
VOLUME 62 2002

VARIATIONS IN THE IODINE VALUES OF FATS FROM FATTY, MUSCULAR AND BONE TISSUES OF THE ROMNEY SHEEP

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SUMMARY

Fats from various tissues of 48 six-year-old Romney ewes and 60 sixteen-month-old Romney wethers were found to have the following mean iodine values:

Depot	*Ewes*	*Wethers*
Perinephric fat	40	44
Omental fat	—	46
Loin subcutaneous fat	42	49
Loin intermuscular fat	44	—
Longissimus dorsi muscle fat	53	60
Femur	45	—
Tibia-tarsus	54	—
Metacarpus	80	—

As indicated above, the iodine values of fats from wethers were higher than those for ewes. Within a given tissue the iodine values showed great variation — *e.g.*, in perinephric fats of the ewes and wethers, the iodine values cover a range of 15 and 12 units respectively. Moreover, the iodine values were, in general, not uniform in the different depots of the same animal but some correlation was found between the iodine values of the subcutaneous and the intermuscular fats and the longissimus dorsi muscle fat in the ewes.

Although the iodine values of the fats have been examined from the point of view of growth rate and order of development of the tissues, and the temperature of the depot, no correlation has been found, nor can any reason be given for the remarkably high iodine value of the fat from the metacarpus.

No significant differences were apparent between the iodine values of fats from wethers which had been grazed on different pastures, namely, perennial ryegrass; short-rotation ryegrass; perennial ryegrass plus white clover; short-rotation ryegrass plus white clover; perennial ryegrass then changed to short-rotation ryegrass plus white clover; short-rotation ryegrass plus white clover then changed to perennial ryegrass.

INTRODUCTION

THE LITERATURE contains many references to the characteristics of body fats of animals. Few papers, however, have been concerned with an examination of material from ruminants of known history nor have many workers attempted to explain the reasons for the variability in the characteristics of the fat. Two sheep experiments in which dissection and chemical analyses of carcasses were undertaken provided the material for the studies reported here. The present paper is an extension of the studies of Shorland, Barton and Rae (1962). It is largely concerned with emphasizing the variability in the iodine value of the fats from these experiments and examining the results in relation to findings made by earlier investigators.

The iodine value or iodine number gives a measure of the composition of a fat in terms of the proportions of saturated and unsaturated acids present. Fats having a high proportion of saturated acids have low iodine values while those with a high proportion of unsaturated acids have high iodine values. Fats with low iodine values are "hard" and they are not as susceptible as "soft" fats (high iodine values) to oxidative spoilage. In addition, iodine values of fat samples can be used as a convenient and simple means of selecting material for a more detailed study involving, for instance, gas-liquid chromatography.

Among the meat animals, soft fat is known to occur in pigs, particularly in those raised in the cornbelt region of the United States of America. When corn is fed to pigs, corn oil is deposited in the carcass fat and if the pigs grow slowly the amount deposited is considerable. In the case of fast-growing pigs, however, the fat is synthesized in considerable degree from the non-fatty constituents of the diet and accordingly the depot fat is relatively hard (Callow, 1935a, b). Thus the characteristics of the fat of the pig are related to its diet (Ellis and Hankins, 1925; Ellis and Isbell, 1926; Ellis *et al.*, 1931; Hilditch, 1956). Similarly, studies with rats have shown that, when soybean oil is fed, the iodine value of the deposited fat is about 132, whereas in rats fed coconut oil under the same conditions, the iodine value is only 35 (Harper, 1961).

The iodine value and the fatty acid composition of the depot fats of ruminants, on the other hand, remain more or less unaffected by the feeding of unsaturated fats (Thomas *et al.*, 1934). The marked difference in the characteristics of the depot fats, in relation to dietary lipids shown to exist between simple-stomached animals and the ruminants, is now generally believed to be due to events taking place in the rumen, notably hydrogenation by rumen micro-organisms. Hydrogenating mechanisms of rumen micro-organisms first indicated by Reiser (1951) are not only responsible for the reduction in iodine value in the ingested fat but also for the formation of *trans* unsaturated acid (Hartman *et al.*, 1954; Shorland *et al.*, 1955; Garton, 1961). It is also of interest that the branch chain fatty acids isolated from the fats of ruminants in the Fats Research Laboratory are now believed to originate from the micro-organisms of the rumen (Saito, 1960; Akashi and Saito, 1960; Keeney *et al.*, 1962). The rumen flora and fauna thus play a considerable role in the modification of dietary lipids but, in addition, it is likely that further changes take place within the tissues of the animal after absorption.

EXPERIMENTAL

Ewes

Nine groups of 6-year-old Romney ewes provided the carcasses from which fats and bones were removed. The sheep had been subjected to undernutrition and various levels of thyroxine treatment for a period of 28 days. Control groups were also analysed. In total, 48 animals were examined (see Kirton and Barton, 1958).

The fat samples were taken from the left side of the ewe carcass. The subcutaneous fat sample comprised all the outer fat of the loin, and the intermuscular fat all the remaining dissectible fat of this joint. The muscle fat was prepared from the longissimus dorsi in the loin region. The metacarpal, tibia-tarsal, and femur bones of eight of the sheep were also analysed.

Wethers

Six groups each comprising 10 sixteen-month-old Romney wethers were slaughtered. Each group had been grazed separately: on perennial ryegrass (P); on short-rotation ryegrass (S); on perennial ryegrass plus white clover (P + C); on short-rotation ryegrass plus white clover (S + C). Two further groups were switched after four months (P to S + C and S + C to P) and remained on these pastures for 2½ months before slaughter.

The omental fat samples of the wethers were obtained at slaughter and the perinephric and subcutaneous fats of the left side of the carcass were obtained at the time of carcass analysis, as was the longissimus dorsi muscle fat sample which was taken from the loin region.

The fatty tissues and the muscular tissues were extracted as described by Shorland *et al.* (1944) and by Barnicoat and Shorland (1952), respectively. A similar extraction procedure was used for the bones after they had been crushed. The fat-free dry matter was determined by heating the fat-free residue to constant weight at 100°C. The iodine values of the extracted fat were determined by the Wijs (1 hr) method using iodine monochloride and measuring the amount in grams of iodine absorbed by 100 g of fat.

RESULTS

The means and ranges of iodine value and percentage fat for the bones and the fatty tissues examined from the ewe carcasses are set out in Table 1. The results are arranged in descending order of mean iodine values. Thus it can readily be seen that the metacarpus has an oily fat with a mean iodine value of 80 and, at the other end of the scale, the perinephric fat is very hard with a mean iodine value of 40. Of the three bones studied it will be noted that:

(a) There is no overlap in the individual iodine values between any three bones.

(b) There is an orderly progression from oily to hard fats moving from the metacarpus (lowest extremity to the tibia-tarsus (middle bone of the three major bones of the hind-limb) to the femur which is the bone at the head of the hind-limb.

As far as the fatty tissues are concerned, mean differences in iodine values are not very great; this is particularly the case for the subcutaneous depot, an "outer" fatty tissue, and the perinephric depot, an "inner" fatty tissue. The

TABLE 1: MEANS AND RANGES FOR IODINE VALUES AND PERCENTAGE FAT FOR VARIOUS DEPOTS OF ROMNEY EWE CARCASSES

Fat Depot	*Iodine Value*		*Fat (%)*	
	Mean	*Range*	*Mean*	*Range*
Metacarpus	80	77–84	24	22–28
Tibia-tarsus	54	51–57	31	27–33
Longissimus dorsi muscle	53	45–58	8	4–12
Femur	45	42–48	40	35–44
Intermuscular fat	44	36–53	71	55–87
Subcutaneous fat	42	35–48	81	58–95
Perinephric fat	40	33–48	88	71–96

NOTE: Bone data based on eight sheep; fatty tissue analyses based on 48 sheep.

TABLE 2: THE EFFECT OF GRAZING DIFFERENT TYPES OF PASTURE ON THE MEAN IODINE VALUES OF THE COMBINED OMENTAL, SUBCUTANEOUS, PERINEPHRIC AND MUSCLE FATS OF WETHERS

Pasture Treatment	*No. of Sheep*	*Iodine Value*
Perennial ryegrass (P)	40	47
Perennial ryegrass plus white clover (P+C)	40	51
Short-rotation ryegrass (S)	40	49
Short-rotation ryegrass plus white clover (S+C)	40	51
Perennial ryegrass (P) changed to short-rotation ryegrass plus white clover (S+C)	38	51
Short-rotation ryegrass plus white clover (S+C) changed to perennial ryegrass (P)	40	50

muscle fat of the longissimus dorsi is the softest carcass fat and it is present in small amounts.

The iodine values of bones vary less (six units in the case of the tibia-tarsus and the femur and seven units in the case of the metacarpus) than the fats in the fatty tissues studied. The extreme variability is shown to a marked degree in the intermuscular fat depot where the iodine values range over 17 units. However, the perinephric fat varies over a 15 unit range, and the muscle fat and subcutaneous fat each have a range of 13 units.

The percentage fat in the different tissues examined also shows extreme variability. Passing mention should be made of the observations on the bones where it is seen that the femur, in particular, has a relatively high fat content and to comment that this fact is often overlooked in many carcass analyses, especially in growth studies in which the various components of the body are being compared.

In Table 2 results are presented for the mean iodine values of the four depot fats examined for each of the six groups of wether carcasses. No significant differences between pasture groups for iodine values were apparent in these data. The liveweight growth of the sheep differed markedly between treatments and there were also pronounced differences in carcass composition (unpublished data).

The mean iodine values for depot fats of the wether carcasses are given in Table 3. The iodine value of the muscle fat differed significantly from the other depots. This was the only significant difference.

TABLE 3: MEAN IODINE VALUES FOR DEPOT FATS OF ROMNEY WETHERS

Fat Depot	*No. of Sheep*	*Iodine Value*
Perinephric	59	44
Omental	60	46
Subcutaneous	60	49
Longissimus dorsi muscle	59	60

TABLE 4: CORRELATION COEFFICIENTS BETWEEN IODINE VALUES OF FATS FROM DIFFERENT TISSUES OF THE LOIN (48 PAIRS OF OBSERVATIONS)
ROMNEY EWES

	Subcutaneous	*Intermuscular*	*Longissimus dorsi muscle*
Perinephric	0.147	0.096	0.077
Subcutaneous	—	0.415**	0.334*
Intermuscular	—	—	0.049

* $P<0.05$.
** $P<0.01$.

Simple correlation coefficients were calculated between the iodine values of the various depots of the ewe carcasses and the results are given in Table 4. The relationships between depots for their iodine values are non-significant except in the case of two correlations, namely, between subcutaneous fat and intermuscular fat and subcutaneous fat and the fat of the longissimus dorsi muscle.

DISCUSSION

Most of the work on variations in the iodine values of fats of meat animals has been done on pigs. Certain relationships have been found with this species between iodine values of the fat and the site and rate of its deposition. Type of diet has also been shown to affect greatly the fat characteristics of the pig. In ruminants, however, similar relationships have not been demonstrated so conclusively.

Henriques and Hansen (1901) were the first to suggest that the composition of the depot fats of pigs is controlled by the temperature of the region. They proposed that regions of high temperature, for example the fat around the kidneys, would have low iodine values. To support their theory they lowered the iodine value of the outer back fat of a pig merely by covering the animal with a sheep's skin. Callow (1935a, b), on the other hand, found that there was considerable variability in the iodine value along the back fat of the pig despite uniformity in temperature. Callow (1935a, b) and Johns (1941) attributed the variability in iodine value of the back fat of pigs to the rate of growth of the fatty tissue and to the thickness of the fatty layer.

Studies by Shorland *et al.* (1944) showed that, while in most instances there was a general connection between the iodine value and growth rate of the fats of the bacon pig, variations in the iodine values of fats from pigs fed copra could not be explained in this manner. Furthermore, the results of fatty acid composition determinations of fats from different regions of the pig or of the sheep carcass did not appear to be consistent with the growth rate of fat hypothesis (Shorland and de la Mare, 1944).

In contrast to the earlier studies already reviewed, Callow and Searle (1956) using cattle, and Callow (1958) with sheep, found no clear-cut relationship between growth rate of fat and its iodine value. In cattle but not in sheep, there was a tendency for the iodine value of the fat in both the fatty and muscular tissues in each anatomical joint to decrease with increasing fat content. The work on sheep and cattle suggested that there might be a correlation between order of development of the fatty tissue and its iodine value but the results were generally much less definite than for pigs. Recently, however, Callow (1962) working with cattle found that a good correlation existed between the average percentage of fat in a joint and the average iodine value. He also showed in this paper that there was an effect due to local temperature of the fat depot examined.

Hartman and Shorland (1961), in considering the differences in composition between internal and external fats of ruminants, have indicated that there are very small differences between these fats in the horse and they are of the opinion that this complicates the formulation of a general theory to explain the changes in composition due to the site of the fat deposition.

The present work emphasizes the difference between non-ruminants and ruminants in regard to the deposition of fat. It will be recalled that the mean iodine value of the subcutaneous fat of ewe carcasses was 42, while the perinephric fat had a mean iodine value of 40. The difference of only two units between these two depots was not statistically significant. A further important aspect is the considerable sheep variability in iodine values within each depot. The ranges presented in Table 1 show the extent of this variability and indicate that there is much overlapping in iodine values between a number of depot fats.

Among the bones, however, it is clear that each bone (*e.g.*, femur) differs from another bone (*e.g.*, metacarpus) in its fat characteristics and that this would indicate that bone fats ought to be regarded as being distinct from the other depots studied. The neatsfoot oil-like nature of the metacarpus fat is of interest but no tenable explanation can be given at this stage for its high iodine value in relation to that of the other bones.

The high iodine value of the muscle fat has been explained by Callow (1962) and Ostrander and Dugan (1962) as being due to the presence of phospholipids extracted from the muscle cells and these have an iodine value of *c.*120 according to Callow and Searle (1956). These last-mentioned workers showed with cattle that muscle fats of high iodine values were associated with animals having low percentages of fat, since the phospholipid fraction tended to be a constant irrespective of the state of fatness of the animal. A preliminary examination of the writers' data failed to show agreement with their findings.

Results presented elsewhere (Shorland *et al.*, 1962) showed that neither thyroxine nor undernutrition significantly affected the iodine values of sheep fats. This finding, together with the results given in Table 2, indicates that there is little or no correlation between rate of fat deposition and the iodine value of the fat. This observation further serves to distinguish the fats of the ruminant from those of the pig.

The lack of significant pasture differences in the iodine values of the fats of wethers is surprising in view of the differences reported by Johns (1962) in the volatile fatty acid composition of the rumen material of sheep in a previous experiment grazed on perennial ryegrass and short-rotation ryegrass plus white clover pastures. The perennial ryegrass gave high percentages of acetic acid and this should result in fats with low iodine values as acetate probably contributes significantly to the synthesis of higher fatty acids in depot and milk lipids (Shorland, 1955; Garton, 1960). While it is true that the wethers grazed on perennial ryegrass had fats of low iodine value, the differences between this group and all other groups was not, however, significant.

In sharp contrast to the present results, Shaw *et al.* (1960), working with two groups of steers each of two animals, stated: "Although values were obtained on only two animals in each group, good agreement was obtained within each group, leaving little doubt that the [iodine] values represented a real difference in unsaturation of fat due to diet".

The correlation coefficients given in Table 4 are generally of a low order. The interpretation therefore is that, if an animal has a high iodine value of one of its fat depots, it will not necessarily have a high value in its other depots. This means that there is a degree of independence between depots, and that a factor affecting the composition or iodine value of one depot may not be responsible for the characterization of the fat in another depot in the same animal.

It is clear from the foregoing that adequate explanations for the variations in iodine values of sheep fats are not yet available. It may now be necessary to examine in greater detail than hitherto the effect which the kind of fermentation occurring in the rumen has on the fatty acid composition of the depot fats of sheep. In addition, it may be profitable to study genetic effects in relation to the characteristics of sheep fats. Current work is concerned with both these aspects.

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DISCUSSION

Q: *Do the proportions of short chain fatty acids in the rumen affect the tissue fat composition, and did not Shaw* et al. *(1960) feed one group of steers a diet containing linseed oil meal and thereby produce differences in the iodine value of their body fat?*

DR F. B. SHORLAND: I know of no exact experiments to determine the effect of short chain fatty acids of the rumen on the composition of the tissue fats, but by analogy with the milk fats such an effect could be anticipated. Obviously some of the fatty acids in the tissue fats of ruminants come from the dietary fats as shown by the presence of *trans* unsaturated acids derived by hydrogenation of the dietary unsaturated fatty acids in the rumen. The final composition of the tissue fat thus depends on the contributions of the dietary fat and of the endogenous fat derived at least in part from short chain fatty acids of the rumen.

R. A. BARTON: Both groups of steers in the experiment of Shaw *et al.* (1960) were fed linseed oil meal. The diets of the two groups differed only in the preparation of the feedstuff — *i.e.*, chopped alfalfa and ground corn plus linseed oil meal was compared with ground and pelleted alfalfa and flaked corn plus linseed oil meal.

Q: *Were the differences in iodine value recorded in Table 1 statistically significant within the bone and tissue groups?*

MR BARTON: Yes, the iodine values of the bones were significantly different. The iodine value of the muscle fat of the ewes differed significantly from the fat of the fatty tissues. This was also the case for the wethers.

Q: *How long is it necessary to feed animals on a particular diet to ensure that the body fat arose from that diet?*

DR SHORLAND: The rate of turnover of fats in the fatty tissues is rapid. Experiments using deuterated fats suggest a half-life of 3 days in a mouse. The depot fats are rapidly affected by changes in composition of the dietary fat. For example, Longenecker (1939; *J. Biol. Chem.*, *130*: 167) showed that, after feeding a ration containing 40% coconut oil, in 14 days the lauric acid content of the depot fat was raised to 7.8% and to 17.2% after 21 days, as compared with 45% in coconut oil and only trace amounts normally found in rat depot fats. The actual time required to produce in an animal a depot fat of the same composition as the dietary fat depends on the composition of the dietary fat and on the species.