

The Manipulation of Milk Secretion in Lactating Dairy Cows^a - Review -

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ABSTRACT : A number of developments have occurred over recent years that are being used commercially or have the potential to increase the milk yield and consequently the efficiency of dairy cows. Bovine growth hormone is the most widely known of several attempts that have been made to alter the metabolic endocrinology of dairy cows to increase the rate of milk secretion. The factors affecting the milk yield response to growth hormone, growth hormone-releasing factor, thyroxine and placental lactogen as well as to the immuno-neutralization of somatostatin are briefly considered. Secondly, the recent greater understanding of the mechanism by which the milk yield is increased following more frequent milking, which has resulted from the identification and characterization of the feedback inhibitor of lactation (FIL) protein, is reviewed. The identification of this protein provides new avenues of research which may lead to a reduction in the rate of decline in milk yield with advancing lactation or to undiminished milk yields despite a reduction in frequency with which the animals are milked. (*Asian-Aus. J. Anim. Sci.* 2000. Vol. 13, No. 2 : 236-243)

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INTRODUCTION

The factors affecting the rate and quality of milk secretion of dairy cows are so great that clearly they can not be even briefly reviewed in such a short paper as this. The effects of nutrition alone on milk yield and composition have been the subject of much research over many years and would require a very extensive review. For example, there has been a great increase in the understanding of the nutritional requirements of dairy cows (e.g., Sniffen et al., 1992; O'Conner et al., 1993). Another example would be the development of feedstuffs which bypass the fermentation of the rumen and thereby directly increase the efficiency with which nutrients are converted into milk (e.g., Sklan et al., 1993). Alternatively, milk secretion can be substantially increased by the amelioration of heat stress in dairy cattle during high ambient temperatures and relative humidities (reviewed by Huber, 1996).

Our aim for this paper was narrower than this: firstly to concentrate on some of the attempts that have been made to directly alter the metabolic endocrinology of dairy animals to increase the rate of milk production. Secondly, we wanted to briefly review research relating to increased rates of milk secretion in lactating animals following an increase in milking frequency. Whilst this latter phenomenon has

been known for a long time, it is only in recent years that progress has been made in determining the mechanism by which it occurs.

ENDOGENOUS HORMONES AND LACTATION

With the adoption in 1994 of bovine growth hormone (GH) in the USA to increase commercial milk production, the manipulation of the endocrine system of dairy cows to increase milk yield entered a new and practical phase. Many attempts to alter the metabolism of farm animals by endocrine means, and thereby increase productive efficiency, have been developed over the years. However, very few have been incorporated into management practice whilst most have been rejected (e.g. feeding thyroprotein, administering anabolic steroids). The aim of the following sections is to briefly review some methods by which milk yield may be increased by the administration of hormones, and the factors affecting the response.

Growth hormone

The administration of GH elicits a profound increase in the production of milk by dairy cows, the milk yield response begins to occur within 24 h, and the maximal response occurs within one week. Elevated responses maintained for the duration of the treatment period (Cohick et al., 1989a, Zinn and Bravo-Ureta, 1996). The typical milk yield response is approximately 6 to 30% greater than that of saline treated control cows, which translates to between 1 to 7 kg of additional milk per day (Zinn and Bravo-Ureta, 1996). The first reported experiment of dairy cows treated for a large part of lactation with

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recombinant bovine GH noted a milk yield response of up to 41% (11.5 kg/d) though this level of response is now thought to be atypically high. A number of experiments have also noted no significant response to the administration of the hormone (Lormore et al., 1990; Hof et al., 1991, Marty and Block, 1992).

Several factors have been shown to determine the extent of the milk yield response. Principal amongst these is the concentration of hormone achieved in the circulation, which is dependent upon the dose and the method of administration (Zinn and Bravo-Ureta, 1996). The milk yield response is also critically dependent upon the adequacy of the nutritional provision. The nutritional requirements of cows treated with GH is a function of the animals maintenance requirements, body condition and requirements for milk synthesis. Thus, the provision of a diet with a sufficient nutritional density is a requirement for a maximal milk yield response (Bauman and Vernon, 1993; Burton et al., 1994; Zinn and Bravo-Ureta, 1996), this notwithstanding, milk yield responses have been observed in cows which have been treated with a range of diets, from pasture only to high energy concentrate-based feeds (reviewed by Burton et al., 1994).

It is apparent that the absolute milk yield response to GH administration is generally similar if treatment starts between the peak and end of lactation (Peel et al., 1983), though the whole lactational response to GH may be reduced if treatment begins very early in lactation. (reviewed by Bauman and Vernon, 1993; Burton et al., 1994). The increase in milk yield following the second or subsequent lactation of GH treatment has been shown to be not as great as that of the first (Phipps et al., 1990), though this is possibly because the cows were not fed to allow an adequate replacement of body reserves between lactations. Increases in milk yield have been reported to be greater in multiparous than primiparous cows (Sullivan et al., 1992) which is possibly related to the continued weight increase observed in cows during their first lactation. The results from experiments examining the effect of genetic merit on milk yield response to GH are often contradictory, with animals of higher genetic merit having lower, higher and the same milk yield response as animals of poorer genetic merit (Zinn and Bravo-Ureta, 1996). Perhaps with the commercial availability and widespread use of GH, this relationship may be determined on statistically meaningful numbers of cows.

Experiments in rats have indicated that the lactogenic activity of human growth hormone may be enhanced as well as inhibited by the binding of monoclonal antibodies of specific sites of the growth hormone molecule (Aston et al., 1986). Modification

of growth hormone in this way may be a method of increasing the average milk yield response observed. It appears that monoclonal antibody binding to specific epitopes prevent the coupling of GH to certain types of receptor while not to others. It is this, rather than allosterically induced conformational changes or an increase in the circulating half life of the hormone which seems to modulate the function of the hormone.

The increased milk yield with GH is dependent upon an increased supply of glucose, which in ruminants are largely met from the products of rumen fermentation via hepatic gluconeogenesis. The appetite of GH treated dairy cows does not increase until after more than 6 weeks of treatment, while the partial efficiencies of energy utilization for maintenance and milk synthesis as well as the efficiency of digestion are thought not to change at all (Tyrrell et al., 1988; Crooker et al., 1990). Thus, the initial increase in milk yield is partly dependent upon the mobilization of body stores, which is in turn dependent upon the level of body reserves present before the start of GH treatment (reviewed by Bauman and Vernon, 1993). With respect to glucose, when GH treatment begins, the whole body utilization of glucose is increased and the whole body oxidation of glucose is decreased (Bauman et al., 1988). Additionally, the use of glucose by the hind limb is reduced (McDowell et al., 1987) while hepatic production of glucose is increased (Cohick et al., 1989b).

To investigate further the relationship between GH and insulin action, insulin was intravenously infused at a number of different rates into late lactation cows while maintaining the plasma concentration of glucose constant with a glucose solution infused at a variable rate (Rose et al., 1996). This so called euglycaemic clamp technique allows the study of the effect of insulin on glucose kinetics without the secondary responses caused by insulin induced hypoglycaemia. In this experiment, the whole body glucose turnover rate was lower in cows with elevated GH levels than in saline treated controls. While this relationship was not apparent for animals in very early lactation or at basal insulin levels, it indicates that in later lactation, elevated plasma concentrations of GH decrease the responsiveness of peripheral tissues to high concentrations of insulin. This would tend to spare glucose for insulin insensitive tissues, principally the mammary gland.

In another series of experiments the effect of GH on non-insulin mediated glucose utilization was determined. By infusing somatostatin into dairy cows for a prolonged period, the pancreatic release of insulin was prevented and the plasma concentration of insulin was reduced to nearly zero (Rose et al., 1997b); under such conditions the whole body glucose utilization rate was assumed to be entirely non-insulin

dependent. By comparing this rate to the rate observed at normal plasma insulin concentrations, it was determined that insulin mediated glucose uptake was only about 8% of normal glucose uptake in dairy cows, precluding a large decrease during GH treatment. Thus, the vast majority of glucose turnover in dairy cows is not under the control of insulin. Indeed, from known rates of mammary glucose uptake (e.g. Kronfeld, 1982) it can be estimated that approximately 80% of total glucose turnover is utilized by the mammary gland in lactating dairy cows. When dairy cows and sheep were treated with GH for 5 days, non-mammary, non-insulin mediated glucose utilization was not affected, as determined using the above techniques (Rose et al., 1997a, 1998). This indicating that overall there is no further sparing of glucose use by peripheral tissues, other than that by insulin sensitive tissues, to increase the availability of glucose to the mammary gland during GH treatment. This suggests that other sources of glucose, specifically glycerol from the hydrolysis of adipose triglycerides and amino acids mobilized from muscle, must supply the additional glucose required in the initial weeks of GH treatment.

Growth hormone-releasing factor

The administration of GH-releasing factor (GRF) to lactating dairy cows causes a substantial increase in the plasma concentration of GH with a concomitant increase in milk yield (Hodate et al., 1990; Binelli et al., 1995; Lapierre et al., 1995). When the effectiveness of GRF and GH were compared, the milk yield of GRF treated cows was shown to be slightly greater than that of GH treated cows, particularly after several weeks of treatment, despite similar plasma concentrations of GH (Dahl et al., 1993). Also, the persistency of the increased milk yield following the end of GRF treatment was greater in GRF treated cows than is usual for that of GH (Dahl et al., 1990). More recently, a comparison of the mechanism of action of GRF and GH (Binelli et al., 1995; Vanderkooi et al., 1995) demonstrated similar milk yield responses for the two hormones. However, the plasma profile of GH was more pulsatile in GRF treated cows, and that the plasma concentration of IGF-1 and IGF-1 binding protein 3 were higher in GH treated cows. However, the similar milk yield responses, and similarity of other measured variables in this study, lead the latter authors to conclude that the mechanism by which milk yield is increased by GH and GRF is largely the same.

Immuno-neutralization of somatostatin

A number of attempts have been made to increase the productive efficiency of farm livestock by immunizing animals against somatostatin (SRIF;

reviewed by Spencer, 1986). Initially, passive immunization against SRIF was designed to prevent the inhibitory effect on pituitary GH release, which would cause an increase in the plasma concentration of GH (Spencer, 1986). The method would have the advantage over even slow release formulations of recombinant GH, in that the number and frequency of injections required is reduced (Sun et al., 1990b). Using this procedure, the milk yield of lactating sheep (Sun et al., 1990b) and goats (Garssen et al., 1987) has been increased. In an experiment where the growth rate of lambs was increased by SRIF immuno-neutralisation, it appeared that the motility of the gut was affected, leading to improved digestion and an increased nutrient supply (Sun et al., 1990a). The contribution of increased digestibility to the increased productive response, relative to increased plasma GH concentrations, however, remains unclear. There have been a number of attempts to increase the growth rate of cattle by actively immunizing against SRIF that have had no effect (e.g., Trout and Schanbacher, 1990; Vicini et al., 1988). Spencer (1986) suggested the difficulty encountered in obtaining consistent effects with this technique might be because of poor or slow antibody responses. However, successful active immunization against SRIF (evidenced by elevated anti SRIF titres) did not result in an increased growth rate in growing lambs or in increased concentrations of GH or food digestibility (Zainurt et al., 1991). Clearly, the problems caused by the variability in the productive response to immuno-neutralization of SRIF will prevent any commercial adoption using the present methods.

Thyroid hormones

While thyroxine (T_4) is the predominant thyroid hormone in the circulation, it has little if any inherent biological activity; the most metabolically active thyroid hormone, triiodothyronine (T_3) is produced by the enzymatic deiodination of T_4 within the thyroid gland and other tissues (Chanoine et al., 1993; Nielsen and Reis, 1993).

The rate of milk production in dairy cows is increased by thyroid hormones, either by subcutaneous injection (Davis et al., 1988) or via the feed as an iodinated protein (Astrup, 1985). However, in contrast to GH, thyroxine treatment is associated with a rapidly increased feed intake and no change in the partial efficiency of conversion of feed to milk (Shaw et al., 1975). Indeed, the additive nature of the milk yield response to simultaneous administration of GH and thyroprotein indicates that GH and thyroid hormones enhance milk yield through different mechanisms (Meits et al., 1961). It appears that thyroid hormones and GH increase cardiac output, though only GH has been shown to increase the proportion of cardiac

output that is delivered to the mammary gland (Davis et al., 1987a). Also, unlike GH, thyroxine has been shown not to affect the plasma concentration of IGF-1 (Davis et al., 1987b). Progress on the understanding of the mechanism by which thyroproteins increase milk yield does not appear to be as advanced as that for GH. The commercial suitability of thyroxine for increasing milk yield is somewhat limited, however. This is principally because of decreased milk yields (below that of control animals; Jayasuriya et al., 1982) and decreased milk quality (Astrup, 1985) following the withdrawal of treatment, and the worry over increased concentrations of thyroid hormones in milk from treated cows.

Placental lactogen

Placental lactogen, produced in the placenta and only present in the circulation during pregnancy, is thought to be an endocrine factor controlling mammary development (reviewed by Byatt et al., 1992a). The milk yield and feed intake of non-pregnant lactating cows has been increased by several days of subcutaneous injection of recombinant bovine placental lactogen for 7 to 9 days (Byatt et al., 1992b). However, the effect caused by even very high doses was much less than the effect of GH on milk yield (the milk yield increase observed with placental lactogen was approximately 5%). This galactopoietic effect may in part be because it is a weak GH agonist; plasma IGF-1 concentrations are increased in a dose dependent manner following its administration (Byatt et al., 1992b). In contrast, however, physiological concentrations of placental lactogen may antagonize the secretion of GH from the anterior pituitary gland, causing the decline in milk yield observed in lactating cows towards the end of gestation. Recombinant bovine placental lactogen has also been shown to be lactogenic *in vivo* and is probably a stimulator of mammary cell proliferation (Byatt et al., 1994). However, it does not stimulate the proliferation of mammary epithelial cells *in vitro*, the effect is unlikely to be direct (Collier et al., 1995).

INCREASED MILKING FREQUENCY

It has long been established that milk production in dairy cows is positively related to milking frequency or the completeness with which milk is removed. This relationship has been observed in all species that have been so far studied, including man (see Peaker, 1995). Additionally, the unilateral milking of one gland of domestic dairy animals more or less often than the other(s) causes an increase or decrease in the milk yield of that gland, demonstrating that the regulation operates on an intra-mammary basis (e.g. Linzell and Peaker, 1971). However it is only in

recent years that progress has been made in understanding how the mammary gland is so able to coordinate the production of milk to meet the requirements of the suckling young.

Extent of the milk yield response

Following an extensive review of literature, Erdman and Varner (1995) concluded that, relative to twice daily milking, there is a fixed milk yield response to increased milking frequency in dairy cows, and that there is no relationship between the milk yield response observed and the initial milk yield of the animal. In his review, the responses to increasing the frequency from twice to three times per day were reasonably consistent. Though ranging from approximately 1 kg to over 5 kg of additional milk per day, they demonstrated an average milk yield response of 3.5 kg/d for thrice daily milking relative to twice daily milking. The number of studies which have examined the effect of reducing milking frequency to once per day or increasing it to 4 times per day are much fewer in number, but again it seems that milk yield response is not related to the milk yield achieved by milking twice per day; the average responses were -6.2 and +4.9 kg/d, respectively, for milking 1 and 4 times per day relative to that for twice per day. Milk fat and protein concentrations tended to decrease with increasing milking frequency, though the effect was not large and in most cases was not significant (Erdman and Varner, 1995). Thus, the increased milk yield ensures that the yields of fat, protein and lactose are also proportionally increased. It was also noted that the extent of the milk yield response, when expressed absolutely, was not different between primiparous and multiparous cows though when expressed as a percentage, that for primiparous cows was greater (Erdman and Varner, 1995).

Feedback inhibitor of lactation

The mammary gland is relatively rare as an exocrine gland that in that it stores its secretion extracellularly. This storage of milk in the gland lumen allows a local level of control on milk secretion in addition to the systemic control imposed by the endocrine system. The extent of this control is indicated by the similar and additive effect on milk yield of bovine GH injections and increased frequency of milking in lactating goats (Knight et al., 1992). Experiments in lactating goats have demonstrated that the distention of the mammary gland caused by milk accumulation, in the short term, does not limit the secretion of milk (Henderson and Peaker, 1984). Though clearly the limited capacity of the mammary gland to store milk does mean that distention eventually disrupts the production of milk (Peaker, 1980). In an series of experiments, cow's and goat's

milk has been screened in tissue and cell culture bioassays for a milk constituent able to inhibit milk secretion (Wilde et al., 1987a, 1995a). The active component was identified as a previously unknown small milk glycoprotein, with a relative molecular mass of 7600, which has been named feedback inhibitor of lactation (FIL). This peptide is synthesized in the epithelial cell (Wilde et al., 1995a), indicating that the cells secrete an inhibitor of their own activity and that the inhibitor acts in an autocrine manner. The protein that has been identified has been shown to inhibit the secretion of milk both *in vitro* (Rennison et al., 1993) and when introduced into the lumen of the mammary gland of goats (Wilde et al., 1995a). Additionally, the inhibition achieved has been shown to be both rapid and reversible as well as dependent, but not to affect the composition of the milk produced (Wilde et al., 1995a). Active immunization of lactating goats against FIL temporarily reduce the decline in milk secretion with advancing lactation, though this was only observed following a third immunization, when the antibody to FIL was present in the milk and not only in the plasma (Wilde et al., 1996). The milk yield following immunization was 3% greater than that pretreatment, compared to an 11% decrease for the treated and control treatment. Further investigation of this technique to manipulate milk secretion on a commercial basis, using a greater number of animals and a more effective immunization technique, is clearly warranted. Possibly of greater commercial relevance was the finding of a slower decrease in the rate of milk secretion following a switch to once daily milking in immunized animals (Wilde et al., 1996). This would allow a reduction in the number of milkings animals undergo, without greatly affecting the total amount of milk produced.

The finding that the autocrine inhibition of milk secretion *in vivo* is dependent upon the concentration of FIL, makes it likely that the concentration of FIL in the milk adjacent to the epithelial cells increases as milk accumulates, and is decreased by milk removal (Wilde et al., 1995b). The kinetics of FIL secretion and removal are not yet known, that is, how the autocrine inhibition of milk secretion is relieved following milk removal, or how changes in the concentration of FIL are achieved during the normal cycle of milk accumulation and removal. Changes in the rate of FIL secretion over this cycle would require that it was regulated independently of the secretion of the other milk constituents and so this is thought to be unlikely (Wilde et al., 1995b). An alternative theory is that the secreted FIL protein is the result of, or undergoes processing in the alveolar lumen following secretion. If this were the case then first order processing would gradually cause the concentration of FIL to increase, even though the

proto-inhibitor were being secreted at a constant rate relative to the other milk constituents (Wilde et al., 1995b). Indeed, proteins structurally similar to FIL have been identified in the whey proteins but have been shown to be inactive in bioassays for FIL (Wilde et al., 1995a). Additionally, if FIL was processed and deactivated following secretion, after the milk was removed, continued metabolism of the protein would cause its concentration to decrease, re-establishing an optimal rate of milk secretion (Wilde et al., 1995b).

The apparently close coordination of the decrease in the secretion rate of the milk constituents, fat, protein and lactose, suggests that the effect of FIL in inhibiting milk secretion occurs at a point common to the synthesis of all three components. This could be achieved for the non lipid components by regulation of the Golgi secretory pathway in mammary epithelial cells (reviewed by Wilde et al., 1995b). There is some evidence to suggest that FIL may block an early stage in the process of Golgi body vesicle exocytosis (Wilde et al., 1995b). The process for the lipid component of milk has also yet to be determined; the situation has been complicated by the finding that a partially purified preparation of the FIL protein caused no observable effect on lipid synthesis from acetate in rabbit mammary explants (Wilde et al., 1987a). An alternative theory (Heesom et al., 1992) suggests that the inhibition of fat production after milk is no longer removed from the gland may be due to an accumulation of non-esterified medium chain fatty acids within the mammary cell, possibly derived from the apical (mammary lumen) side of the mammary epithelium. Non-esterified medium chain fatty acids have been shown to inhibit the conversion of glucose to fatty acids in rats, both *in vivo* and *in vitro* (Heesom et al., 1992; Souza and Williamson, 1993). With respect to the dairy cow, it is unknown whether medium chain fatty acids prevent lipid synthesis following an accumulation of milk in the mammary lumen, not least because of the differences in metabolism between ruminants and non-ruminants; lipid synthesis in the mammary gland of ruminants differs from that of rodents in that the major precursor of lipid is acetate, not glucose. Whatever is the case, the unaltered milk concentration of fat following intra-luminal administration of purified FIL to goats (Wilde et al., 1995a) suggests that FIL is competent to control the secretion of all milk components coordinately.

In addition to the acute effects of FIL on milk secretion, partially purified preparations of the peptide have been shown to decrease secretory-cell differentiation in rabbit following intra-ductal injection (Wilde et al., 1987b) and to inhibit mouse mammary cells on floating collagen gels (Wilde et al., 1991). These observations made *in vitro* reflect the decreased

cell differentiation observed in the mammary glands of goats milked once per day compared to that seen in the other glands continuously milked twice per day (Wilde and Knight, 1990). It is possible that these longer term effects of FIL may be partially due to the down regulation of galactopoietic hormone receptors: total prolactin binding to rabbit mammary glands was reduced 24h after FIL was injected through the teat canal (Bennett et al., 1992). In contrast, when the milking frequency of goats was increased from 2 to 3 times per day, total prolactin binding to the mammary gland was increased (McKinnon et al., 1988).

Clearly, future research in this field must concentrate on the kinetics of FIL once it has been released from the mammary epithelium. That is how the FIL protein, or the product of its modification, interacts with the mammary epithelium how the message to limit milk component synthesis is transmitted through the cell and the locus in the process of milk synthesis where the inhibition occurs. Additionally, the longer-term effects of FIL on mammary physiology will need to be elucidated.

REFERENCES

- Astrup, H. N. 1985. The effect of two kinds of rapeseed meal and of thyroxine upon milk quality. *Acta Agriculturae Scandinavica*. 35:310-314.
- Bauman, D. E., C. J. Peele, W. D. Steinhour, P. J. Reynolds, H. F. Tyrrell, A. C. G. Brown and G. L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: influence on rates of irreversible loss and oxidation of glucose and non-esterified fatty acids. *J. Nutr.* 118:1031-1040.
- Bauman, D. E. and R. G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. *Ann. Rev. Nutr.* 13:437-461.
- Bennett, C. N., C. J. Wilde and C. H. Knight. 1992. Changes in prolactin binding with milk accumulation in the lactating rabbit. *J. Endocrinology*. 132(Suppl.):292.
- Binelli, M., W. K. Vanderkooi, L. T. Chapin, M. J. Vandelaar, J. D. Turner, W. M. Moseley and H. A. Tucker. 1995. Comparison of growth hormone-releasing factor and somatotropin: Body growth and lactation of primiparous cows. *J. Dairy Sci.* 78:2129-2139.
- Burton, J. L., B. W. McBride, E. Block, D. R. Glimm and J. J. Kennelly. 1994. A review of bovine growth hormone. *Canadian J. Anim. Sci.* 74:167-201.
- Byatt, J. C., P. J. Eppard, L. Munyakazi, R. H. Sorbet, J. J. Veenhuizen, D. F. Curran and R. J. Collier. 1992b. Stimulation of milk yield and feed intake by bovine placental lactogen in the dairy cow. *J. Dairy Sci.* 75:1216-1223.
- Byatt, J. C., P. J. Eppard, J. J. Veenhuizen, T. L. Curran, D. F. Curran, M. F. McGrath and R. J. Collier. 1994. Stimulation of mammarygenesis and lactogenesis by recombinant bovine placental lactogen in steroid-primed heifers. *J. Endocrinology*. 140:33-43.
- Byatt, J. C., W. C. Warren, P. J. Eppard, N. R. Staten, G. G. Krivi and R. J. Collier. 1992a. Ruminant placental lactogens: structure and biology. *J. Anim. Sci.* 70:2911-2923.
- Chanoine, J. P., L. E. Braverman, A. P. Farwell, M. Safran, S. Alex, S. Dubord and J. L. Leonard. 1993. The thyroid gland is the major source of circulating T3 in the rat. *J. Clin. Investigation*. 91:2709-2713.
- Cohick, W. S., K. Plaut, S. J. Sechen and D. E. Bauman. 1989a. Temporal pattern of insulin like growth factor-1 response to exogenous bovine somatotropin in lactating cows. *Domestic Animal Endocrinology*. 6:263-274.
- Cohick, W. S., R. Slepatis, M. Harkins and D. E. Bauman. 1989b. Effects of exogenous bovine somatotropin (bST) on net flux rates of glucose and insulin across splanchnic tissues of lactating cows. *FASEB Journal*. 3:A938.
- Collier, R. J., J. C. Byatt, M. F. McGrath and P. J. Eppard. 1995. Role of placental lactogen. In: *Intracellular Signalling in the Mammary Gland*. Edited by Wilde, C. J., M. Peaker and C. H. Knight. Plenum Press, New York, USA.
- Crooker, B. A., M. A. McGuire, W. S. Cohick, M. Harkins, D. E. Bauman and K. Sejrsen. 1990. Effect of dose of bovine somatotropin on nutrient utilization in growing dairy heifers. *J. Nutr.* 120:1256-1263.
- Dahl, G. E., L. T. Chapin, W. M. Moseley and H. A. Tucker. 1993. Galactopoietic effects of recombinant somatotropin and growth hormone-releasing factor in dairy cows. *J. Dairy Sci.* 76:1550-1557.
- Dahl, G. E., L. T. Chapin, S. A. Zinn, W. M. Moseley, T. R. Schwartz and H. A. Tucker. 1990. Sixty-day infusions of somatotropin-releasing factor stimulate milk production in dairy cows. *J. Dairy Sci.* 73:2444-2452.
- Davis, S. R., R. J. Collier, J. P. McNamara, H. H. Head and W. Sussman. 1987a. Effects of thyroxine and growth hormone treatment of dairy cows on milk yield, cardiac output and mammary blood flow. *J. Anim. Sci.* 66:70-79.
- Davis, S. R., P. D. Gluckman, I. C. Hart and H. V. Henderson. 1987b. Effects of injecting growth hormone or thyroxine on milk production and blood plasma concentrations of insulin-like growth factors I and II in dairy cows. *J. Endocrinology*. 114:17-24.
- Erdman, R. A. and M. Varner. 1995. Fixed yield responses to increased milking frequency. *J. Dairy Sci.* 78:1199-1203.
- Garssen, G. J., A. M. A. W. Welling and G. S. G. Spencer. 1987. Active immunisation against somatostatin increases milk yield in goats. *Netherlands J. Agricultural Science*. 35:84-86.
- Heesom, K. J., P. F. A. Souza, V. Ilic and D. H. Williamson. 1992. Chain-length dependency of interactions of medium-chain fatty acids with glucose metabolism in acini isolated from lactating rat mammary glands: a putative feed-back to control lipid synthesis from glucose. *Biochem. J.* 281:273-278.
- Henderson, A. J. and M. Peaker. 1984. Feedback control of milk secretion in the goat by a chemical in milk. *J. Physiology*. 351:39-45.
- Hodate, K., T. Johke, A. Ozawa and S. Ohashi. 1990. Plasma growth hormone, insulin like growth factor-1, and milk production responses to exogenous human growth hormone-releasing factor analogs in dairy cows.

- Endocrinologica Japonica. 261-273.
- Hof, G., P. J. Lenaers, S. Tammenga, S., L. J. Jonker and A. I. Koffeman. 1991. Bovine somatotropin and feed interactions in dairy cows. *Livest. Prod. Sci.* 28:21-36.
- Huber, J. T. 1996. Amelioration of heat stress in dairy cattle. In: *Progress in Dairy Science*. Edited by Phillips, C. J. C. CAB International, Wallingford, UK. pp. 211-243.
- Jayasuriya, M. C. N., H. B. Perdok, H. M. Ross-Parker and M. F. J. Van Houtert. 1982. Effect of alkali-treated rice straw supplemented with spent tea leaf and thyroprotein on milk yield, milk composition and certain physiological parameters of dairy cows. *Anim. Feed Sci. Technol.* 7: 201-216.
- Knight, C. H., J. E. Hillerton, M. A. Kerr, R. M. Teverson, A. Turvey and C. J. Wilde. 1992. Separate and additive stimulation of bovine milk yield by the local and systemic galactopoietic stimuli of frequent milking and growth hormone. *J. Dairy Research.* 59:243-252.
- Kronfeld, D. S. 1982. Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. *J. Dairy Sci.* 65:2204-2212.
- Lapierre, H., G. Pelletier, T. Aribat, K. Fournier, P. Gaudreau, P. Brazeau and D. Petitclerc. 1995. The effect of feed intake and growth hormone-releasing factor on lactating dairy cows. *J. Dairy Sci.* 78:804-815.
- Linzell, J. L. and M. Peaker. 1971. The effects of oxytocin and milk removal on milk secretion in the goat. *J. Physiology.* London. 216:717-734.
- Lormore, M. J., L. D. Muller, D. R. Deaver and L. C. Griel. 1990. Early lactation responses of dairy cows administered bovine somatotropin and fed diets high in energy and protein. *J. Dairy Sci.* 73:3237-3247.
- Marty, B. J. and E. Block. 1992. Effects of dietary fat supplementation and recombinant bovine somatotropin on milk production, nutritional status and lipid metabolism of dairy cows. *Canadian J. Anim. Sci.* 72:633-649.
- McDowell, G. H., J. M. Gooden, D. Leenanuruksa, M. Jois and A. W. English. 1987. Effects of exogenous growth hormone on milk production and nutrient uptake by muscle and mammary tissues of dairy cows in mid-lactation. *Aust. J. Biological Sci.* 40:295-306.
- McKinnon, J., C. H. Knight, D. J. Flint and C. J. Wilde. 1988. Effect of milking frequency and efficiency on goat mammary prolactin receptor number. *J. Endocrinology* 119(Suppl.):167.
- Meits, J. 1961. Farm animals: Hormonal induction of lactation and galactopoiesis. In: *Milk: The mammary gland and its secretion*. Volume 1. Edited by Kon, S.K. and Cowie, A. T. Academic Press, London. UK. pp. 321-367.
- Nielsen, M. O. and C. J. Riis. P. M. 1993. Somatotropin, insulin-like growth factor-1 and the mammary gland in the regulation of nutrient and energy metabolism during early lactation. *Acta Veterinaria Scand.* 89 (Suppl.) 47-54.
- O'Connor, J. D., C. J. Sniffen, D. G. Fox and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *J. Anim. Sci.* 71:1298-1311.
- Peaker, M. 1995. Autocrine control of milk secretion: development of the concept. In, *Intracellular Signalling in the Mammary Gland*. Edited by Wilde, C. J., M. Peaker and C. H. Knight. Plenum Press, New York, USA. pp. 193-202.
- Peaker, M. 1980. The effect of raised intramammary pressure on mammary function in the goat in relation to the cessation of lactation. *J. Physiology.* 301:415-428.
- Peel, C. J., T. J. Fronk, D. E. Bauman and R. C. Gorewit. 1983. Effect of exogenous growth hormone in early and late lactation on lactational performance of dairy cows. *J. Dairy Sci.* 66:776-782.
- Phipps, R. H. 1990. A review of the influence of somatotropin on the health, reproduction and welfare of lactating dairy cows. In: *Use of Somatotropin in Livestock Production*. Edited by Sejrsen, K., Vestergaard, M and Neimann-Sorensen, A. Elsevier Applied Science, New York, USA. pp. 88-119.
- Rennison, M. E., M. Kerr, C. V. P. Addey, S. E. Handel, M. D. Turner, C. J. Wilde and R. D. Burgoyne. 1993. Inhibition of constitutive protein secretion from lactating mouse mammary epithelial cells by FIL (feedback inhibitor of lactation), a secreted milk protein. *J. Cell Sci.* 106:641-648.
- Rose, M. T., F. Itoh, M. Matsumoto, Y. Takahashi and Y. Obara. 1998. Insulin independent glucose uptake in growth hormone treated dairy cows. *J. Dairy Res.* 65: 423-431.
- Rose, M. T., Y. Obara, F. Itoh, H. Hashimoto and Y. Takahashi. 1997a. Growth hormone does not affect non-insulin-mediated glucose uptake in sheep. *Experimental Physiology.* 82:749-760.
- Rose, M. T., Y. Obara, F. Itoh, H. Hashimoto and Y. Takahashi. 1997b. Non-insulin- and insulin-mediated glucose uptake in dairy cows. *J. Dairy Res.* 64:341-353.
- Rose, M. T., Y. Obara, H. Fuse, F. Itoh, A. Ozawa, Y. Takahashi, K. Hodate and S. Ohashi. 1996. Effect of growth hormone-releasing factor on response to insulin of cows during early and late lactation. *J. Dairy Sci.* 79: 1734-1745.
- Shaw, G. H., E. M. Convey, H. A. Tucker, E. P. Reineke, J. W. Thomas and J. J. Byrne. 1975. Bovine serum thyroxine, prolactin, growth hormone, glucocorticoid, and thyroxine binding globulin response to thyroprotein. *J. Dairy Sci.* 58:703-708.
- Sklan, D. and M. Tinsky. 1993. Production and reproduction responses by dairy cows fed varying undegradable protein coated with rumen bypass fat. *J. Dairy Sci.* 76: 216-223.
- Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562-3577.
- Souza, P. F. A. and D. H. Williamson. 1993. Effects of feeding medium-chain triacylglycerols on maternal lipid metabolism and pup growth in lactating rats. *Br. J. Nutr.* 69:779-787.
- Spencer, G. S. G. 1986. Immuno-neutralization of somatostatin and its effects on animal production. *Domestic Animal Endocrinology.* 3:55-68.
- Sullivan, J. L., J. T. Huber, S. K. DeNise, R. G. Hoffman, L. Kung, S. E. Franson and K. S. Madsen. 1992. Factors affecting response of cows to biweekly injections of somatotropin. *J. Dairy Sci.* 75:756-763.

- Sun, Y. X., G. L. Drane, S. D. Currey, N. D. Lehner, J. M. Gooden, R. M. Hoskinson, P. C. Wynn and G. H. McDowell. 1990a. Immunization against somatotropin release inhibiting factor improves digestibility of food, growth, and wool production of crossbred lambs. *Aust. J. Agric. Res.* 41:401-411.
- Sun, Y. X., S. E. Sinclair, P. C. Wynn and G. H. McDowell. 1990b. Immunization against somatotropin release inhibiting factor increases milk yield in ewes. *Aust. J. Agric. Sci.* 41:393-400.
- Trout, W. E. and B. D. Schanbacher. 1990. Growth hormone and insulin-like growth factor-1 responses in steers actively immunized against somatostatin or growth hormone-releasing factor. *J. Endocrinology.* 125:123-129.
- Tyrell, H. F., A. C. G. Brown, P. J. Reynolds, G. L. Haaland, D. E. Bauman, C. J. Peele and W. D. Steinhour. 1988. Effects of bovine somatotropin on the metabolism of lactating dairy cows: Energy and nitrogen utilization as determined by respiration calorimetry. *J. Nutr.* 118:1024-1030.
- Vanderkooi, W. K., M. J. Vandehaar, B. K. Sharma, M. Binelli, H. A. Tucker, R. M. Akers and W. M. Moseley. 1995. Comparison of growth hormone-releasing factor and somatotropin: The somatotropic axis in lactating primiparous cows. *J. Dairy Sci.* 78:2140-2149.
- Vicini, J. L., J. H. Clark, W. L. Hurley and J. M. Bahr. 1988. The effect of immunization against somatostatin on growth and concentration of somatotropin in plasma of Holstein calves. *Domestic Animal Endocrinology.* 5:35-45.
- Wilde, C. J., C. V. P. Addey, L. M. Boddy and M. Peaker. 1995a. Autocrine regulation of milk secretion by a protein in milk. *Biochem. J.* 305:51-58.
- Wilde, C. J., C. V. P. Addey, L. M. Boddy and M. Peaker. 1995b. Autocrine control of milk secretion: from concept to application. In: *Intracellular Signalling in the Mammary Gland.* Edited by Wilde, C. J., Peaker, M and Knight, C. H. Plenum Press, New York, USA. pp. 227-237.
- Wilde, C. J., C. V. P. Addey and M. Peaker. 1996. Effects of immunization against an autocrine inhibitor of milk secretion in lactating goats. *J. Physiology.* 491:465-469.
- Wilde, C. J., D. R. Blatchford and M. Peaker. 1991. Regulation of mouse mammary cell differentiation by extracellular milk proteins. *Experimental Physiology.* 76: 379-387.
- Wilde, C. J., D. T. Calvert, A. Daly and M. Peaker. 1987a. The effect of goat milk fractions on synthesis of milk constituents by rabbit mammary explants and on milk yield *in vivo*. *Biochem. J.* 242:285-288.
- Wilde, C. J., D. T. Calvert and M. Peaker. 1987b. Effect of a fraction of goat milk serum proteins on milk accumulation and enzyme activities in rabbit mammary gland. *Biochemical Society Transactions.* 15:916-917.
- Wilde, C. J. and C. H. Knight. 1990. Milk yield and mammary function in goats during and after once daily milking. *J. Dairy Res.* 57:441-447.
- Zainurt, A. S., R. M. Hoskinson and R. C. Kellaway. 1991. Production responses of lambs immunized against somatostatin. *Anim. Prod.* 53:339-344.
- Zinn, S. A. and B. Bravo-Ureta. 1996. The effect of bovine somatotropin on dairy production, cow health and economics. In: *Progress in Dairy Science* (Ed. C. J. C. Phillips). CAB International, Wallingford, UK. pp. 59-85.