minerals used in the basal diet were ample even when the total feed intake was reduced by increasing the energy level. The responses to the increases in dietary fat in this experiment are in general agreement with those of Experiment 1. It was noted that poor feathering did occur at the 30% level with soybean oil. Previously it had been reported by Donaldson et al. (6) that poor feathering in birds occurred on diets containing over 4% fat.

Comparison of corn oil and rice oil with soybean oil (Table IV) showed that all the oils produced comparable increases in growth and food efficiency except that 30% rice oil produced a drop in feed efficiency as compared to 10 or 20%. It also produced the highest mortality.

Data in Table V show that the increase in growth rate which occurred with the increase in fat in the diet cannot be attributed to any unknown factor which may also be present in the above named sources of unidentified factors. Despite the presence of excess quantities of each of these factors, the response to increasing levels of soybean oil was still obtained.

Apparently the chick is capable of utilizing high levels of energy in the form of fat provided that the TABLE IV

a 95% confidence interval.

• Letter to the Editor

The Relationship Between Optical Activity and Structure of Natural Triglycerides

THERE HAS BEEN considerable recent discussion in $\mathbf L$ the literature concerning the analysis of natural fat triglycerides to determine which fatty acids are esterified to which hydroxy groups of the glycerol molecule. Partial hydrolysis by pancreatic lipase can be used to determine which fatty acids are esterified at the 2-position of natural triglyeerides (1). Comparison of these results with the fatty acid composition of the total fat allows calculation of which fatty acids are esterified at the combined 1- and 3-positions (the terminal positions). VanderWal (2) and Gunstone (3) have referred to the absence of suitable techniques for determining whether or not the fatty acids esterified at the 1- and 3-positions of natural triglycerides are equivalent. Apparently, the relationship between the optical activity of triglycerides and the equival-

TABLE V Influence of Unidentified Nutrient Factors on the Nutritive Value of
Soybean Oil

Group no.	Supplement to basal diet	4 wk body wt	Re- sponse over basal	Feed effi- ciency	Im- prove- ment in feed eff. over basal	Mor- tal- ity
$\frac{1}{2}$ 3	None 10% Soybean oil 585.9 \pm 23.18 $ 20\%$ Soybean oil 592.7 \pm 31.11	g 508.8 ± 21.10 a	% . 15.2 16.5	g feed/g $_{\text{body}}$ wt 1.55 1.27 1.17	$\%$ 18.1 24.5	% $\bf{0}$ $\mathbf{0}$

^a Contains 6% distillers dried solubles, 0.5% antibiotic fermentation residue, 0.5% whey product, 5% fish meal, 3% fish solubles.

protein level of the diet is adjusted accordingly, and the physical condition of the feed is such that all nutrients in the diet are available. When the fat is in the form of a liquid oil, as in the present diet, it is impossible to test the effects of over 30% fat because of the physical nature of the mixture. The oil floats on top of the solid parts of the feed, making the latter unavailable to the chick.

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ence or non-equivalence of their terminal positions has not been considered. We wish to point out that optical activity determinations can be used to establish whether natural triglyeerides have equivalent or non-equivalent fatty acids esterified at the 1- and 3-positions of the glycerol.

The term "equivalence or non-equivalence of the 1- and 3-positions in natural triglyeerides" refers to whether nature has differentiated between these two positions during the biosynthesis of triglyeerides. Symmetrical mono- and diaeid triglyeerides (such as trilaurin and 2-oleodipalmitin) will, of course, have equivalent terminal positions since both are esterified to the same fatty acid. The 1- and 3-positions of a single triglyeeride molecule can be esterified to different fatty acids, however, and the total fat containing

that molecule can still have equivalent terminal positions. This will occur if each fatty acid molecule has an equal chance of being esterified at either the 1 or 3-position of a triglyeeride molecule during biosynthesis. Equal placement on the 1- and 3-positions would make these two positions interchangeable. Therefore, equivalence or non-equivalence of the terminal positions in a natural fat can be defined on the basis of whether the 1- and 3-aeyl groups are interchangeable, In a mixture of triglyeerides, the 1- aud 3-positions can be considered equivalent if *intramolecular* exchange of the 1- and 3-acyl groups on each individual nmlecule does not change the composition of the total system. In this context, a change in molecular symmetry (i.e. from D-form to L-form) is considered a change in composition.

If the two terminal hydroxy groups of glycerol are esterified to two different optically inactive fatty acids, then the middle carbon of the glycerol becomes asymmetrically substituted, and an optically active glyeeride results. If the two terminal hydroxy groups are esterified with the same fatty acid, the resulting glyceride is optically inactive.

1-palmito-2-oleo-3-stearin 1-stearo-2-oleo-3-palmitin

If the fatty acids esterified at the 1- and 3-positions of 1-palmito-2-oleo-3-stearin are interchanged, the molecule becomes 1-stearo-2-oleo-3-palmitin. (It is essential that the carbon atoms of the glycerol retain their original numbering while such an exchange is made.) Examination of the schematic formulas for these two triglycerides shows them to be mirror images and therefore enantiomers. In a racemic mixture, both of these isomers are present in equal quantities, and the mixture is optically inactive. If one of the two isomers is the predominant or exclusive component present, the mixture is optically active.

For the 1- and 3-positions of a natural palmitooleo-stearin to be equivalent, intramoleeular exchange of palmitic and stearic acids must not alter its composition. If equal quantities of 1-palmito-2-oleo-3 stearin and 1-stearo-2-oleo-3-palmitin arc originally present, then intramolecular exchange of the terminal acyl groups converts each molecule into its enantiomer. Therefore, the composition of the total mixture would not change and it would remain racemic and optieally inactive. Stearie and palmitic acids are equally divided between the 1- and 3-positions.

Conversely, when unequal quantities of the 1-palmito-2-oleo-3-stearin and 1-stearo-2-oleo-3-palmitin arc present, then intramolecular exchange of terminal aeyl groups does change the composition and molecular symmetry of the system. Such a mixture must be optically active, and stearic and palmitic acids are unequally divided between the 1- and 3-positions.

We conclude, therefore, that the equivalence or non-equivalence of the terminal positions in natural fat triglycerides is directly related to whether the same triglycerides are optically active. Optical activity indicates non-equivalence of the 1- and 3-positions. Optical inactivity indicates equivalence of the 1- and 3-positions.

Because the asymmetry of an optically active triglyeeride is extremely small, its rotation of polarized light cannot usually be measured by present techniques. This has been shown to be true for synthetic asymmetric triglyeerides of common long chain fatty acids (4,5). Sehlenk (5), however, has reported that x-ray diffraction patterns of solid long chain fatty acid triglycerides can differentiate optically active from raeemic triglycerides. Schlenk has isolated palmito-oleo-stearin from cocoa butter and shown it to be a racemate. This indicates that equal quantities of 1-palmito-2-oleo-3-stearin and 1-stearo-2-oleo-3 pahnitin must be present in cocoa butter, and that the 1- and 3-positions of this natural triglyceride arc equivalent.

On the other hand, Maier and Holman (6) have reported that the ability of *Sapium sebiferum* and *Sebastiana lingustrina* seed fats to rotate polarized light is due to the presence of optically active triglycerides containing 2,4-deeadienoic acid as a terminal ester group. This indicates that the unusual 2,4-deeadienoic acid is not equally distributed between the 1-and 3-positions of the triglycerides of these fats.

It still remains to be proven whether most natural triglycerides are optically active or racemic. Should most prove to be raeemie, then their]- and 3-positions would be equivalent. VanderWal (2), Gunstone (3) and Coleman and Fulton (7) have assumed this to be true in their hypotheses for the distribution of fatty acids in natural triglycerides. Should some natural triglycerides prove raeemic and others optically active, then each fat must be studied individually, unless some consistent pattern can be found.

Schlenk's procedure for determining the optical activity of a natural triglyceride involves the isolation of a single triglyeeride from a natural fat and comparison of its x-ray diffraction pattern with those of similar synthetic active and racemie materials. Since techniques for isolating single triglycerides from natural sources are currently inadequate (except in special cases like palmito-oleo-stearin from cocoa butter) better purification techniques will be necessary before widespread use of Schlenk's method will be possible. It is conceivable, however, that future studies of the physical properties and enzymatic reactions of optically active triglycerides may reveal some easier method for determining if any optically active triglycerides are present in a total natural fat.

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