FAT DEPOSITION IN RUMINANTS

E.F. ANNISON

SUMMARY

Fat deposition inescapably accompanies skeletal growth and muscle accretion. In early growth deposition of fat and protein occur simultaneously, but beyond a certain body weight protein accretion becomes negligible, and fat gain becomes a large and constant fraction of weight gain. This pattern of tissue development forms the basis of the most successful current strategy for minimising carcass fat, namely the use of large, late-maturing animals. At slaughter weight, these animals are much leaner than smaller, early-maturing animals. Other strategies that may be used to reduce fat deposition include the administration of natural and synthetic growth promotants, and β-adrenergic agonists, but two of the most effective agents, growth hormone and P-agonists, have not been approved for use in livestock production. Immunological approaches, which include enhancement of the activity of endogenous growth hormone and the reduction of fat deposits by injection of antibodies to the animals' own adipocytes, show great promise, and may provide the meat industry with efficient and consumer acceptable methods of producing leaner animals.

INTRODUCTION

Lipids have many diverse functions in the body, but their major roles are as structural components, mainly in cell membranes, or as energy reserves. Lipids involved in membrane function are mainly glycerophospholipids, but adipose tissue consists almost entirely of triacylglycerols. The high energy density of triacylglycerols, and their hydrophobicity which prevents co-storage of water and electrolytes, makes them a preferred storage form of energy (see Stryer 1988).

In mature ruminants, absorbed energy yielding nutrients in excess of current energy requirements are converted to long-chain fatty acids which, after esterification to form triacylglycerols, are deposited as adipose tissue. Absorbed long-chain fatty acids, which circulate as the triacylglycerol component of chylomicra and lipoproteins are also deposited, after complete hydrolysis and re-esterification, in adipose tissue. This process makes it possible to influence the fatty acid composition of adipose tissue, as discussed in a later section. When inflows of nutrients from the alimentary tract fail to meet current energy needs, the energy deficit is met by the mobilisation of adipose tissue in the form of non-esterified fatty acids (NEFA), which are transported to tissues bound to plasma albumin. The hydrolysis of triacylglycerols during the transfer of fatty acids from circulating lipid to adipose tissue, and the reverse process during adipose tissue mobilisation is effected by lipoprotein lipase, an enzyme widely distributed in adipose tissue (see Vernon 1981).

In growing animals, skeletal development and muscle accretion are inescapably accompanied by varying degrees of fat accretion. Consumer pressure to reduce the fat content of fresh meat and meat products has encouraged intensive research to minimize fat deposition. The economic importance of the development of systems which produce low-fat meat may be gauged from estimates that in the U.S.A. the industry spends about

Department of Animal Science, University of Sydney, Camden N.S.W. 2570

$2 billion annually to deposit fat on animals and another $2 billion to remove the fat from meat products (Bergen and Merkel 1991). Current strategies to manipulate body composition in favour of protein production include feeding and selection practices, and the use of exogenous agents, the most important of which are hormones and β-adrenergic agonists

GROWTH AND DEVELOPMENT OF ADIPOSE TISSUE

White adipose tissue is widely distributed throughout the body. Contrary to earlier views, there is now good evidence that the cellular development that accompanies adipose tissue growth involves both cellular hyperplasia and hypertrophy at all stages of growth, and that both processes can be influenced by nutritional manipulation (see Cryer et al. 1992). The various fat depots do not develop at the same time, however, or at the same rate, but the mechanisms for this asynchronous pattern of development are not known. Adipocytes, which appear not to divide, are derived from small, undifferentiated cells found in the stromal-vascular fraction of adipose tissue. These undifferentiated cells can be isolated and cultured, and under appropriate conditions will proliferate and differentiate. Adams et al. (1992) isolated preadipocytes from various adipose tissue depots of sheep of different ages, but when incubated in a medium capable of supporting optimal growth, there were no detectable differences in the rates of growth of any of the cells, suggesting that there are no intrinsic differences in the cells which could account for differential development. In. further studies, rates of proliferation responded to a number of hormones/growth factors, but responses were unrelated to the depots from which the cells were derived.

Van der Walt (1984) pointed out that the various adipose tissue depots in ruminants are not equally active metabolically. The de novo synthesis of long chain fatty acids, for example, is 2-7 fold higher in backfat than in mesenteric fat. Furthermore, relative metabolic activities of different adipose tissues may change with age, plane of nutrition and physiological status.

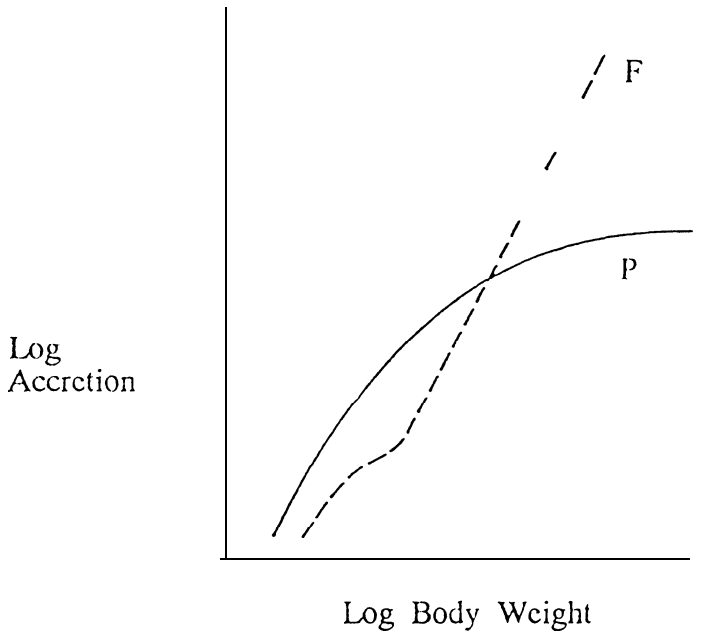


Figure 1. Idealized log–log plot of fat (F) and protein (P) accretion against body weight for sheep, rat, pig and cattle. Adapted from Bergen (1974).

In normally growing animals, skeletal growth and protein accretion is accompanied by fat accretion Beyond a certain body weight, fat gain becomes a large and constant fraction of weight gain (Searle et al. 1972). This implies that whereas the accretion of both fat and protein occur simultaneously in early growth, in later growth the rate of protein accretion becomes negligible (Fig. 1). Differences in the relative rates of fat and protein accretion between late maturing and early maturing animals form the basis of efforts to minimize the fat content of meat producing animals at slaughter, as discussed later (p. 55).

SYNTHESIS OF ADIPOSE TISSUE

In ruminants, adipose tissue is the only important site of fatty acid synthesis in the non-lactating animal, in contrast to other species where almost all fatty acid synthesis occurs either in the liver,, or in both liver and adipose tissue (see Vernon 1981). Adipose tissue fatty acids are either synthesized de novo from simple precursors, or are derived from dietary lipids. The contribution of newly synthesized fatty acids to lipid deposition is variable, but unlikely to exceed 30-35% (Vernon 1981).

Fatty acid synthesis in adipose tissue

Acetate, and not glucose, is the major precursor of long-chain fatty acids This striking difference between ruminants and non-ruminants stems from the roles of glucose and acetate in acetyl CoA formation. In non-ruminants, acetate is largely derived from glucose via pyruvate produced in the mitochondrion and transferred to the cytoplasm, the site of fatty acid synthesis, by the citrate cleavage pathway (see Annison 1984). The enzymes involved in this pathway, however, are present at low levels in ruminant tissues. In addition, reduced activities of key glycolytic enzymes also restrict the utilization of glucose carbon for fatty acid synthesis in ruminant tissue.

Fatty acid synthesis in the cytosol requires the generation of reducing equivalents in the form of nicotinamide adenine dinucleotide phosphate (NADPH), which in non-ruminants is derived from the oxidation of glucose by the pentose phosphate pathway, and by the coupled operation of NAD-malate dehydrogenase and NADP-malate dehydrogenase. In ruminant tissues the latter source of reducing equivalents is largely absent, and there is evidence that the oxidation of acetate via the isocitrate dehydrogenase pathway, together with pentose cycle activity, provides the required NADPH for fatty acid synthesis (see Annison 1984).

Lactate and pyruvate both contribute to fatty acid synthesis in ruminant adipose tissue (see Annison 1984). Propionate and methylmalonate are utilized only slightly for fatty acid synthesis, except in sheep and goats fed diets that result in a greatly increased proportion of propionate in ruminal VFA, when the proportion of odd-numbered and branched-chain fatty acids in depots may rise to 10-20% (Garton 1977).

The desaturation of long-chain saturated fatty acids to Δ9, 10, cis-monounsaturated derivatives occurs in ovine and bovine adipose tissue (Vernon 1981). There is evidence that fatty acids synthesized de novo are more readily desaturated than exogenous fatty acids (Wahle 1974). Fatty acid chain elongation activity, readily demonstrated in vitro by the formation of labelled stearic and oleic acids from [1-^{14}C] acetate,, accounts for the high proportions of these C-18 fatty acids in adipose tissue. In a recent study St. John et al. (1991) showed that the activities of the desaturase and chain elongation systems in the adipose tissue of Angus and Braford heifers were identical.

Contribution of exogenous fattv acids to adipose tissue

Lipids account for a small, but not negligible proportion of ruminant diets. Green herbage contains 5-10% lipid (dry matter basis) mainly as mono- and digalacto-1, 2-diacylglycerols, whereas in cereals most of the lipid is in the form of triacylglycerols. Both classes of lipid contain high proportions of C-18 unsaturated fatty acids.

In the rumen, complex lipids are rapidly hydrolysed by bacterial lipases, and free unsaturated fatty acids undergo extensive biohydrogenation, with the production of trans-fatty acids, and a wide range of geometrical and positional isomers. About 90% of linoleic and linolenic acids are hydrogenated to stearic acid (Bickerstaffe et al. 1972).

The free fatty acids produced by hydrolysis and biohydrogenation pass out of the rumen adsorbed on to particulate matter. There is virtually no absorption of long-chain fatty acids from the rumen, omasum and abomasum, and free acids, together with some structural lipid of microbial origin enter the small intestine. The processes of lipid digestion and absorption from the small intestine are essentially similar to those of non-ruminants, except that the amphiphile in micellar fat is lysophosphatidyl choline and not monoacylglyceride (see Garton 1977). There is no selectivity either in the absorption of cis- and trans-isomers from the small intestine or of their incorporation into triacylglycerides in intestinal epithelium. There is some desaturation of stearic acid, with the formation of cis-A9 oleic acid, in intestinal cells during uptake (Bickerstaffe et al. 1972).

It is generally recommended that the amount of fat in ruminant diets should not exceed 6-7%. Higher levels may reduce cellulose digestibility, change acetate:propionate ratios and depress methane formation (see Scott and Ashes 1993). Most of the published studies on the effects of high levels of dietary fat on rumen metabolism, however, record only the levels of fat added to a basal diet, which itself may contain 2-3% fat (see Palmquist 1984). Measurement of the fat content of forage based diets is difficult, since special extraction procedures are required to account for the more polar glycolipids and phospholipids components. In effect, there are few reliable data on the total fat content of diets reported to have adverse effects on rumen function. Furthermore, the influence of type and composition of fat has rarely been systematically studied. These considerations imply that there is considerable uncertainty concerning constraints to the use of added fat in ruminant diets.

Absorbed fatty acids, after incorporation into triacylglycerols, enter the circulation in the form of lipoproteins via lymph. Their transfer into adipose tissue occurs after complete hydrolysis by lipoprotein lipase (Vernon 1981).

Regulation of fat accretion

Adipose tissue accretion occurs when lipogenesis exceeds lipolysis. The balance between these processes is subject to hormonal control, which in turn is influenced by overall energy status, as outlined earlier. The key hormone in the short-term regulation of adipose tissue metabolism is insulin, which stimulates fatty acid synthesis and glucose utilization in adipose tissue (Vernon 1981), and inhibits lipolysis (Vernon 1992). Long-term regulation of the partitioning of nutrients, and carcass composition, termed homeorhesis (Bauman and Currie 1980) is dominated by growth hormone, which influences adipose tissue metabolism by depressing lipogenesis and stimulating lipolysis- This implies that rates of lipogenesis and lipolysis in adipose tissue are controlled primarily by the relative activities of insulin and growth hormone. This is best illustrated by the changes in adipose tissue metabolism in ruminants in late pregnancy, and during lactation (Bauman 1984).

The regulation of lipolysis involves acute and chronic responses (see Vernon 1992). The lipase involved in the hydrolysis of triacylglycerols is influenced by a variety of hormones, which include adrenaline, glucagon, ACTH and noradrenaline, each of which may trigger a cascade of signals which ultimately stimulate lipolysis (Vernon 1992). Insulin may also modulate lipolysis acutely or when acting alone or with thyroid hormones, glucocorticoids, sex steroids and growth hormones can exert chronic modulatory effects (Vernon 1992).

Modification of the fatty acid composition of adipose tissue by feeding fats protected from ruminal attack

The extensive biohydrogenation of dietary fats in the rumen prevents significant modification of the degree of unsaturation of adipose tissue fatty acids, or milk fat, unless the fat is protected from ruminal attack (Scott and Ashes 1993). The first process to protect fat, developed by Scott et al. (1970), involved emulsification of the fat with sodium caseinate and treatment of the spray-dried powdered product with formaldehyde. Fats treated in this way escaped attack in the rumen, but were digested and absorbed normally from the small intestine. This process overcomes the usual constraints on the use of fats in ruminant diets as discussed earlier (p. 54).

Protected vegetable oils, rich in polyunsaturated fatty acids, were used successfully to increase the unsaturated fatty acid content of both adipose tissue and milk fat. The linoleic acid content of bovine subcutaneous adipose tissue, for example, increased from about 2% to 25% when protected safflower oil was fed for 8 weeks (Scott and Cook 1975).

A new process has been developed whereby whole oilseeds, after dehulling, are treated in slurry form with formaldehyde (Scott and Ashes 1993). Both the protein and lipid components of the oilseed are protected, and products based on canola oil seed and sunflower meal both improved feed conversion (feed/gain ratio) by about 10%, and significantly improved dressing percentage when fed as supplements to feedlot cattle (Scott and Ashes 1993). The effects on the fatty acid composition of subcutaneous adipose tissue were less marked than when protected oils were fed, but the significantly increased levels of linoleic acid may influence favourably the organoleptic properties of meat. As stressed by the authors, the use of protected oil seeds makes it possible to manipulate protein:energy ratios in feedlot rations for optimum feed conversion efficiency.

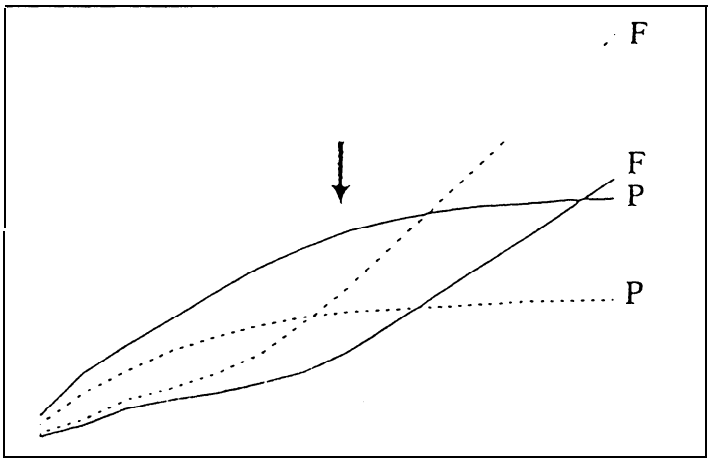
MANIPULATION OF BODY COMPOSITION TO MINIMIZE FAT DEPOSITION

Feeding and selection practices

Feed restriction, whether deliberate or dictated by circumstances, produces lean animals, but the longer time required to reach acceptable market weight makes this practice costly in intensive systems. In the extensive operations that characterise much of beef production in Australia, the limited availability of good quality feed, particularly in the dry season, lengthens the time required to reach market weight. Presumably the lower costs of production of these lean animals, however, is to some extent offset by the variable quality of the product.

The most successful strategy to date for reducing the fat content of meat carcasses has been the breeding and selection of large, late-maturing animals (see Bergen and Merkel 1991). This system of animal production takes advantage of the patterns of protein and fat deposition during growth and development (Fig. 2). Weight gain after protein accretion reaches a plateau is largely due to fat deposition (Bergen 1974). In early-

maturing animals maximum protein accretion and onset of fattening occurs at lower body weights than in late-maturing, larger animals (Fig. 2). Late-maturing animals are slaughtered at a relatively earlier physiological age, before the phase of accelerated fat deposition.



Body Weight

Figure 2. Patterns of fat (F) and protein (P) accretion during the growth and development of early-maturing (----) and late-maturing (____) animals. At slaughter weight (↓), late-maturing animals contain less fat than early-maturing animals. Adapted from Bergen and Merkel (1991).

Use of exogenous agents

Hormones and growth promotants Intact (non-castrated) males exhibit greater weight gain and protein accretion, and deposit less fat than either castrated or female animals (Buttery and Dawson 1990). Treatment with androgenic or oestrogenic steroids, which promote protein deposition, is widely practiced in Australia and overseas. The mechanism of action of these anabolic steroids is not fully known, but androgens appear to act by binding directly to specific muscle receptors to boost protein synthesis and deposition. Oestrogens increase the secretion of both growth hormone and insulin, and there is some evidence of direct effects at the level of the muscle cell (see McDowell and Annison 1990).

Both classes of anabolic steroids affect carcass composition by partitioning nutrients away from fat deposition in favour of muscle production. In recent years about 45% of eligible cattle in Australia have been treated either with anabolic steroids or growth promotants with equivalent activities (Ralph 1989). Treatment is largely based on ear implants which minimize levels of hormone or drug residues at slaughter.

The natural hormones, testosterone, progesterone and oestradiol are the active ingredients, often in combination, of a range of commercial products, which are used as growth promotants for steers and heifers (Ralph 1989). Two other synthetic products, which appear to be more effective than natural hormones in some circumstances, are

zeranol and trenbolone acetate (TBA). The latter drug, although banned in EEC countries,, has been cleared for use in Australia, and is currently marketed, combined with 14% oestradiol, under the trade name Revalor (Hoechst Pty. Ltd.). Intensive studies have shown that TBA acts mainly by influencing protein turnover. Although both protein breakdown and synthesis are reduced, the effects on protein breakdown are greater, and net protein deposition is increased (see Buttery and Dawson 1990). This effect of TBA on protein turnover has been shown to reduce nitrogen losses to an extent that may be significant when nitrogen intakes are low (see Ralph 1989). Another useful response to TBA is the 10% reduction in metabolic rate, which is significant when dietary energy intakes are low, as occurs in a drought-affected grazing environment.

A matter of crucial importance in the use of growth promotants is plane of nutrition Nutrient supply must be adequate in terms of essential amino acids, energy and micronutrients to allow the tissue to express it capacity for increased growth.

Growth hormone Advances in fermentation technology, coupled with those in recombinant DNA procedures, have made it possible to produce large quantities of bovine growth hormone, now termed somatotropin. The stimulus for somatotropin production was largely the substantial increases in milk yield achieved by somatotropin administration (McDowell and Annison 1991), but growth hormone has also been shown to increase N retention, lean tissue growth and efficiency of feed conversion in cattle and pigs (Buttery and Dawson 1990). As with the growth promotants discussed above, these effects in themselves assist in the production of leaner carcasses, since animals will reach slaughter weight at a younger age when fat deposition is less, as discussed earlier.

Administration of growth hormone, however, has direct effects on fat deposition in cattle and pigs (Table 1). This reduction in carcass fat is mediated through increased lipolysis and depressed lipogenesis (see Bergen and Merkel 1991).

Proposed mechanisms to account for the effects of growth hormone on lipolysis and lipogenesis are independent of IGF-1, the peptide believed to be involved in the effects of growth hormone on protein metabolism. Growth hormone functions as a counter-regulatory hormone to oppose the actions of insulin on carbohydrate and lipid metabolism (Davidson 1989). The effects on adipose tissue include decreases in the activities of the major lipogenic enzymes, reduced glucose uptake and oxidation, decreased insulin sensitivity and stimulation of lipolysis (see Bergen and Merkel 1991).

The effects of growth hormone on fat deposition depend on the stage of growth of animals. In young growing animals, growth hormone acts mainly to depress fat synthesis, whereas in slower growing, older animals, lipolysis of stored fat may be more important (Bergen and Merkel 1991).

Energy balance also influences responses to growth hormone. When animals are in positive energy balance, growth hormone administration reduces lipogenesis, but effects on lipolysis are small. In contrast, in animals in negative energy balance, when lipogenesis is inevitably low, growth hormone increases rates of adipose tissue mobilisation (see Etherton and Louveau 1992).

In spite of proven efficacy and absence of adverse effects in treated livestock, or any evidence of potential harm to consumers, regulatory authorities in U.S.A., Europe and Australia have continued to defer the granting of licences for the use of growth hormone in livestock production.

The availability of slow-release preparations of growth hormone (Eppard et al. 1991) has eliminated the need for the daily injection of livestock, but the low potency of growth hormone has prompted the search for more active products (Ballard et al. 1993). An alternative approach to the direct administration of growth hormone is to seek to enhance

the effects of endogenous hormone by immunological means (Aston et al. 1991). These include the induction of neutralizing anti-somatostatin antibodies to increase circulating growth hormone levels, and enhancement of the activity of growth hormone releasing factor by site-directed antisera to the peptide. Two other approaches are based on either immunologically enhancing hormonal activity, or mimicking this effect with anti-idiotypic vaccines (Aston et al. 1991).

Adrenergic agonists A number of synthetic β-adrenergic agonists, given orally, increase protein accretion and reduce fat deposition (see McDowell and Annison 1991). The mechanism by which β agonists increase muscle protein accretion is unclear (see Bergen and Merkel 1991), but part of the reduction in the carcass fat of treated animals stems from the diversion of energy to support both increased protein synthesis and a higher basal metabolic rate. The major effects of β agonists on adipose tissue production, however, are accounted for by increased lipolysis, reduced lipogenesis and reduced triacylglyceride synthesis (Yang and McElligott 1989).

Increased lipolysis in adipocytes was shown by Yang and McElligott (1989) to be directly related to increased triacylglycerol lipase activity. In the same studies, decreased lipogenesis was shown to be due to lowered activity of lipogenic enzymes. There is evidence that the effects of β agonists on fatty acid synthesis and triglyceride synthesis, and on lipolysis, are all mediated by regulation of cAMP-dependent protein kinases, and that reduced lipogenesis is accounted for by depressed expression of genes for lipogenic enzymes (see Bergen and Merkel 1991).

The effectiveness of β agonists in reducing carcass fat deposition is shown by the data of Bergen and Merkel (1991) in Table 1. As with growth hormone administration, β agonists reduce fat deposition by about 20% and increase feed conversion efficiencies.

At this time synthetic β agonists, which include the compounds clenbuterol (Boehringer), cimaterol (Cyanamid) and ractopamine (Eli Lilly) have not been approved for use in livestock production by regulatory authorities in U.S.A., Europe or Australia. Drawbacks to their use are that, unlike growth hormone, they are orally active and have been shown to stimulate cardiac function. The rapid clearance of these agents, however,, suggests that short withdrawal periods before slaughter would ensure consumer safety (Buttery and Dawson 1988).

Table 1 Production responses (% of control) of cattle and pigs to dietary β-adrenergic agonists and growth hormone injections expressed as average daily gain (ADG), feed conversion efficiency (FCE), lean gain (LG) and fat gain (FG). From Bergen and Merkel (1991)

Growth promotant	Species	Production Response			
		ADG	FCE	LG	FG
Beta agonists	Cattle	116	118	120	80
	Pigs	117	117	120	82
Growth hormone	Cattle	109	110	110	80
	Pigs	112	112	112	76

Immunological control of fat deposition

The enhancement of growth hormone activity by immunological procedures was discussed earlier (p. 57). Attempts to increase growth hormone release by blocking the activity of somatostatin by immunization were successful in a non-improved breed of sheep, but subsequent efforts in commercial breeds of ruminants have been less successful (see Futter and Flint 1990).

Direct efforts to decrease fat deposition by producing antibodies capable of destroying adipocytes have been successful in rats (Flint 1990). Antibodies against rat adipocytes were produced in sheep, and shown to be capable of lysing rat adipocytes in vitro. When injected into rats, the antibodies reduced body fat, with long lasting effects Body fat was reduced by up to 50% for six months in rats given antibodies for several days. The treated rats showed increased appetite accompanied by higher growth rates and improvements in food conversion efficiency of about 15%. These increases in protein accretion at the expense of fat deposition suggest that this immunological approach has great promise for the meat producing industries.

The significance of immunological procedures to improve the quality of meat, or to increase overall profitability of production goes beyond technological innovation. Public disquiet at the use of growth promotants may lead to the indefinite deferment of clearance by statutory authorities of growth promotants of proven efficacy and safety, such as growth hormone. In contrast, it is likely that the public would view immunological procedures as akin to immunization against disease. If this proves to be so, clearance of immunological procedures for general use in livestock may follow quickly from proof of efficacy.

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