Emerging Metabolic Modifiers

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Introduction

The major challenge facing livestock industries today is the production of leaner, healthier meat products. Additionally, meat and dairy producers are expected to meet the demand for higher productivity on a decreasing land mass in an era when environmental concerns, such as ground water contamination and solid waste accumulation, must be addressed. Because current recommendations of the biomedical community have underscored the need to provide leaner meat products, the use of nutrient partitioning agents such as somatotropin and β-agonists to reduce fat and enhance protein deposition have received considerable attention during the past two decades. Moreover, the economic integrity of these industries hinges on an integration of programs and technologies that promote more efficient animal production. Therefore, strategies that influence both the environmental, and biological aspects of animal production must be considered and utilized. In addition, dietary inputs and control of the metabolic processes that regulate feed intake, production efficiency and rate of growth, must be better understood, so that we can optimize and manipulate growth of all livestock species.

Any dietary supplement or hormone that impacts upon nutrient intake and/or partitioning may be broadly considered as a metabolic modifier. In addition to fine-tuning specific nutrient requirements for optimal growth, animal scientists have been actively attempting to alter meat quality and production efficiency through the use of genetic manipulation, hormone intervention and feeding orally active nutrient partitioning agents. The aim of this review is to examine and update some of the hormone strategies that have been used to alter nutrient partitioning, as well as, discuss three classes of dietary nutrients (additives) which have recently shown promise in growth manipulation.

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Somatotropin

One of the most thoroughly studied hormones involved in the regulation of growth and metabolism is pituitary growth hormone or somatotropin. The control of metabolism and the impact of administering supraphysiological levels of somatotropin to livestock have been thoroughly reviewed (Steele and Evock-Clover, 1993; NRC, 1994; Etherton and Bauman, 1998; Buyse and Decuypere, 1999; Bauman, 1999). Since the early pioneering studies with somatotropin in pigs (Machlin, 1972) and cows (Asimov and Krouze, 1937) significant contributions to the understanding of the function of the somatotrophic axis and its manipulation in livestock have led to a marked impact on dairy and meat industries. The biology and economic impact of the use of bovine somatotropin which was commercially introduced in 1994 were recently reviewed (Etherton and Bauman, 1998; Bauman, 1999), and they have clearly demonstrated the challenges and rigor involved in bringing a growth enhancing substance from the laboratory to commercial production environment.

Likewise, the recent commercial introduction of Reporcin (recombinant porcine somatotropin or pST) into the swine industry of Australia, New Zealand and Malaysia has made a significant impact on the sale of pork, based on carcass merit. The recent purchase of distribution and production rights of Reporcin by an animal health firm that has a significant presence in the United States may bring this regulatory hormone into the forefront of the American pork industry. Because of the impact on growth and the potential to reduce feed intake and biological waste, it is not unreasonable to assume that pST will likely be considered for FDA approval in the United States. At present, the delivery system for pST requires daily injection of each animal. A clear challenge facing the research community exists to optimize injection devices, develop effective sustained release technology, or pursue potential genetic or immunological approaches to treatment. Alternative strategies to enhance production of somatotropin by introduction of DNA to specific tissues have been recently proposed and reviewed (Wray-Cahen et al., 1998; Etherton, 1999); these include direct injection or gene gun technology, infection with reconstructed viral vectors and, intravenous introduction of encapsulated DNA for targeting specific cells. While

these strategies have been tried in experimental animals their potential use in commercial animal production is unknown. The advantage to these approaches is that hyper-expression of a gene of interest may be attained in somatic cells during a specific period of growth without permanently altering germ line cells or interfering with growth in utero.

Ractopamine

The FDA has recently approved the use of ractopamine for swine production in the United States (Muirhead, 2000). Ractopamine is one member of a class of compounds known as β-receptor agonists (BA) that have been shown to enhance lean meat production in many animal species including pigs, chickens and ruminants. The mechanism of action of BA and their efficacy have been recently reviewed (NRC, 1994; Bell et al., 1998; Mersmann, 1998). Briefly, BA are analogs of catecholamines that can bind to appropriate adrenergic receptors in both muscle and adipose tissue. In general, BA act to enhance lipolysis and decrease lipid synthesis in fat; although differences in potency and activity have been noted for individual BA (NRC, 1994). Indeed, many studies in livestock species have clearly shown a marked decrease in carcass fat following the feeding of BA (Mersmann, 1998); while in pigs, ractopamine apparently has little effect on fat deposition (Dunshea et al., 1993). In contrast, protein deposition was markedly elevated by ractopamine, which was only evident at higher levels of protein intake (Dunshea et al., 1993). It has been suggested that ractopamine-treatment may alter hormone sensitivity or act to desensitize adipose β-receptors (Dunshea and King, 1995). Ractopamine also induces muscle hypertrophy (Mersmann, 1998) without altering efficiency of dietary amino acid utilization (Dunshea et al., 1993), in contrast to what has been demonstrated for pST-treated pigs (Caperna et al., 1995). Thus, growth enhancement is related to the adequacy of the nutrient environment. As the first meat leanness enhancer approved in the United States, the impact of this metabolic modifier will be closely monitored to determine the acceptability of such technology to American consumers and pork producers.

Conjugated Linoleic Acid (CLA)

Conjugated linoleic acids are naturally occurring linoleic acid derivatives which have recently been shown to have a wide array of functions in vivo. Ruminant meat and dairy products are especially rich sources of CLA isomers (Chin et al., 1992). Evidence gathered over the last decade has suggested that feeding CLA has significant health benefits in experimental animals, including reduced tumorigenesis, increased oxidative protection and enhanced immune functions (Belury, 1995; Banni et al., 1998; Hayek et al., 1999; Pariza, 2000). Recent studies have further defined the roles of CLA in lipid metabolism and suggest that CLA are associated with reduced lipid synthesis and enhanced ketogenesis, and may also play a role in insulin responsiveness and glucose homeostasis (Loor and Herbein, 1998; Sakono et al., 1999; Houseknecht et al., 1998a). Of particular interest to animal production are studies which have demonstrated improved growth performance and reduced body fat in rodents fed CLA (Chin et al., 1994; Park et al., 1997 and 1999; Delany et al., 1999). Based on these reports, several experiments have been conducted in swine and dairy animals. Dugan et al. (1997) reported that feeding finisher pigs 2% dietary CLA was associated with a small reduction in feed intake and improved feed conversion ratio, however, the primary effects observed were alterations in body composition such that dissectable subcutaneous fat was reduced in all cuts while lean tissue was increased in the loin and total carcass. In a more comprehensive study with finishing pigs, Ostrowska et al. (1999) reported a significant decrease in fat deposition rate which was linearly related to the CLA concentration in the diet; lean gain was concomitantly increased as the level of CLA was increased. However, in this study feed intake was not influenced by CLA. In dairy cows, CLA were shown to inhibit lipid synthesis while enhancing the overall levels of CLA in milk (Loor and Herbein, 1998). Taken together, it is clear that CLA are attractive metabolic modifiers that can be used to both manipulate growth in a positive way and produce meat products that contain higher levels of CLA which are believed to have positive health effects for consumers. There are many mechanistic questions which still need to be answered with regard to dietary CLA and perhaps more importantly, an assessment of the function of each of the major isoforms which appear in meat of treated animals and in commercial preparations of CLA.

Betaine

Betaine is an amino acid (trimethyl-glycine) formed by oxidation of choline (Kidd et al., 1997). It is present in most living organisms and is concentrated in high levels in the sugar beet, from which it can be commercially extracted (Virtanen, 1995). The use of betaine as an ingredient in livestock diets is not a new concept; it was initially introduced to the feed industry as a replacement for methionine and choline in poultry diets where it was presumed to act as a methyl donor and as an osmoprotectant.

Compounds known as lipotropes, including methionine, choline, and betaine contribute significantly to transmethylation reactions. Methyl donor reactions are vital to biological processes and are involved in the synthesis of many metabolites including phosphatidylcholine, creatine and carnitine, as well as, regulation of DNA activity. Initial studies suggested that dietary betaine might also reduce carcass fat and alter distribution of carcass fat in broilers (Saunderson and MacKinley, 1990), fish (Virtanen et al., 1989), and pigs (Cadogan et al., 1993; Virtanen and Campbell, 1994). Betaine also improved average daily gain in finishing pigs (Webel, 1994; Haydon et al., 1995; Lawrence et al., 1995; Smith et al., 1995) and was associated with decreased feed intake and improved feed:gain ratio in gilts, but not in barrows (Cera and Schinckel, 1995; Haydon et al., 1995). Haydon et al. (1995) also reported that betaine decreased daily gain and feed intake when fed in low-energy diets, but found the opposite effect with high-energy diets. There are however, mixed reports on effects of betaine on loin eye area and backfat thickness (Lawrence et al., 1995; Smith et al., 1995; Haydon et al.,

1995). Matthews et al. (1998) also reported mixed results of growth criteria, but have observed reduced levels of plasma and urine urea nitrogen in betaine-fed pigs, which suggests that protein status may be affected; these effects are dependent on the crude protein and energy content of the diet. It is not clear how betaine could promote these responses. The effect of fat accretion may be mediated via allocation of amino acids between lean growth, visceral growth and metabolic breakdown rather than by lipid metabolism per se (Virtanen and Campbell, 1994). In support of this hypothesis, we have found very little evidence to suggest that betaine influences lipid oxidation or metabolism by direct analysis either in vivo or in vitro (Caperna et al., unpublished). Clearly, more definitive work is required to fully understand under what conditions betaine will have consistent effects on nutrient partitioning.

In contrast to studies with swine, the influence of betaine in poultry appears to be more consistent. Betaine as a single feed supplement significantly improved chicken live weight, and tended to improve feed conversion ratio (Waldenstedt et al., 1999). Coccidiosis challenge has been used as a model of gastrointestinal osmotically stressed gut and initial studies showed that in combination with the ionophore coccidiostat salinomycin, betaine had a positive effect on bird performance during coccidial challenge (Virtanen et al., 1993; Augustine and McNaughton, 1996; Augustine et al., 1997). The mode of action is not completely understood, but Augustine et al. (1997) have suggested that betaine may contribute to the improved performance of coccidia-infected chickens directly, by partial inhibition of coccidial invasion and development, and indirectly, by support of intestinal structure and function in the presence of coccidial infection.

Leptin: Its Role in Regulation of Feed Intake and Energetics

The recent discoveries of genetic defects that are responsible for obesity in lines of inbred mice (Zhang et al., 1994) have led to a vast body of new biomedical research on the regulation of feed intake and obesity in humans and rodents. These studies have identified leptin, a 16 kD protein as the gene product involved in several aspects of energy metabolism. Leptin is produced primarily in fat cells and has direct influence on hypothalamic control of feed intake and thermoregulation (Hamann and Matthaei, 1996). It is also believed that leptin is a critical link in the communication between fat cells and the central nervous system . The pleiotypic nature of leptin function has been further documented in studies that have focused on the role of leptin in the regulation of cellular differentiation and reproduction (Cioffi et al., 1996; Gainsford et al., 1996). In addition, leptin also appears to influence the action of insulin in peripheral tissues (Cohen et al., 1996), and may enhance synthesis of the mitochondrial uncoupling proteins which also regulate cellular energetics (Scarpace et al., 1998).

Energy balance regulation is a complex process involving several controlling systems, and not surprisingly, the control mechanisms are complex and involve different biochemical pathways (Spiegelman and Flier, 1996; Schwartz and Seeley, 1997; Flier and Maratos-Flier, 1998). Tartaglia and coworkers (Tartaglia et al., 1995) reported the cloning of the leptin receptor from the mouse choroid plexus which bears homology to the signaling subunits of the cytokine receptor family members. Several splice variants of the leptin receptor (Ob-Ra through Ob-Re) with different tissue distributions have been subsequently described (Chen et al., 1996a; Lee et al., 1996), but their function is still unknown. The extensive distribution of leptin receptors is consistent with the growing evidence of a multiplicity of functions. The Ob-Ra variant is thought to be a leptin transporter (Mercer et al., 1996a) involved in the transport of leptin through the blood-brain barrier, while the Ob-Re form appears to be a soluble binding protein. The long form of the receptor (Ob-Rb) is expressed in various regions of the brain and is thought to be responsible for the central actions of leptin (Tartaglia et al., 1995; Glaum et al., 1996). However, there are reports of the identification of Ob-Rb in an increasing number of tissues, including the kidneys, adrenal medulla, pancreatic β-cells, placenta, ovaries, adipose tissue, lung, liver and hematopoietic cells (Lee et al., 1996; Masuzaki et al., 1997; Emilsson et al., 1997; Hoggard et al., 1997; Karlsson et al., 1997; Sierra-Honigmann et al., 1998). Signal transduction from the Ob-Rb receptors involves cytoplasmic protein kinases known as JAKs and protein transcription factors kinase substrates of the STAT molecule family.

Leptin has been implicated in causing peripheral insulin resistance by attenuating insulin action and perhaps insulin signaling in various insulin-responsive cell types (Cohen et al., 1996), inhibition of insulin secretion from the pancreas (Emilsson et al., 1997), and inhibition of cortisol synthesis in isolated adrenocortical cells (Bornstein et al., 1997). Stimulation of glucose uptake (Kamohara et al., 1997), adipocyte lipolysis (Fruhbeck et al., 1997) and alteration of lipid partitioning (Muoio et al., 1997) in skeletal muscle have also been described. In addition, leptin appears to be a regulatory signal for somatotropin secretion (Carro et al., 1977; Barb et al., 1998).

In the fed, steady-state, leptin expression and secretion reflect body fat mass in rodents and humans (Frederich et al., 1995; Maffei et al., 1995) and are highly correlated with adipocyte size (Houseknecht et al., 1998b). This correlation with fat mass is drastically altered, however, with changes in energy balance. Short term food deprivation results in a rapid decrease in leptin gene expression (Cusin et al., 1995; Trayhurn et al., 1995b), while excessive caloric intake results in upregulation (Considine et al., 1996). Thus, leptin not only signals the status of body energy stores to the brain, but also functions as a sensor of energy balance. Leptin treatment of animals has been shown to cause a dose-dependent decrease in food intake, loss of body weight and loss of fat depots suggesting a specific lipoatrophic activity of leptin (Chen et al., 1996b). Leptin also induces an increase in energy expenditure or maintains energy expenditure after a reduction in feed intake (Stehling et al., 1996). This is in sharp contrast to the reduced metabolic rate associated with feed restriction. In addition leptin is associated with increased behavioral activity and a rise in core temperature when administered to leptin

deficient mice (Pelleymounter et al., 1995). Therefore, leptin not only causes a reduction in feed intake, but also potentiates body weight losses due to an increased metabolic rate.

A number of factors that acutely influence the production of leptin have been identified. Physiological influences include exercise and exposure to cold, each of which results in a decrease in ob gene expression, and a corresponding reduction in circulating leptin levels (Trayhurn et al., 1995a; Hardie et al., 1996; Trayhurn et al., 1998). Adrenergic stimulants (catecholamines) and thiazolidinediones down-regulate leptin expression and secretion by adipocytes (Trayhurn et al., 1995a; De Vos et al., 1996; Trayhurn et al., 1998). This has led to the view that the sympathetic nervous system provides a negative feedback loop to the white adipose tissue while glucocorticoids and insulin stimulate leptin production (De Vos et al., 1995; Saladin et al., 1995; Leroy et al., 1996).

Leptin is a peripheral signal which interacts with a multiplicity of neuroendocrine targets. The central effect of leptin, at least in part, is through the inhibition of neuropeptide Y (NPY). NPY stimulates food intake and decreases energy expenditure (Stephens et al., 1995; Mercer et al., 1996b). Leptin also interacts with glucagon-like peptide-1 (Goldstone et al., 1997), corticotrophin releasing hormone (Uehara et al., 1998) and the pro-opiomelanocortin system (Schwartz et al., 1996). In addition, recent evidence suggests that leptin may also play a role in the regulation of orexin synthesis. Orexins are a family of newly described peptides that stimulate appetite in rats (Sakurai et al., 1998), humans (Sakurai et al., 1999) and pigs (Dyer et al., 1999). Leptin treatment of rats was associated with inhibition of both prepro-orexin and orexin receptor mRNA (Lopez et al., 2000).

Clearly, a better understanding of the function of leptin at the tissue level will open new possibilities in regulating growth and energy utilization. Further, the manipulation of leptin may provide a new and exciting tool to enhance feed intake in young-growing pigs in which growth is limited by appetite. Thus, understanding the basic mechanisms that regulate fat cell differentiation and adiposity, feed intake, and energy metabolism in livestock may lead to new technologies that will enhance animal performance and health. Further, regulation of feed intake and whole body energy balance in livestock species is important for optimizing animal growth, reproduction and lactation.

Conclusions

The last two decades have provided us a window into the future of animal agriculture. We have gained substantial background knowledge in regulation of growth processes and a great deal has been elucidated concerning the molecules that are involved in the regulation of energy and protein metabolism. Unfortunately, the picture is becoming increasingly more complex which makes the challenge of regulating growth and body composition an even more formidable task. Nevertheless, the acceptance of bovine somatotropin and ractopamine as metabolic modifiers has clearly paved the way for others in the future. While the promise for genetic enhancement of growth may surpass many of the steps we have already taken, a true understanding of growth processes and adaptation of sound nutritional practices will be the key to maximizing growth and production efficiency in the future.

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