Biotechnologies for Improving Animal Metabolism and Growth - A Review

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ABSTRACT : Biotechnology will play critical role in improving animal productivity. Animal growth rate and muscle deposition potential can be greatly improved by the application of biotechnology and biotechnological products. Administration of recombinant somatotropin (ST) or other compounds such as IGF-1 and growth hormone-releasing peptides (GHRPs) can enhance growth rate and carcass lean percentage. Gene transfer offers a powerful approach to manipulate endocrine system and metabolic pathways toward faster growth and better feed efficiency. Biotechnology is also extensively used for improving metabolism and activity of gut microorganisms for better nutrient digestibility. Knockout of growth-inhibiting genes such as myostatin results in considerable acceleration of body weight and muscle growth. Animal growth can also be improved by the use of gene therapy. Immunomodulation is another approach for efficient growth through controlling the activity of endogenous anabolic hormones. All the above aspects will be discussed in this review. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1794-1802)*

Key Words : Biotechnology, Animal Growth, Physiology, Endocrinology, Review

INTRODUCTION

Animal agriculture has made great contributions to human being in the past century. Animal products provide one-sixth of human food energy and more than one-third of the protein on a global basis (Bradford, 1999). Further increases in per capita and total demand for animal food products are forecast for the globe, mainly developing countries for next decades. However, animal agriculture is increasingly suffering challenges from both environmental protection (Chen, 2001) and public health concerns (Barton and Hart, 2001). Future animal agriculture will be subject to more and more legal restrictions. One example is that all except four antibiotics and some animal-origin feeds such as meat and bone meal have been prohibited as animal feeds in European Union. As a result, the further improvement or even maintenance of current productivity of animal agriculture may become more difficult than ever by applying only known technologies. Therefore, new technologies are not only essential but extremely urgent. As the development of molecular biology and genetic engineering, biotechnology has become a practical or potential approach for solving the problems of animal agriculture. This review is intended to introduce the emerging biotechnologies for improving animal growth rate and efficiency.

ADMINISTRATION OF RECOMBINANT SOMATOTROPIN AND RELATED COMPOUNDS

Recombinant somatotropin (ST)

Administration of recombinant somatotropin can accelerate muscle growth and reduce fat deposition in most animals. However, ST is most effective in pigs than in other animals. Growing-finishing pigs have greater response to ST than young pigs (table 1). Administration of ST may have some effects on meat quality. Pork tenderness may be decreased because of the lower intramuscular lipid (Bonneau, 1991). The incidence of boar taint in entire male pigs is reduced by ST (Bonneau et al., 1992; Hagen et al., 1991).

IGF-1

IGF-1 is a potent mitogen that mediates the action of growth hormone (GH) on cell proliferation (Jones and Clemmons, 1995). Administration of IGF-1 can produce effects similar to those observed with ST administration. Evidences from rodent and human studies indicate that there may be a direct action of IGF-1 and (or) synergism of GH and IGF-1 on growth and growth regulation (Guler et al., 1988; Elahi et al., 1993; Clark et al., 1994). Klindt et al. (1998) found administration of IGF-1 in Meishan pigs for 28 days increased growth rate 22%, carcass protein accretion 33% and trimmed lean cuts 5%. However, the improvement made by IGF-1 was much less than that by pST. There was no synergism between IGF-1 and pST on the measurements.

Current study focuses on IGF-1 analogs which are more effective than IGF-1 itself, and on manipulation of IGF-1 binding proteins in plasma which appear important in determining tissue-specific biological effects of IGF-1.

Growth hormone-releasing peptides (GHRPs)

Growth hormone-releasing peptides (GHRPs) are a family of synthetic oligopeptides, which specifically stimulate the release of GH in many species as well as in humans. The first synthesis of GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) was found to have the biological

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Table 1. Summary of levels of performance and accretion rates of protein and lipid and responses to exogenous pST across different phases of growth in pigs

Phase of growth	pST, μg/kg	Gain, g/day	Accretion rate, g/day		
			Gain/feed	Protein	Lipid
10-25 kg	0	680	0.61	96	89
	120	680 (0)	0.61 (0)	113 (+17)	61 (-31)
20-50 kg	0	900	0.43	120	207
	150	990 (+10)	0.49 (+13)	150 (+25)	122 (-41)
50-100 kg	0	1,140	0.33	135	340
	150	1,334 (+17)	0.44 (+33)	235	61 (-82)

Dose of pST represents daily dose. Values in parentheses are response to pST treatment (in %). Etherton and Bauman, 1998.

effect of stimulating GH release in many species (Walker et al., 1990). Based on the structure of GHRP-6, a second generation of GHRP, initially known as GHRP-1 (D-Ala-His-D-βNal-Ala-Trp-D-Phe-Lys-NH_2), was developed. Recently, a new generation of GHRP, GHRP-2 (D-Ala-D-βNal-Ala-Trp-D-Phe-Lys-NH_2), was synthesized. The GH-releasing activity of GHRP-2 has been found to be two to three times more active in rats and humans than GHRP-6 and GHRP-1 (Bowers, 1993). At present, GHRP-2 seems to be the most potent member of the family of GHRPs.

In domestic animals, the effects of GHRP-2 on the release of GH *in vivo* have been examined in calves (Hashizume et al., 1997b) and goats (Hashizume et al., 1997a) and significant stimulating effects have been found in both species. But the effect of GHRP-2 on growth performance of farm animals have not been examined until recently. Phung et al. (2000) injected GHRP-2 subcutaneously into pigs of 69 kg body weight (BW) at dose of 30 μg/kg BW once daily for 30 days and found that average daily gain and feed efficiency was increased by 22.35% ($p<0.05$) and 20.64% ($p<0.01$) respectively compared to the saline injection control. Plasma GH peak concentration was enhanced by about 12-15 folds. The mechanism for GH-releasing effect of GHRP-2 is not clearly understood. *In vitro* study suggests that GHRP-2 may partially act via GH-releasing factor receptor and kinase C and cAMP pathways (Roh et al., 1997). The above researches implicate a great potential of GHRPs as a growth promoter.

TRANSGENESIS

Gene transfer offers a powerful approach not only for studying the molecular mechanisms of animal growth and development but also for developing manipulatory techniques of animal growth and growth efficiency. Palmiter et al. (1982) developed a "gigantic" mouse by introducing rat GH into mouse genome through microinjection. The transgenic mice had extraordinarily high level of GH in the serum (up to 800-fold) and showed substantially higher body weight (up to almost 2-fold) than the controls. The same approach was first extended to produce farm animals in 1985 (Hammer et al., 1985). Since then, a lot of experiments have been conducted to test the efficacy of altering growth efficiency and body composition of farm animals by transgenesis (Muller and Brem, 1996). The most frequently used gene is GH. Other genes including growth hormone-releasing factor (GRF), IGF-1, Chicken Ski (cSki) have also been tested (Ward, 1999). The gene construct consists mostly of the regulatory element of a metallothionein (MT) gene fused to the coding sequence for growth-promoting genes. MT is widely used because its expression can be regulated by the level of circulating zinc. However, the expression of transgenes can not be well regulated by MT mediation (Wall, 1996). Some other regulatory approaches, such as the use of tetracycline or its analogs to regulate transgene expression, have been explored (Corcoran et al., 1996). The method of introducing transgenes into an animal genome is microinjection procedure developed in mice in 1980 (Gordon et al., 1980). Although this method has many disadvantages and some new approaches are being explored (Yang et al., 2000), it is by far the only approved and the most successful method to produce transgenic farm animals.

The transfer of GH genes has been performed in many species of animals, but has showed much more successful in fish and pigs than in other farm animals such as cattle, sheep, goats and poultry. The transgenic fish (Brem, 1993; Maclean and Rahman, 1994) and pigs (Pursel et al., 1989) obtained responses similar to those with the administration of exogenous GH. According to the review by Pursel and Solomon (1993), growth rate and feed efficiency were improved by 13% and about 18% respectively in GH-transgenic pigs. At 92 kg bodyweight, GH-transgenic pigs had 85% less total fat in carcass, and 85% less saturated fatty acids, 91% less monounsaturated fatty acids, and 66% less polyunsaturated fatty acids in fat compared to control pigs. In primal cuts of GH-transgenic pigs, intramuscular fat was reduced 43% in ham, 66% in loin, 64% in shoulder, and 69% in belly. However, meat tenderness was not significantly affected. Similar patterns of carcass lipid content and fatty acid profiles were also observed at early

growing stages (smaller body weight) for transgenic pigs (table 2). Two points should be pointed out from table 2. One is that fat deposition trend is different in transgenic pigs compared to control. As body weight increased, carcass fat content decreased in transgenic pigs, whereas increased in non-transgenic pigs. Another point is that the decrease of carcass fat deposition occurs from early stage through whole growing and finishing period for transgenic pigs. In comparison, administration of exogenous GH was more effective in finishing stage than growing stage.

GH-transgenic pigs had severe health and reproductive problems (Pinkert et al., 1994) because of the unregulatable GH expression. The potential solution is to target the expression of GH transgene to specific tissues and to control the level and duration of GH expression by modified gene constructs (Nottle et al., 1997; Pursel et al., 1997)

Transgenic fish expressing GH or IGF-1 grows 30-50% faster and convert feed 6-19% more efficiently than non-transgenic controls (Chen and Lu, 1998).

Transgenic mice expressed human IGF-1 specifically in muscle exhibited myofiber hypertrophy (Coleman et al., 1995a). However, in transgenic pigs, 80% of the expressed IGF-1 protein was not biologically active (Coleman et al., 1995b).

Other genes that have been used in transgenic approach to growth manipulation include growth hormone releasing factor (GRF) gene and cSki gene. Transfer of GRF gene may avoid some of the side effects observed in GH-transferred animals. Transgenic pigs (Pursel et al., 1992) and calf (Bowen et al., 1994) expressed c-ski gene showed some degree of muscle hypertrophy.

Genes involved in intermediary pathways, such as those for essential amino acid biosynthesis and glyoxylate cycle genes, have been tested as transgenes for improving nutrient utilization (Ward, 1999). When cys E and cys K genes encoding serine transacetylase and o-acetylserine sulph-hydrylase for cysteine biosynthesis were transferred and expressed in rumen epithelium of sheep, the transgenic sheep were able to synthesize *de novo* cysteine from inorganic sulphur (Ward and Nancarrow, 1992). Rees et al. (1990) tried the transfer of genes for lysine and threonine biosynthesis from aspartic acid into mouse 3T3 cells. Saini et al. (1996) introduced bacterial glyoxylate cycle genes in the liver and intestine in mice and found that mice were able to convert acetate into glucose. When the same technology is applied to ruminants, acetate produced in rumen could become a gluconeogenic precursor and in turn feed efficiency, particularly for forage feed, could be enhanced.

Gene transfer seems more difficult and complicated in farm animals than in mice. As showed in table 3, The efficiency of producing transgenic farm animals is very low and varies with animal species. The percentage of gene-injected embryos that develop into transgenic animals varied from 0.31% to 4.03% for pigs, 0.1% to 4.45% for sheep, and 0.34% to 2.63% for cattle (Pursel and Solomon, 1993). Because of the low efficiency, production of transgenic farm animals requires large intellectual, technical and financial investment, huge animal resources, and great

Table 2. Lipid composition of carcass ground tissue from GH-transgenic pigs

Component	Weight group (W), kg								significance	
	Transgenic (T)				Control					
	14	26	48	88	14	26	48	88	T	W
Total lipid, %	6.19	7.62	7.54	3.27	10.03	12.32	15.50	19.55	*	*
Total SFA, %	1.90	2.19	2.26	0.81	3.17	3.64	4.92	6.59	*	*
Total MUFA, %	1.99	2.47	2.35	0.81	3.74	4.69	6.48	7.73	*	*
Total PUFA, %	1.08	1.81	1.75	0.93	1.50	2.38	2.54	2.51	*	*
PUFA/SFA	0.57	0.83	0.77	1.23	0.47	0.65	0.52	0.38	*	*
Cholesterol, mg/100 g	106.5	100.0	85.6	75.5	100.9	95.0	85.6	75.1	NS	*

Solomon, 1992

Table 3. Efficiency of producing transgenic animals

Species	No. Injected	No. Transferred	Fetuses or born		Transgenic animals produced		
			No.	% of injected	No.	% of born	% of injected
Mice	12,314	12,314	1,847	15.0	321	17.3	2.61
Pigs	19,397	19,397	1,920	9.9	177	9.2	0.91
Sheep	5,242	5,242	556	10.6	46	8.3	0.88
Goats	1,058	782	173	16.4	12	6.9	1.11
Cattle	11,206	1,018	193	1.7	7	3.6	0.06

Bondioli and Wall, 1998

time expenditure. Therefore, the commercial applications of transgenic farm animals for improving growth and growth efficiency won't be possible until the efficiency of transgenic techniques is dramatically enhanced.

BIOTECHNOLOGIES FOR GUT MICROORGANISMS

Biotechnology can be extensively used for improving metabolism and activity of gut microorganisms, which is very important for animal health and growth. This can be done by three categories of biotechnological approaches. One is the application of biotechnological products to improve the gut ecosystem and promote the growth of beneficial bacteria. Pre- or pro-biotics (Salminen et al., 1998) and oligosaccharides (Fishbein, 1998) belong to this category. The second approach is to genetically modify microorganisms naturally present in the gut to enhance their capacity of defined functions or to add new functions (Chang, 1996). Introductions of diverse genes into gut microorganisms have been extensively explored (McSweeney et al., 1999). The genetically modified microorganisms are able either to digest fibrous components and lignins of forages, to degrade toxins, to synthesize essential amino acids, to reduce methane formation, or to tolerate acids (Forsberg et al., 1993). The third approach is to introduce new species or strains of microorganisms into the gut (Stewart et al., 1988). Application of the three approaches has a great potential to increase digestibilities of feedstuffs and to improve animal health and growth. However, as many other biotechnologies, biotechnology for gut microorganisms is far from being commercially applicable because of the technical problems and public concerns (McSweeney et al., 1999).

GENE KNOCKOUT

Gene knockout or gene disruption is a molecular approach that specifically silences a target gene. There are many genes that involved in the regulation of growth and nutrient deposition. Some genes have a general or local (tissue-specific) growth-inhibiting function that causes retarded growth of whole body or specific tissue. Knockout of these genes could reverse the retardation or even lead to overgrowth (Sellier, 2000).

Type II IGF receptor gene (Igf2r) may be an example of genes which have a general growth-inhibiting function. Knockout mice lacking Igf2r showed the increase of IGF-2 levels. Prenatal growth was improved with birth weight being 1.4 times higher than that of wild-type controls (Ludwig et al., 1996).

Myostatin (MS) is a good example of genes having local growth-inhibiting effects. MS is a member of the transforming growth factor (TGF-β) superfamily. It is expressed preferentially in developing and adult skeletal muscle. MS is transcribed as a 2.9 kb mRNA species and translated as disulfide-linked dimer that is subsequently secreted, processed and functioned in an autocrine/ paracrine manner similar to that of other TGF-βsuperfamily proteins (McPherron et al., 1997). McPherron and Lee (1997) found MS gene is highly conserved among vertebrate species. The sequences of murine, rat, human, porcine, chicken and turkey MS are 100% identical in the C-terminal region following the putative proteolytic processing site. Baboon, bovine, and ovine MS contain only one to three amino acid differences in the mature protein. Zebrafish MS is more diverged and is only 88% identical to the others in this region.

The presence of MS in animals may act as a negative regulator of skeletal muscle mass. McPherron et al. (1997) provided the evidence for the first time by mutating MS gene in mice. They found MS-knocked -out mice showed a dramatic and widespread increase in skeletal muscle mass. Individual muscles of mutant mice weigh 200-300% more than those of wild-type mice due to the combination of muscle cell hyperplasia and hypertrophy (table 4). Recently, Taylor et al. (2001) examined the effects of recombinant MS protein on metabolisms of mouse skeletal muscle *in vitro* and found that MS protein inhibited cell proliferation, DNA synthesis and protein synthesis in a dose-dependent manner, providing a direct evidence of inhibitory role of MS on muscle growth.

The degree of sequence conservation of MS across species suggests that MS may play a similar role in regulating muscle mass in other animals as it does in mice. McPherron and Lee (1997) found that the double muscling of two cattle breeds, Belgian Blue and Piedmontese, was due to MS mutation. In Belgian Blue, 11 nucleotides in the third exon were deleted, which resulted in null activity of

Table 4. Weights of individual muscles from mutant (-/-) and wild-type (+/+) animals

	Weight (g)*		% of +/+
	+/+ (n=9)	-/- (n=9)	
Body	30.3±3.3	40.9±4.2	135
Diagastric	0.022±0.005	0.045±0.007	205
Pectoralis	0.178±0.042	0.467±.0.067	262
Triceps brachij	0.158±0.036	0.372±0.039	235
Quadriceps	0.232±0.052	0.470±0.053	203
Gastrocnemius/ plantaris	0.150±0.033	0.328±0.020	219
Tibialis cranialis	0.047±0.005	0.095±0.017	202
Soleus	0.006±0.002	0.012±0.001	200

* Significant difference between +/+ and -/- with p<0.001.

McPherron et al., 1997

MS protein. In Piedmontese, one guanosine in exon 3 sequence was replaced by adenosine, resulting in tyrosine substitution for cysteine. The same mutations were not, or much less frequently detected in other breeds. Double-muscled cattle grow 20-25% more muscle mass than other cattle. In pigs, Ji et al. (1998) found MS expression in longissimus muscle was 65% higher ($p<0.04$) for low-birth-weight piglets (0.57 kg body weight) than that for piglets with higher birth weight (1.37 kg).

The discovery of MS and its regulatory role on muscle growth opens up a new means to control muscle mass of farm animals. Muscle mass may hopefully be enhanced by gene knockout. Success of this approach in increasing muscle mass in mice (McPherron et al., 1997) demonstrates the possibility. However, commercial application of this technique won't be feasible until all the biological impacts of MS knockout in farm animals are evaluated.

GENE THERAPY

Gene therapy is an approach of introducing exogenous genes into animals without the use of transgenesis. Gene therapy is originally developed as a means to correct genetically-based diseases in humans. However, this technique may also be used to deliver genes which can alter growth and body compositions. Since it is still at its formative stage, there is little information in the referred literatures about its use in growth manipulation. In laboratory animals, after genetically modified myoblasts expressed human GH were delivered systemically, circulating GH concentration was increased (Barr and Leiden, 1991; Dhawan et al., 1991; Heartlein et al., 1994). If this approach succeeds in domestic animals, repeated injection of recombinant GH would be avoided.

Another potential application of gene therapy for growth control may be the direct injection of plasmid DNA encoding growth-promoting genes into muscle tissue. Since Wolff et al. first found (1990) and confirmed (1992) that foreign genes could be taken up and expressed persistently in mouse muscle by direct injection, transfection of skeletal muscle by plasmid DNA has been well demonstrated in several vertebrate species (Hansen et al., 1991; Jiao et al., 1992, Manthorpe et al., 1993). Skeletal muscle has been become a most attractive site for expression of foreign genes. It is promising to use skeletal muscle as an artificial endocrine tissue to produce a variety of physiologically active proteins for therapeutic and production purposes (MacColl et al., 1999). A *in vivo* study in adult mice showed that a single intramuscular injection of 100 ug plasmid DNA encoding GH-releasing hormone resulted in 3-4 fold increase in serum GH for up to 2 weeks and 10% increase of body weight (DraghiaAkli et al., 1997). Delivery of plasmid encoding GH produced a similar effect (Anwer et al., 1998). GH deficiency can be corrected in murine models by injection of GH-secreting modified myoblasts or adenovirus encoded GH cDNA (MacColl et al., 1999). More recently, expression of plasmid DNA encoding IGF-1 was confirmed possible in pig muscle by injection (Reichel et al., 2000). Furthermore, methods of controlled release of GH in muscle have been developed (Rivera et al., 1996). All these accomplishments offer a strong evidence that muscle growth or whole-body growth of farm animals may be manipulated by gene therapy targeting skeletal muscle.

IMMUNOMODULATION

Technology of immunization is to utilize the animal's immune response to alter endocrine pathways that control growth rate and body composition. This appears to be a more acceptable approach to control growth rate and lean /fat deposition than hormone injection. Information accumulated to date shows that either active immunization using vaccines or passive immunization using monoclonal or polyclonal antibodies against some endogenous hormones provides a potential approach for improving growth performance.

Activity of endogenous GH could be enhanced by active immunization (Buttery, 1993). GH binding protein can attenuate the expression of GH response, immunomodulation of the binding proteins may enhance the anabolic action of GH (Flint, 1992). Monoclonal antibodies to GH could potentiate the activity of endogenous GH (Pell et al., 1989) and increase growth rate and protein gain in pigs (van der Hel et al., 1994). Wang et al. (1996a) found that a monoclonal antibody specific to the rat ST receptor could bind to the receptor and resulted in a growth response in hypophysectomized rats. Antibodies against somatostatin incresed ST synthesis in the pituitary (Dubreuil et al., 1991).

A monoclonal antibody,designated PS-7.6, was generated in mice by immunizing mice with recombinant porcine growth hormone (pGH). This antibody has been shown to enhance the growth-promoting activity of pGH in an experimental hypophysectomized rat model. The possible mechanism of PS-7.6 in enhancing animal growth is to prevent pGH from being bound to GH binding protein in circulation, thus making more pGH available to tissue receptors (Wang et al., 1993). Based on the immunological properties of PS-7.6, Wang et al. (1994) developed a monoclonal anti-idiotypic antibody, designed 2A6, which was able to mimic pGH in promotion of animal growth. According to the amino acid sequence (54-95) of pGH recognized by PS-7.6, a synthetic peptide, pGH (54-95) was developed by the same group of researchers (Wang et al., 1995). Polyclonal antibodies produced in swine immunized

with pGH (54-95) had a similar function to PS-7.6. Daily weight gain during growing stage of pigs and leaf fat and loin eye muscle at 110-120 kg were significantly improved by the peptide immunization (Wang et al., 1996b). These studies suggest that both active and passive immunization to GH may be a potential approach to improve animal growth and carcass composition.

In chicken, immunization against IGF-1 alone had not effect on growth performance, efficiency of energy utilization, and carcass composition. However, immunization against both IGF-1 and IGF-2 resulted in a lighter body weight, less abdominal fat, and heavier spleen weight at 5 weeks of age (Spencer et al., 1995). Immunization against IGF-2 alone had not effect on body weight gain and feed efficiency, but abdominal fat decreased by 27% (Spencer et al., 1997). In lambs, immunization against somatostatin increased daily gain by 15%. Plasma IGF-1 concentration increased significantly for lambs immunized from birth. Dry matter intake and carcass composition were not influenced. (Ingvartsen and Vestergaard, 2000)

Immunization against luteinizing hormone releasing hormone could remove boar taint and avoid surgical castration, at the same time maintain the superior leanness and feed efficiency of entire male pigs (Bonneau and Enright, 1995; Lobley et al., 1992).

Flint (1992) developed a passive immunization with antibodies to adipose plasma membranes. Lean gain increased by 12%, while fat deposition decreased by 25%. Passive immunization of sheep with anti-ovine adipocyte membrane serum decreased fat deposition, indicating the potential of this approach as a means of decreasing adiposity in farm animals (Moloney et al., 1998).

Passive immunization with antiserum against adrenocorticotropin increased weight gain in rats (Silence et al., 1992). The reason may be that high concentrations of corticosteroid hormones could antagonize the effects of ST.

The major limitation of immunomodulation by an active immunizaton is that the level and duration of the immune response and the magnitude of physiological response are poorly controlled, while a passive immunization approaches using antibodies is more expensive although it may provide better control (Bonneau and Laarveld, 1999).

ACKNOWLEDGEMENTS

This paper was presented at 3rd CJK Symposium on Biotechnology and Animal Production held in Jinju, Korea on June 28-29, 2001. The author is grateful to Sichuan Provincial Academic and Technical Personnel Foundation for covering partial expense of trip to Korea.

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