

Meals of the brown seaweeds *Ascophyllum nodosum*, *Fucus vesiculosus* and *Fucus serratus* may contain considerable amounts of β-carotene, violaxanthin and fucoxanthin. Addition of 10 to 15 percent of such meals to rations deficient in carotenoids resulted in a considerable increase in egg yolk color when given to the laying hen. This increase was caused partly by a change in carotenoid composition and partly by an increase in pigment concentration.

The addition of 10 percent of *F. vesiculosus* meal and 15 percent of *F. serratus* meal to the basal diet resulted in a 10-fold and a 7.5-fold increase respectively in the carotenoid content of the yolks.

The violaxanthin and fucoxanthin of the seaweed meals were not carried to the yolks as such but were partly converted to furanoid and other epoxidic derivatives of the original pigments.

REFERENCES

Black, W. A. P., 1953. Seaweed as a stockfood. Agriculture, 60: 126–130.

Brockmann, H., and H. Schodder, 1941. Aluminiumoxyd mit abgestuftem Adsorptionsvermögen zur chromatographischen Adsorption. Chem. Ber. 74: 73–78.

Brockmann, H., and O. Völker, 1934. Der gelbe Federfarbstoff des Kanarivogels und das Vorkommen von Carotenoiden bei Vögeln. Zeitschrift f. physiol. Chem. 224: 193–215.

Høie, J., and F. Sannan, 1960. Experiments with seaweed meals as supplements to rations for chicks and laying hens (II). Scientific Reports from The Agricultural College of Norway, 39: (19): 1–76.

Jensen, A., and S. L. Jensen, 1959. Quantitative Paper Chromatography of Carotenoids. Acta Chem. Scand. 13: 1863–1868.

Karrer, P., and E. Krause-Voith, 1948. Einige weitere Beobachtungen bezüglich der Verbreitung der Carotenoide, insbesondere Carotenoid-epoxyde. Helv. Chim. Acta 31: 802.

Olsson, N., 1941. Försök med ekollon, ängsmossa och tång som hönsfoder. Lantbrukshögskolans Husdjurförsöksanstalt. Särtryck och förhandsmeddelanden. Nr. 10 (Stockholm): 1–9.

Strain, H. H., 1938. Leaf Xanthophylls. Carnegie Institution of Washington Publication No. 490, p. 93.

A Method of Growing Chick Embryos *In Vitro*[1]

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INTRODUCTION

THE interest in growing chick embryos *in vitro* is not new. Romanoff (1943) used 50 ml. beakers and obtained consistently good results to 48 hours, if the embryos were not shaken. The main problem was to obtain enough fresh air without drying out the embryo. Kosin (1945) employed a 30 ml. tall form beaker. He used this method of confirming the macroscopic method of identifying fertile, unincubated germ disks. Quisenberry and Dillon (1962) employed a plastic shell and obtained normal embryonic development up to six days of age. Williams and Boone

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