Growth promotion in farm animals

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The use of anabolic steroids has indicated clearly that it is possible to enhance the growth of farm animals, especially ruminants. Enhancement of growth rate normally reduces the cost of producing meat. In recent years the importance of reducing the fat intake of the human population has been fully recognized and methods have been developed to enhance the deposition of lean at the expense of fat, usually by alteration of the animal's endocrine status, for example by the application of beta-agonists. Advances in biotechnology, especially during the last decade, have enabled the scientific community to develop methods to enhance the performance of animals without direct application of hormones or their analogues. However, the application of these scientific achievements is attracting more and more attention from a vocal segment of the public who oppose the use of what they consider to be unnatural methods of food production, and from certain politicians, especially in Europe, who wish to control the production of food so as to reduce surpluses which occur in some of the developed countries of the world. In addition, both the relative abundance of food supplies, at least for the developed world, together with advances in scientific knowledge have increased the rigour with which the safety of food products is judged. The previously described statements combine to make the quest to produce acceptable methods of safely and humanely enhancing the efficiency of meat production from animals a most interesting challenge.

ANABOLIC STEROIDS

Many studies have reported the efficacy of anabolic agents since Dinusson *et al.* (1950) first demonstrated that the oestrogenic substance diethyl stilbesterol when implanted into heifers increased growth rate. Since that time a series of implants based on the natural sex steroids has been developed to enhance the growth of animals, especially ruminants. Oestradiol, oestradiol benzoate plus progesterone, and oestradiol benzoate plus testosterone are among the most commonly used preparations of the natural sex steroids used in many parts of the world and have been shown to increase growth rate by 8–15% and feed conversion efficiency by 5–10%. In addition to these endogenous hormonal implants, several analogues have been developed. The two which received licences in various countries are the testosterone analogue, trenbolone acetate, and zeranol, an oestrogenic substance derived from the fungal metabolite zeralanone. Numerous studies were conducted on the safety of these materials both to the consumer of the meat and to the treated animal. In the European Economic Community (EEC) an expert committee concluded that at least for the natural hormones and compounds which are rapidly metabolized to yield natural hormones, provided they were used according to the officially approved manufacturers' instructions, they presented no hazard especially to the eventual consumer of the meat (Lamming *et al.* 1987). Among the pieces of evidence available is the comparison between the quantity of an implanted hormone,

e.g. oestradiol in 250 g meat and the endogenous daily hormone production in a prepubertal child. A 250 g steak from a steer treated with oestradiol contains about 0.005 μg oestradiol compared with a child's daily endogenous oestradiol production of 42 μg. Similar convincing findings are available for testosterone and for progesterone (Heitzman *et al.* 1984). Despite the scientific evidence the use of all hormone implants to promote growth of animals within the EEC is now banned (directive 85/649 EEC). However, use of anabolic implants remains a permitted practice in animal production in many other countries of the world, e.g. the USA.

Many studies on the mode of action of anabolic steroids have been conducted and some have yielded unexpected observations. For example, treatment of ruminants and rats with the androgenic substance trenbolone acetate enhances the rate of muscle accretion but this is achieved by a small reduction in the rate of muscle protein synthesis and a much larger decrease in the rate of muscle protein catabolism (Vernon & Buttery, 1978; Sinnett-Smith *et al.* 1983). Testosterone works by enhancing the rate of both reactions (Martinez *et al.* 1984). Oestradiol implants are particularly effective in cattle and its mode of action has been suggested to involve the enhancement of endogenous growth hormone secretion. The precise mode of action of trenbolone acetate has yet to be elucidated but, as with oestradiol, part of its effects is likely to be associated with changes in the circulating concentrations of several of the endogenous hormones (Buttery & Sinnett-Smith, 1982). Hunter & Vercoe (1987) have recently described an alternative application of trenbolone acetate which exploits its mechanism of action. As this compound reduces the rate of muscle protein turnover and consequently reduces basal energy expenditure, this group of Australian workers were able to reduce the weight loss of cattle fed on very-poor diets. They suggest that trenbolone acetate may enable cattle to withstand better the marked fluctuations in nutrient availability often seen in the tropics and sub-tropics.

It is unlikely that the full extent of the growth-promoting properties of anabolic steroids will be seen unless the animal is adequately fed. For example, studies with silage-fed steers treated with oestradiol demonstrate that a significant growth response is only seen when animals are given supplementary protein (Gill *et al.* 1987; Newbold *et al.* 1988). A possible explanation for this comes from the work of Breier *et al.* (1988*a,b*), see p. 461. A similar interaction with diet, although judged not to be statistically significant, has been reported for the combined implant of trenbolone acetate plus oestradiol (Galbraith *et al.* 1983).

GROWTH HORMONE

The ability to produce bovine growth hormone by recombinant technology has resulted in a detailed investigation of the potential of using exogenously applied growth hormone to enhance growth in farm livestock. Although the response to growth hormone varies, on average an increase in growth rate of 12% is obtained in cattle but some increases up to 25% have been reported. This increased growth is usually associated with a reduction in fat and an increase in the lean content of the carcass (Enright, 1989). This repartitioning effect can mask overall effects on growth rate (Pell, 1989).

Growth hormone treatment causes an increased rate of muscle protein synthesis in the muscle (Pell, 1989). Pell *et al.* (1989) investigated the interaction between diet and response to growth hormone in lambs and were able to show that the reduction in fat

content is related to the extent to which the control animals are depositing fat, the biggest reductions being seen in animals on a high plane of nutrition. Although some increases in muscle mass are seen in animals on a low plane of nutrition (Peters, 1986) maximal effects will only be seen in animals receiving an adequate protein intake (Enright, 1989). The work of Breier *et al.* (1988*a,b*) and of Elsasser *et al.* (1989) gives some insight into the mechanism of the interaction between plane of nutrition and response to growth hormone. These workers showed that plasma insulin-like growth factor (IGF)-1 concentrations increased more in response to growth hormone when animals were on a high plane of nutrition than when poorly fed. A low plane of nutrition results in a reduction in hepatic growth hormone receptors, thus reducing the extent of IGF-1 release from the liver in response to circulating growth hormone. This dietary influence on the hepatic growth hormone receptor could also explain in part the failure of oestradiol to promote growth in poorly-fed animals.

An interesting development in the use of growth hormone to enhance growth has been the discovery that some monoclonal antibodies to a hormone may enhance rather than inhibit its activity (Holder & Aston, 1989). The possibility exists, therefore, that treatment of an animal with an antibody to growth hormone may increase lean deposition without the need to give growth hormone exogenously, i.e. the antibody interacting with endogenously produced growth hormone (Holder & Aston, 1989).

The use of growth hormone to promote lean deposition in ruminants and other farm animals has not been approved to date within the EEC.

GROWTH HORMONE-RELEASING FACTOR (GRF) AND OTHER FACTORS INDUCING GROWTH HORMONE RELEASE

Treatment with GRF has similar effects to growth hormone treatment so far as can be judged from the experiments that have been reported in the literature (Enright, 1989). GRF is a smaller peptide than growth hormone and this, coupled with its greater potency, should in theory make it more attractive as a growth-promoting agent than growth hormone itself. However, the molecule is highly conserved between species especially in residues 1–27, the active portion of the molecule (Baile & Buonomo, 1987); thus, it is most likely that any preparation used to enhance animal growth would also be active in humans. One point often made in support of bovine growth hormone as an enhancer of animal growth is its inactivity in humans.

A number of other biologically active peptides have been shown to induce growth hormone release including the opioid peptides (beta-endorphin and the enkephalins) and bombesin (a peptide isolated from the skin of the frogs *Bombina bombina* and *Bombina variegata variegata*; Millard, 1989). Thus, the possibility exists that these and related materials may potentially have uses as repartitioning and growth-promoting agents.

SOMATOSTATIN

Somatostatin is a peptide of fourteen amino acids and its structure is conserved across species. It is synthesized as a ninety-two amino acid prosomatostatin molecule from which six peptides including somatostatin itself and a twenty-eight amino acid form of somatostatin are known to be derived (Patel, 1987). Somatostatin inhibits growth hormone secretion, but in addition it also inhibits the secretion of thyrotropin from the

pituitary and glucagon and insulin release from the pancreas (Patel, 1987). The concept of autoimmunizing animals against somatostatin to enhance growth hormone release was initially explored in sheep by Spencer *et al.* (1983). These initial studies reported enhanced growth in autoimmunized animals, but subsequent studies by these and other workers have often yielded equivocal results. Bass *et al.* (1987) more recently obtained findings which again highlighted the importance of diet in the response to a growth-promoting regimen. Lambs were fed on either a good-quality pellet diet or cut pasture and some animals from each group were autoimmunized against somatostatin. Although plasma growth hormone concentrations were higher in both groups of immunized animals, only the animals fed on the pellets showed an increase in weight gain. Presumably the pasture-fed animals which were on a low plane of nutrition had livers which were partially refractory to growth hormone and consequently IGF-1 release (Breier *et al.* 1988*a*). It is interesting to note that the findings of Bass *et al.* (1987) indicate that in animals showing an enhanced growth that there was some evidence, albeit not judged to be significant, for an enhanced deposition of fat; enhanced growth hormone activity would normally be expected to be associated with an increase in lean content (see p. 460). The possibility that the changes in digesta flow seen on immunization against somatostatin also play a major role in the response of animals needs to be considered further (Fadalla *et al.* 1985). Although autoimmunization against somatostatin does not seem to be a viable method of growth promotion, the use of the immune response to promote growth may be more acceptable to the legislation and the general public than direct application of hormones.

BETA-AGONISTS

To date numerous reports have shown that a variety of substances with selective binding to the beta-2-adrenergic receptor when included in the diet of mammals reduce body fat and increase lean. The response in chickens is smaller than that seen in mammals but may also be of commercial significance (Hanrahan, 1987). The mode of action of these materials is not fully understood. The reduction in lipid content of the carcass is probably largely due to an increased rate of lipolysis since routinely an increase in plasma-free fatty acids is observed following treatment (e.g., Mersmann *et al.* 1989). Some in vitro studies have suggested that the main influence of beta-agonist treatment is a reduction in lipogenesis (e.g. Coleman *et al.* 1988). Whether this effect also occurs in vivo is unknown. There is also debate over the influence of beta-agonists on muscle protein metabolism. Some reports have suggested an increase in muscle protein synthesis (Emery *et al.* 1984; Claeys *et al.* 1989). In contrast, Reeds *et al.* (1986) reported only a small transient increase in protein synthesis with rats, but concluded that overall the major effect was a reduction in the rate of muscle protein breakdown. This reduction in muscle protein breakdown has also been observed in ruminants (Borohov *et al.* 1987; Dawson *et al.* 1988). The cause of this reduced protein catabolism can at present only be a matter of conjecture. One possible explanation lies in the changes in the activity of the calcium-activated neutral protease (calpain; *EC* 3.4.22.17) system seen in the muscle of treated sheep (Higgins *et al.* 1988). As illustrated in Table 1 there is a marked increase in the activity of the natural inhibitor of the calpains, calpastatin. However, caution should be exercised in relying too strongly on this explanation since there is evidence that the main role of the calpain system in the cell is associated with degradation of hormone

Table 1. *Effect of dietary restriction or clenbuterol treatment (2 mg/kg diet) on calpains I and II (EC 3.4.22.17) and calpastatin activity in sheep Longissimus dorsi muscle**

(Mean values with no. of animals in parentheses)

Dietary treatment . . .	Control (6)	Restricted (4)	Clenbuterol (6)	Pooled SED (df 12)
Average daily wt gain (kg)	0·347	0·048	0·360	0·037
Longissimus dorsi wet wt (g)	635	377	788	53
Calpastatin (units/kg muscle)	2740	2340	5440	420
Calpain I (units/kg muscle)	570	430	490	60
Calpain II (units/kg muscle)	1250	1500	2880	250

SED, standard error of difference.
* For details, see Higgins *et al.* (1988).

receptors rather than structural muscle proteins (Higgins *et al.* 1988). It would be most interesting to see if the calpain system were modified following trenbolone acetate treatment which also reduces muscle protein breakdown (see p. 460).

The repartitioning effects of beta-agonists are reported to occur with both adequate- and restricted-feeding conditions (Kim *et al.* 1987). Their effects in enhancing growth rate, however, appear to be variable (Williams, 1987), but findings obtained from this laboratory have indicated that in young steers fed on a high-quality diet (dried grass–barley (0·7:0·3, w/w)), inclusion of cimaterol (1·5 mg/kg) can increase growth rate by >30% (from 0·86 kg/d to 1·13 kg/d; J. M. Dawson, unpublished results).

At the time of writing, beta-agonists have not been approved for use as animal feed additives within the EEC.

OTHER METHODS OF REDUCING THE FAT CONTENT OF THE CARCASS

As implied previously, the use of the immune response may turn out to be an acceptable method of enhancing the lean deposition and growth rate of animals. Flint *et al.* (1986) demonstrated that antibodies raised to adipocyte plasma membranes reduce the body fat. Passive immunization against cholecystokinin-8 (CCK-8) increased the feed intake of lean Zucker rats which resulted in an increased growth rate (McLaughlin *et al.* 1985). Recently active immunization against CCK-8 has been reported to reduce the fat content of sheep carcasses with no significant effect on total body weight gain (Trout *et al.* 1989).

The use of enhanced concentrations of some natural metabolites in the diet may not be viewed by the public with as much suspicion as the use of hormones or the injections needed for most immunization procedures. One such treatment is the inclusion of dihydroxyacetone and pyruvic acid in the diet which has been reported to reduce the fat content of rats (Stanko *et al.* 1983) and pigs (Newman *et al.* 1987).

TRANSGENIC ANIMALS

Several reports have appeared describing the effects of increasing the copy number of the growth hormone gene in farm livestock. Unfortunately many of the animals have not

been suitable, or produced in sufficient numbers, for meaningful nutritional studies let alone available for carcass dissection. Evidence available does, however, indicate that many of the responses seen on incorporating extra copies of growth hormone genes into the genome are similar to those observed with exogenously applied growth hormone, including the failure to see an enhanced growth rate unless the animal is adequately fed (Campbell, 1988). Now that transgenesis of farm livestock is a reality, further work is required not only to perfect the control of the expression of the inserted gene(s), but also into the control of the growth process itself. Without a detailed knowledge of these processes it will be very difficult to select which genes are suitable candidates for manipulation.

CONCLUSIONS

A wide range of techniques are now known to be effective in enhancing the growth rate and extent of lean deposition in animals, but in most cases increases in weight gain are only seen in well-fed animals. Repartitioning of fat and lean, however, can be observed in animals on a lower plane of nutrition. The application of most if not all the techniques discussed previously, at least to the European livestock industry, is currently being constrained both by legislation and by a vocal sector of public opinion. This antagonism is often overriding scientifically sound evidence on the safety of the techniques to the animal, to the consumer of the meat, to those producing the materials and to those involved in the husbandry of the treated animals.

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