THE EFFECT OF UNSATURATED FATS ON CARCASS COMPOSITION AS INFLUENCED BY THE PRESENCE OR ABSENCE OF RUMEN PROTOZOA

by

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"Amo, Ergo Sum."

E.T.C.

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INTRODUCTION

The nutritional importance of dietary animal fats and their alleged association with human circulatory abnormalities have stimulated research in the composition of animal fats. Students of nutrition are taught that the fatty acid composition of an animal's fat lies somewhere between that synthesized from carbohydrates and that of the fatty acid composition of the diet.

While ruminants and non-ruminants may consume diets quite similar in lipid composition, differences in their lipid metabolism leads to the production of depot fat that is more saturated in cattle and sheep and more unsaturated in swine. These differences are attributed in part to differences in the anatomy and physiology of their digestive tract. During the last two decades it has been found that lipids can undergo changes in the rumen. That is, glycerides can be hydrolyzed and unsaturated fatty acids can be hydrogenated or otherwise modified by the formation of isomers. More recently it was found that rumen microorganisms greatly influence the extent of hydrogenation, although a complete and accurate account of the lipogenic activity of these microorganisms has not been established.

Within the scope of the data thus far available, one can conclude that rumen microorganisms (bacteria and/or protozoa) alter dietary lipids to the extent that a somewhat homogenous product is found in the adipose tissues of cattle and sheep. Interest, therefore, lies in determining which one of these microbial classes produce this effect and if an alteration in the microbial population, by removal of one class (rumen protozoa), produces a significant change in the depot fat of cattle. If, in fact, this change is accomplished by reducing the

number of rumen protozoa the importance of such a find may lead to a better understanding of the factors influencing carcass quality.

The following experiments were undertaken to determine the changes in lipid metabolism related to the removal of rumen protozoa and the effect of such changes on depot fat composition.

LITERATURE REVIEW

Role of Lipids in the Diet of Ruminants

Much of the plant material consumed by herbiverous animals consists of grass leaves, the dry matter of which contains from 4 to 6 percent lipids, with the major portion in the form of glycerides. It has been shown that the main dietary fatty constituents of pasture-fed animals is linolenic acid (Shorland, et al., 1955). Also, it has been shown that the total fatty acids of pasture lipids contains very high proportions of saturated acids, including only very small amounts of saturated C18 (stearic acid) (Garton, 1960). The lipid content of concentrates depends on the source and whether they have been extracted with solvent. In the most commonly used plant materials, such as soybean meal, linseed meal, corn, barley or wheat, the oil contains glycerides rich in unsaturated C18 fatty acids. In animal products such as fishmeal, unsaturated acids of chain length longer than C18 are present (Garton, 1960). However, these polyunsaturated fatty acids appear only in trace amounts in the depot fat of ruminants.

Dietary lipids present in natural plant source apparently have no effect upon ration intake or digestibility. However, when fats or oils are added to the ration in excess of that normally found in plant material, feed intake is noticeably reduced (Cameron and Hogue, 1968; Cameron, et al., 1966) with the effect being more severe on high concentrate diets (Putnam, et al., 1969). The addition of fats (oil or lard) may further exert a depressing effect upon the digestion of proteins and fibrous materials of the ration (Brooks, et al., 1954). This affect may be alleviated by the addition of calcium carbonate (Davison and Woods, 1963a, 1963b). The nature of the ruminant digestive process, unlike that

of simple-stomach animals, provides a more or less continuous flow of digesta to the lower intestinal tract following feed intake. This apparent "hold up" of digesta in the rumen has been reported to enhance the digestibility of dietary fatty acids (Felinski, et al., 1964).

The majority of rations fed to the ruminant animal are usually low in dietary fat, indicating that much of the depot fat observed in the animal is derived from that synthesized from dietary carbohydrates. For this reason it is apparently quite difficult to produce any fat difficiency within these animals. Lambs fed a fat-free diet for seven months showed no signs of skin lesions or other symptoms typical of a fat deficiency (Cunningham and Loosli, 1954). Analysis of the rumen contents indicated the presence of trace amounts of linoleic and linolenic acids. Babatunde and co-workers (1968) indicated a considerable net synthesis of linoleic acid at the tissue level.

As a general rule, the data on the metabolism of long-chain acids by rumen microorganisms, would indicate little metabolic activity other than hydrogenation and isomerization. While the catabolism and resynthesis of dietary fat does not occur to any great extent in the rumen, published data on rumen metabolism of dietary lipids indicates a rapid hydrolysis of triglycerides to the glycerol and free fatty acid components (Church, 1969). A large percent of the unsaturated fatty acids may then be hydrogenated to their saturated analogue (Ward, et al., 1964; Shorland, et al., 1955; Babatunde, et al., 1968; Lassiter, 1968). For example, on incubation of linoleic acid in the artificial rumen, 93% was converted to stearic acid in the first 90 minutes and within 5 hours was nearly 100% saturated acids (Ward, et al., 1964). In subsequent experiments Ward and

associates (1964) found that the less fluid portion, consisting apparently of undigested feedstuffs was exceptionally rich in saturated fatty acid, particularly stearic and palmitic acid, whereas the percentage of unsaturated acids was small. Some research, however, suggests that in addition to dietary lipids, fat may be synthesized in the rumen (Hungate, 1966) or enter the alimentary tract from some endogenous source (Felinski, et al., 1964).

The production and absorption of volatile fatty acids (VFA), mainly acetic, propionic and butyric acids, has been the subject of many reviews (Baker, et al., 1947; Oxford, 1955). The addition of fats or oils to the ration has been reported to increase the ruminal VFA concentration although the differences were not significant (Putnam, et al., 1969; Shaw and Ensor, 1959; Esplin, et al., 1963). The apparent effect of dietary lipids on ruminal volatile fatty acid production is to decrease the molar proportion of acetic and increase that of propionic acid (Shaw and Ensor, 1959). Concentrations of total volatile fatty acids would be expected to be closely related to rate of fermentation, but would also reflect such factors as removal from the rumen by absorption and passage to the lower gastro-intestinal tract or utilization of these acids by rumen microorganisms. Other factors which have been reported to affect ruminal fatty acid ratios include: proportions of roughage to concentrate, pelleting, particle size, heat treatment, various oils, protein level, environment, frequency of feeding and mineral adequacy of the diet (Shaw, et al., 1960; Thompson, et al., 1965; Weiss, et al., 1967; Wheaton, et al., 1970).

It has been suggested that the incorporation of propionic acid, rather than acetic acid, in the first condensation step of fatty acid synthesis, will result in the production of long-chain, odd-numbered fatty acids.

According to Brooks, et al. (1954) and Tove and Matrone (1962), the total number of microorganisms within the rumen are not as great when fat is present in the diet as compared to the control diets. However, some data indicates there is a change in the microbial species present when fat is added to the ration (Brooks, et al., 1954). Many other dietary factors may influence the type and concentration of microorganisms within the rumen. These factors include: roughage level of the diet, water intake, physical form of the ration, ration processing and length of time after feeding (Masson, 1950; Hungate, 1966; Dehority and Purser, 1970).

Role of the Rumen Microorganisms as Related to Lipid Metabolism

The hydrogenating action of the rumen microorganisms was first demonstrated by Reiser in 1951 and later confirmed by others (Hoflund, et al., 1955; Shorland, et al., 1957; Tove, 1960). Many studies have shown that ruminant depot fat, unlike that of the monogastric (Miller and Rice, 1967; Koch, et al., 1968), is not extensively altered by dietary lipid, saturated or unsaturated (Shorland, et al., 1957; Tove and Matrone, 1962). The difference in the digestive processes, or more correctly the presence of a large microbial population within ruminants as compared to non-ruminants, contributes greatly to the observed effect. Tove and Matrone (1962) fed lambs a diet in which 20% of the ingested fat

was comprised of linoleic (C18:2) acid. Rumen samples taken at 3 hours after feeding revealed only traces of the acid present. This eightfold difference between the dietary and rumen levels of linoleic acid is indicative of the rapidity at which hydrogenation by rumen microorganisms can occur.

Some results suggest that the hydrogenase present in rumen microorganisms are to some extent specific, with a more complete hydrogenation of linolenic acid occuring over that of linoleic acid (Shorland, et al., 1957). Controversy exists, however, over whether the extent of lipogenic activity occuring in the rumen is accomplished by the bacteria or protozoa population.

Much of the data presented refers to the hydrogenation of lipids by rumen bacteria (Reiser, 1951; Garton, 1960) or rumen microflora (Tove, 1960, Tove and Matrone, 1962). Other research using washed suspensions of rumen bacteria suggest that the bacteria do not contribute significantly to the hydrogenation of dietary unsaturated fats (Wright, 1959).

In addition to the lipids of the diet, very small amounts of lipids are released from rumen microorganisms as they pass from the rumen to the lower parts of the digestive tract (Garton, 1960). Because of the possible need of ruminants for "essential" fatty acids such as linoleic acid, Cunningham and Loosli (1954) raised the question whether or not such acids are present in rumen bacteria. They were unable to demonstrate bacterial synthesis in vitro. Garton and Oxford (1955) have confirmed their findings. Other researchers (Akashi and Sartie, 1960; Felinski, et al., 1964) suggest that the lipolytic activity of ruminal bacteria contribute to the presence of odd-number or branch-chain higher fatty acids by microbial synthesis or from fermentation of amino acids.

The number of bacteria in defaunated animals are greater than in normal faunated ones (Hungate, 1966). The difference can be due either to the competition for food or to consumption of bacteria by the rumen protozoa.

The establishment of a normal rumen protozoa population will result in a significant decrease in the viable bacteria concentration. Although, it can be concluded that the overall response to establishing a protozoa population is a general shift in the fatty acid concentration from unsaturated acids to their more saturated analogue (Wright, 1959; Tove and Matrone, 1962; Klopfenstein, et al., 1966), the major effect of protozoa in the rumen upon dietary lipids is a relative shift from linoleic to oleic acid (Klopfenstein, et al., 1966). Defaunation of the rumen will result in a larger proportion of these unsaturated fatty acids reaching the lower digestive tract (Tove and Matrone, 1962; Lassiter, 1968).

As related to the volatile fatty acid production, ruminal protozoa have been shown to increase the proportion of butyric acid (Klopfenstein, et al., 1966) and thus lower the acetic to propionic acid ratio (Christiansen, et al., 1965; Klopfenstein, et al., 1966) while not effecting the total volatile fatty acid concentration.

It is generally believed that neither rumen bacteria nor rumen protozoa can synthesis fats to any great extent. It must be reported, however, that one researcher (Keeney, et al., 1962) has estimated that at least 24 grams of bacterial lipids and 113 grams of protozoal lipids are available to the digestive processes for the mature cow during each 24 hour feeding period.

Response of Blood and Body Tissues to Absorbed Lipids

Within the normal, intact ruminant animal the serum lipid values appear to be unresponsive to dietary lipid levels or breed of the animal (Banks and Hilditch, 1932; Veum, et al., 1970). Age, sex, gestation, parturition and lactation will cause a fluctuation in plasma lipid values (Garton, 1960). While the concentration of plasma lipid increased with the age of the animal, the lack of response to additional dietary fat indicated a high degree of homeostatic control over these parameters. When animals were removed from feed, there was a significant difference associated with the length of fast and blood lipid levels (Veum, et al., 1970). This increase in serum lipid levels is probably related to mobilization of tissue lipids to meet energy needed during fasting.

It would appear that only a small portion of the unsaturated fats reach the lower intestinal tract and are available for absorption into the blood vascular system (Duncan and Garton, 1962; Miller and Rice, 1967). This is undoubtedly due to prior hydrogenation in the rumen. It has been suggested that the role of the unsaturated fatty acids in ruminant bile, and presumably that already within the digesta, is to facilitate absorption of long-chain, saturated fatty acids (Leat, 1965). If this is indeed true, one would expect the proportion of unsaturated fats reaching the blood to be extremely low.

Moore, et al. (1968) found that the plasma cholesterol esters and phospholipids contained polyunsaturated fatty acids in appreciable amounts. The plasma triglycerides and unesterified fatty acids contained

low concentrations of linoleic acid, and only trace amounts of linolenic and arachidonic acids (Duncan and Garton, 1962; Moore, et al., 1968). There is, however, considerable variation among the animals in their fatty acid composition of serum and liver triglycerides (Miller and Rice, 1967). The plasma triglycerides were found to resemble depot fat in that stearic, palmitic and oleic acids were the major components, accounting for more than 80% of the total acids. Linoleic acid accounted for only 1.5% of the acid and neither linolenic nor arachidonic acids were present (Garton, 1960). In contrast, the fatty acids of the cholesterol ester, amounted to about half of the total fatty acids in plasma lipids. They contained only 8.9% saturated, while the unsaturated acids present were mainly linoleic and linolenic acids, with smaller amounts of palmitoleic, oleic and arachidonic acids (Veum, et al., 1970). Thus it appears that the polyunsaturated C18 acids, after escaping hydrogenization in the rumen, are selectively esterified with cholestrol during or after their intestinal absorption (Duncan and Garton, 1962; Garton, 1960).

Although the ruminant carcass fat has not been shown to be significantly associated with all plasma lipids, the results would imply that the concentration of plasma lipids is positively related to the type of adipose tissue and degree of finish of the carcass (Keeney, et al., 1962; Brumgardt and Bray, 1966).

A considerable amount of data has been collected relating to the composition and areas of deposition of adipose tissue in the ruminant animal and to the effects that ration, age, sex, temperature and a number of other parameters have on the type and amount of fat deposited.

Ruminant adipose tissue is found under the skin, especially over the back, around the kidney and in the abdominal cavity. Generally the amounts and distribution varies from breed to breed and to some extent, from animal to animal. The adipose tissue fatty acids are characterized by a high proportion of saturated and monoenoic acids and a very low proportion of polyunsaturated fatty acids (Miller and Rice, 1967). Although ruminant depot fat may contain more than 35 fatty acids, 6 usually comprise more than 90% of the total fatty acids. These are myristic (C14:0), palmitic (C16:0), and stearic acid (C18:0) of the saturated acid series, and palmitoleic (C16:1), oleic (C18:1) and linoleic (C18:2) of the unsaturated series (Tove, 1960; Terrell, et al., 1967). All these acids, except linoleic acid, which can only arise from dietary fat, can be synthesized by the animal from carbohydrate or other fatty acids (Tove, 1960). Frazer (1952) suggested that the reason for the larger amount of long chain than short chain acids in adipose tissue was associated with the method of absorption. The short chain fatty acids being absorbed via the lymphatic system.

Adipose tissue consists mainly of triglycerides (Miller and Rice, 1967). It would appear also that triglycerides reflect the effect of hydrogenation more so than any other class of lipids. Phospholipids are found in their greatest concentration within the muscle and may reach levels of 1/4 to 1/3 that of the total lipids present (Hornstein, et al., 1961).

Banks and Hilditch (1932) have reported that, "whatever the degree of saturation of depot fat, its molar content of C18 acids is in the neighbourhood of 70% (rising to about 73% with very unsaturated fats and

falling to about 65% in the more saturated fats)." The increase in stearic acid is mainly at the expense of oleic, and not linoleic acid (Banks and Hilditch, 1932; Tove and Mochrie, 1963). The amount of linoleic acid increased only slightly with the increase in unsaturation of depot fat. Most of the unsaturated depot fat from ruminants does not contain more than 1.2 to 3.2 percent linoleic acid and 0.3 to 0.8 percent linolenic acid (Holmberg and Sellmann, 1956). Unsaturated acids containing two or more double bonds make up less than 10% of the triglyceride fraction and 50% of the phospholipid fraction, with an overall ratio of 4:1 triglyceride to phospholipid in ruminant animals (Hornstein, et al., 1961; Hornstein, et al., 1967; Terrell and Bray, 1969). The data indicates that a broad spectrum of values may be obtained for individual observations with some tendency for grouping around a central mean. Although some variation in the fatty acid composition of adipose tissue may be closely related to the diet of the animal, animal age and environmental effects, much of it is due to the selective deposition of specific fats in preferred areas of the body (Marchello and Cramer, 1963; Terrell, et al., 1967).

Shorland (1953) has postulated that the difference in composition of various fats may result from differential rate of growth of fatty tissues and from distribution of the dietary fatty acids between various fatty tissues. Samples of fat collected from different areas of the carcass appear to vary considerably in some chemical characteristics. The outer layer of subcutaneous fat contains more unsaturated fatty acids than the inner layer (Terrell, et al., 1969a). While within the areas of subcutaneous fat the brisket fat had the highest percent of myristic, palmitoleic

and oleic acids and backfat contained more odd-numbered (C15 and C17) chain acids (Lassiter, 1968).

The predominant fatty acids observed in the intramuscular lipids are palmitic, stearic and oleic acids. These acids accounted for about 30, 15 and 40%, respectively, of the total fatty acid profile (Marchello, et al., 1968). Reports indicate that the extent of intramuscular fat and type of fatty acids present varies from muscle to muscle (Terrell, et al., 1967, Marchello, et al., 1968, Terrell, et al., 1969b), with the highest level of intramuscular lipid located proximate to the 11-12 thoracic vertibrae (Cook, et al., 1964). Further studies indicate that as the amount of intramuscular lipids increased the level of unsaturated fatty acids decreased (Lawrie, et al., 1963), although the reverse has been reported (Cook, et al., 1965). The kidney or perirenal fat has the highest degree of saturated fatty acid of all body components (Roberts and McKirby, 1964; Church, et al., 1967).

From data on composition of various adipose tissues in the body it can be seen that the degree of saturation generally decreases from the internal most parts (kidney) to the external (subcutaneous) depot sites. The change may be associated with a similar change in body temperature and possibly with a difference in the rate of triglyceride deposition (Garton, 1960). Studies have shown that an increase in temperature causes a more saturated type of fat (Marchello and Cramer, 1963; Marchello, et al., 1967; Marchello, et al., 1969). For this reason the length of time exposed to a colder environment may be important in obtaining carcass fat of more unsaturated fatty acid deposition.