Nutritive Value of Sperm Whale Oil and Finback Whale Oil.

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(Received February 10, 1933.)

Though nutritional studies of fats or oils have already been carried out by many investigators, little is yet attempted in the feeding experiments with such oils as sperm whale oil which contains a large amount of waxes.

The present author, therefore, carried some feeding experiments of rats with synthetic diets containing large amounts of the oils obtained from sperm whale (*Physeter macrocephalus* L.) and finback whale (*Balaenoptera physalus* L.) for the purpose of contributing something to this nutrition problem.

Through the previous work of M. Tsujimoto⁽¹⁾ and Y. Toyama⁽²⁾, the chemical properties of the oils prepared from sperm whale and finback whale have already been determined as follows:

	The head oil from sperm whale (M. Tsujimoto)	The body oil from sperm whale (M. Tsujimoto)	Finback whale oil (Y. Toyama).
Sp. gr.	$d_4^{15} = 0.8848$	$d_4^{20} = 0.8806$	$d_4^{15} = 0.9231$
Refr. index	$n_D^{20} = 1.4633$	$n_D^{20} = 1.4620$	$n_D^{40} = 1.4727$
Acid value	0.99	1.24	2.21
Saponification value	147.1	131.6	196.6
Iodine value	71.4	82.4	112.9
Reichert-Meissel value	0.57	—	—
Unsaponifiable matter	36.0%	36.4%	1.09%
Fatty acids	65.0%	64.13%	—
Glycerol	3.52%	—	—
Cholesterol	0.18%	—	—

The sperm whale oils consisted chiefly of mixed waxes. The unsaponifiable matter (wax alcohols) contains cetyl alcohol and oleic alcohol in nearly equal proportion. Small amounts of octadecyl and tetradecyl alcohol appear to be present. The total percentage of cholesterol was found to be 0.18% of the sperm oil. The fatty acids consisted of about 19% solid and about 81% of liquid acids. Among the solid (saturated) acids palmitic and miristic have been identified. A small quantity of caprylic or capric acids was also present. The liquid (unsaturated) acid consisted mainly of the oleic acids series: physetoleic and oleic acids. Not more than 1% of highly unsaturated acids was also present. On the contrary, the finback whale oils contained fatty acids of the same composition which occur in other animal or vegetable

oils as common glycerides.

The present author carried out feeding experiments with a genuine head oil and an intestine oil obtained from sperm whale (male: body length about 14 meters) and with a blubber oil and an intestine oil prepared from a finback whale. These samples were kindly supplied from Tōyō Hogeï Kaisha.

Experimental.

(1) *Experiments with diets containing whale oils in sufficient quantity.*

The analysis of the samples used in the experiments:

	Sperm whale		Finback whale	
	The head oil	The intestine oil	The blubber oil	The intestine oil
Sp. gr.	—	—	$d_4^{15} = 0.916$	$d_4^{15} = 0.916$
Refr. index	—	$n_D^{25} = 1.464$	$n_D^{25} = 1.466$	$n_D^{25} = 1.467$
Acid value	0.9	2.0	0.2	0.9
Sap. value	145.4	144.4	185.5	180.5
Iod. value	62.7	88.6	92.7	97.6
Vitamin A	—	—	—	—

For the experiments: Albino rats weighing 40~50 g each, were previously fed for several days on a complete diet until they reached 50~60 g. Then they were divided into several groups, each consisting of four animals, and were fed on various experimental synthetic diets. (Fig. 1~8)

The diets consisted of:

Potato starch (Japanese Pharmacopoea IV)	65 g	60 g
Fish meat protein (bonito meat freed from fat)	15 "	15 "
McCollum's salt mixture	4 "	4 "
Oryzanin solution (Sankyo & Co.)	5 cc	—
Dried yeast (supplied from Oriental Company extracted with ether)	—	2 "
Whale oils	15 g	20 g
Biosterin, dissolved in olive oil, given per os daily.	1 mg	1 mg

Fig. 1. The growth curves of the rats fed on diets containing whale oils.

(II) *Experiments with waxes and purified sperm oils.*

In order to investigate the characteristic symptom of rats fed on the diets containing sperm oils as described above, the author attempted to purify the waxes from sperm oils.

i) *Spermaceti (cetin)*: When the head oil from sperm whale was cooled at $0\sim 5^{\circ}\text{C}$, a large quantity of the solid wax which consisted chiefly of cetin separated at the bottom as white crystalline mass. It was collected, freed from the mother liquor (so-called winter sperm oil) by centrifugal machine, and recrystallized from hot alcohol.

ii) *Liquid waxes*: 100 g said winter sperm oils freed from the above solid waxes were boiled with 400 c.c. of a 3% Na_2CO_3 solution for 3 hours. The impurities in the oils are thus completely dissolved. After cooling, the liquid oils were separated and washed with water until the wash water contained no trace of sodium carbonate. Analysis of waxes:

	Solid waxes	Liquid waxes		
	Spermaceti (cetin) (head oil)	Winter sperm oil, freed from spermaceti (head oil)	Winter sperm oil, treated with Na_2CO_3 (head oil)	Winter sperm oil, treated with Na_2CO_3 (intestine oil)
Sp. gr.	—	$d_4^{15} = 0.887$	$d_4^{15} = 0.856$	$d_4^{15} = 0.917$
Refr. index	—	$n_D^{25} = 1.459$	$n_D^{25} = 1.460$	$n_D^{25} = 1.470$
Acid value	0	0.5	0.2	0.2
Sap. value	135.3	152.8	153.4	148.3
Iod. value	17.0	76.9	66.2	90.6

iii) *Finback whale oils purified with Na_2CO_3* : The analysis of the samples treated with Na_2CO_3 in the above method (ii):

	Purified finback whale oils	
	The blubber oil	The intestine oil
Sp. gr.	$d_4^{15} = 0.919$	$d_4^{15} = 0.918$
Refr. index	$n_D^{25} = 1.474$	$n_D^{25} = 1.474$
Acid value	0.2	0.2
Sap. value	184.3	182.5
Iod. value	93.0	102.5

The results indicated that the glycerides are not destroyed.

Feeding experiments: Feeding experiments were carried out in the similar manner as described above. The diets consisted of:

Potato starch (J. P. IV.)	70 g	65 g
Fish meat protein (bonito meat)	15 "	15 "
McCullum's salt mixture	4 "	4 "
Yeast as described above	2 "	—
Oryzanin solution	—	5 cc
Waxes prepared as described above	10 g	15 g
Biosterin dissolved in olive oil per os daily	1 mg	1 mg

Fig. 9. The growth curves of the rats fed on diets containing purified whale oils.

Besides, a little linoleic acid was added to each diet, because it was proved to be an indispensable food factor, the absence of which causes a disease like avitaminosis.

From the above experiments it can be seen that the pure waxes have no noticeable nutritive value upon rats, and seborrhoea seems sometimes to be produced by other fats or oils which contain waxes either in small amount or free from it (Fig. 9~14).

(III) Experiments with fatty alcohols.

Pure waxes had neither any pronounced nutritive value upon rats, nor seemed to produce seborrhoea. So, the authors carried out some experiments with purified fatty alcohols prepared from the head oil (sperm whale) by saponification. As it was shown by M. Tsujimoto and Y. Toyama, the principal components of the head oil from sperm whale are cetyl, octadecyl and oleic alcohols, so the authors prepared these alcohols in pure state and separately tested on rats.

Cetyl alcohol: The solid waxes isolated from sperm oil was saponified with alc. KOH in the usual way, and the fatty acids were precipitated as Ca-soaps with hot alcoholic solution of CaCl_2 ; filtered and the filtrate, after the removal of ethyl alcohol, was extracted with ether, and the etherial extract after washing with water was evaporated to dryness. The residue consisted chiefly of cetyl and octadecyl alcohols. It was recrystallized from acetone, and converted into the corresponding acetate by boiling with three times its amount of acetic anhydride for three hours. The acetylated products thus obtained were fractionally distilled in vacuum. The fraction boiling at

166~7°C (2 mm) having the iodine value 7.4~10 was saponified and subjected to distillation and the distillate was used for animal experiments; b p (3 mm)=161~2°C. In order to determine the purity of this preparation, it was again converted into acetate and the iodine value was determined with the following results.

0.1215 g Subs. gave 1.1 c.c. $\text{Na}_2\text{S}_2\text{O}_3$ (1 c.c.=12.58 mg iodine) iodine value=11.4
0.1206 " " " 1.1 " " " =11.4

Oleic alcohol: Oleic alcohol was prepared in the similar manner as that of cetyl alcohol from the acetone-soluble portion of the nonsaponifiable matter, separated from the liquid waxes in sperm oils. After repeated distillation of the acetylated product, the preparation gave the following constants: b p (4 mm)=180~5°C, $d_4^{15}=0.894$, $n_D^{20}=1.4532$, iodine value=69.5~79.5 (cal. 81.9). The preparation used in the animal experiment was also prepared from the above one by saponifying and redistilling: b p (4 mm)=178~180°C. The purity of the preparation was confirmed by converting it into the acetate: b p (3 mm)=180~182°C.

0.1148 g Subs. gave 6.5 c.c. $\text{Na}_2\text{S}_2\text{O}_3$ (1 c.c.=12.58 mg iodine) iodine value=71.2
0.1185 " " " 6.7 " " " =71.1

Oleic alcohol (synthetic): Synthetic oleic alcohol was prepared from ethyl oleate by reduction with Na in dry ethyl alcohol. After the removal of alcohol the sample thus obtained was washed with alkali, dried over anhydrous Na_2SO_4 , and distilled in vacuo: b p (4 mm)=177~8°C. Then, the preparation was analyzed as the acetate: b p (3 mm)=180~2°C, $d_4^{15}=0.889$, $n_D^{20}=1.4543$.

0.1139 g Subs. gave 7.8 c.c. $\text{Na}_2\text{S}_2\text{O}_3$ (1 c.c.=12.58 mg iodine) iodine value=86.1
0.1133 " " " 7.8 " " " =86.6
(cal. 81.9)

Feeding experiments: The feeding experiment of rats with the fat-deficient diet supplemented with 5% and 10% fatty alcohols were as follows: The diets consisted of:-

Potato starch (J. P. IV.)	70 g	75 g	80 g
Bonito meat protein	15 g	15 g	15 g
McCullum's salt mixture	4 "	4 "	4 "
Yeast as above described	2 "	2 "	2 "
Fatty alcohol (cetyl or oleic alcohol)	10 g	5 g	0 g
Biosterin dissolved in olive oil per os daily	1 mg	1 mg	1 mg

Preparation used in the expt.	Body wt. at the beginn. of the expt. (av. of 4 rats)	Body wt. at the end of the expt. (av. of 4 rats)	Days of living (av. of 4 rats)
Cetyl alcohol 10%	59 g	44.0 g	11.0
(the head oil f. sperm whale) 5%	58.5 g	45.5 g	10.0

Oleic alcohol	10%	58 g	44.0 g	3.5
(")	5%	59 g	45.7 g	3.7
Oleic alcohol (synthetic)	10%	58 g	40.0 g	5.0
Control	0%	61 g	105.0 g	50.0

Moreover, in order to determine the noxious effect of oleic alcohol on rats, when fed on a diet containing butter, rats were fed on a basal diet consisting of starch 75 g, bonito meat protein 15 g, butter 5 g, McCollum's salt mixture 4 g, and commercial oryzanin solution 5 c.c.. The sample oleic alcohol 0.1 g was supplied for each rat per os daily.

The preparation used in the expt.	Initial body wt.	Max. body wt. during the exp.	Body wt. at the end	Duration for expt.
Oleic alcohol	61 g	66 g	63 g	20 (days of living)
(the head oil sperm whale)	57 g	63 g	53 g	21 (")
Control	54 g	69 g	69 g	25

From the above result, it was confirmed that free fatty alcohols especially oleic alcohol gave toxic effects upon animals.

(IV) *Injection experiments with oleic alcohol.*

This experiment was conducted with the object of determining the possible role of oleic alcohol in the production of seborrhoea in rats fed on the diet supplied with sperm oils. 2 c.c. oleic alcohol or its acetate prepared from the sperm oil was injected to the peritoneal cavity of rats which were previously fed on an adequate diet. No seborrhoea was produced in any of the rats (4 rats); and three of them died after 10~40 hours and the last one injected with 2 c.c. oleic acetate died after 6 days. So it was also concluded that oleic alcohol itself would not produce seborrhoea in rats.

(V) *Effect of linoleic acid upon seborrhoea of rats supplied with sperm whale oils.*

An additional experiment was repeated to see the effects of linoleic acid and yeast on the sick rats, because they were recently recognized to be an indispensable food factor like vitamins. Rats were fed on the similar diet as above described: potato starch 65 g, fish meat protein 15 g, McCollum's salt mixture 4 g, dry yeast extracted with ether 2 g, sperm whale oil or fin-back whale oil 15 g, and in addition to the basal diet biosterin 1 mg and

pure linoleic acid* 50~100 mg were supplied to every rat per os daily. The rats given the head oil from sperm whale exhibited seborrhoea after 3 weeks; but on the contrary, the one given the intestine oil from sperm whale and finback whale oils was not observed to show the skin symptom for the entire feeding period of about 100 days. (Fig. 15~16).

Fig. 15 The growth curves of rats fed on diets containing 15% sperm whale or finback whale oils, supplemented with a little linoleic acid and dry yeast.

Summary

1. The sperm whale oils produced seborrhoea in rats and exhibited the retarding action upon the growth of rats. Finback whale oils (especially the intestine oil), on the contrary, indicated a pronounced nutritive value.

2. In order to determine whether waxes might exhibit any nutritive activity in rats, the head oil from sperm whale was freed from spermaceti (cetin) at a temperature of 0~5°C, and expressed oil was used as the sample of winter sperm oil for investigation. Moreover, the above four kinds of oils were separately treated with Na_2CO_3 in boiling water, and the refined oils so obtained were used for the experiments. Pure waxes prepared from sperm oil when given per os did not show the said symptom, especially when a little linoleic acid and dry yeast were administered with the waxes; so that, seborrhoea developed in rats fed on the diets containing large amount of sperm oils does not seem to be due to the high content of waxy substances in the materials.

3. Fatty alcohols especially unsaturated fatty alcohols like oleic alcohol which is a component of sperm whale waxes, have marked toxic property, but do not produce seborrhoea in rats when given per os or injected. As the waxes as such had no noxious effect, it was concluded that the waxes were not assimilated by the animals; otherwise, the diet containing sperm

* Pure linoleic acid was specially prepared by the present author from soy bean oil by the bromination method.

Fig. 2—Head oil from sperm whale
15% No. 363, body wt. 49 g (after 9 days)

Fig. 3—Intestine oil from sperm whale
15% No. 390, body wt. 55 g (after 11 days).

Fig. 4—Blubber oil from finback whale
15% No. 353, body wt. 102 g (after 48 days).

Fig. 5—Intestine oil from finback whale
15% No. 355, body wt. 121 g (after 48 days).

Fig. 6—Blubber oil from finback whale
15% No. 351, body wt. 64 g (after 75 days).

Fig. 7—Intestine oil from finback whale
15% No. 355, body wt. 152 g (after 75 days).

Fig. 10—Spermaceti (cetin) 10% added
with yeast 2% No. 452, body wt. 89 g (after
52 days).

Fig. 8—Intestine oil from finback whale
15% No. 355, body wt. 180 g (after 128 days).

Fig. 11—Spermaceti (cetin) 15% Oryzanin 5 cc added No. 428 body wt. 65 g (after 30 days).

Fig. 12—Head oil from sperm whale 15% Oryzanin 5 cc added No. 490 body wt. 51 g (after 30 days).

Fig. 13—Intestine oil from sperm whale 15% treated with Na_2CO_3 Oryzanin 5 cc added No. 496 body wt. 61 g (after 30 days).

Fig. 14—Blubber oil from finback whale 15% treated with Na_2CO_3 Oryzanin 5 cc added No. 504 body wt. 62 g (after 50 days).

Fig. 16—Head oil from sperm whale 15% added with linoleic acid (2 drops) and yeast No. 595 body wt. 53 g (after 3 weeks).

Fig. 17—Intestine oil from sperm whale 15% added with linoleic acid (2 drops) and yeast No. 592 body wt. 69 g (after 5 weeks).

whale oils in sufficient quantity should give more noxious effect.

Moreover, the author has observed that diets supplied with fat consisting of ordinary glycerides have frequently produced the similar symptom in the skin of rats; and on the other hand, that the symptom was prevented by the addition of a small amount of yeast and linoleic acid even when given sperm oils.

The author expresses his sincere thanks to Prof. U. Suzuki for his kind advice and encouragement throughout the work. The author is also indebted to Mr. K. Tago and to the Tōyō Hogeï Kaisha for the kind supply of reliable whale oils in fresh condition.

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On the Ergosterin Content of Various Edible Mushrooms in Japan.

By Midzuho SUMI.

(Received February 17, 1933.)

Some years ago⁽¹⁾, the author had isolated ergosterin from *Cortinellus Shiitake*, carefully studied its chemical nature, converted it into vitamin D by irradiation with ultra-violet rays, and proved it to be identical with the preparations obtained from ergot or from yeast by other authors. Later, it was observed that this substance is widely distributed in many other mushrooms, either cultivated or grown wild in various districts of Japan. As these mushrooms are not only consumed to a large extent as a favourite food by our people but also some of them are exported to other countries in a notable amount, it seems desirable, from the stand point of nutrition and public health, to determine their ergosterin content quantitatively. As there is not yet reliable method known for this purpose the author has adopted the digitonin method which is commonly used for the determination of sterins.

Since there is no noticeable amount of sterin other than ergosterin, detected in these fungi, the author presumes that the result obtained by the above method will suffice to represent the approximate amount of ergosterin.