Prolactin and androgens — their role in the utilisation of nutrients 227

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SUMMARY

This paper is concerned with evaluating the evidence for and describing the mechanisms whereby prolactin and the androgens may modify nutrient utilisation in the ruminant. Specifically nutrient utilisation refers to net effects on the synthesis of fat and protein by the growing or lactating animal.

Prolactin is an anterior pituitary hormone whose plasma concentration responds positively to daylength and to a lesser extent to ambient temperature. Such changes are frequently correlated with changes in food intake, body growth and milk yield such that it has been suggested that prolactin may play a role in mediating the stimulatory effect of long daylength of these parameters. Experiments involving various methods of prolactin manipulation are considered but overall the data are not convincing, with the exception of the situation with lactating ruminants where prolactin is necessary for the initiation of lactation. Critical experiments probably involving combinations of prolactin suppression with prolactin supplementation, are clearly required to clarify the role of prolactin in nutrient utilisation.

Androgens are substances which produce and/or maintain male sexual characters; they are characteristically higher in males than females. The most common manipulation of the level of androgens is via castration. Castration depresses food intake, overall rate of growth and muscle growth but increases the relative rate of fat accretion. Exogenous administration of natural or synthetic androgens can counteract the effects of castration. In contrast to prolactin, there is clear evidence of a cause/effect relationship between androgens and the utilisation of nutrients for growth. However the actual mechanisms of response to the androgens is poorly understood.

INTRODUCTION

In this paper we are concerned with the possible roles of prolactin and the androgens in the regulation of nutrient utilisation with particular reference to the net effects on the synthesis of fat and protein by the ruminant animal.

PROLACTIN

Prolactin is an anterior pituitary hormone which is very widely distributed among the vertebrates being found in amphibians, fish, birds and the mammals. The rate of prolactin secretion is regulated mainly by a prolactin inhibiting factor (probably dopamine) originating largely from the hypothalamus. According to Nicoll and Bern (1972), more than 100 physiological actions have been ascribed to prolactin in vertebrates. These they divided into five categories:

(i) effects related to reproduction;
(ii) effects on water and electrolyte balance;

(iii) effects on integumentary structures or ectodermal derivatives;
(iv) actions involving synergism with adrenal or gonadal steroids;
(v) effects on somatic growth or metabolism.

In this paper we are concerned especially with the last category. Nicoll and Bern (1972) regard prolactin as a hormone which may condition the responses of the target organ to the trophic effects of other hormones and that a common mechanism might operate, perhaps by affecting permeability of the cell membrane. In order for a hormone to exert effects at the tissue level then receptors for that hormone must be present; in this respect, specific binding sites for prolactin are widespread among a variety of tissues including the liver, mammary gland, ovary, testis and adrenal gland (Posner *et al.*, 1974). In non-ruminants there is evidence of a role for prolactin in growth. For example, both bovine and ovine prolactin have been shown to stimulate growth and/or nitrogen retention in mice (Wallis and Dew, 1973), rats (Bates *et al.*, 1964) and bone growth in the hypophysectomised rat (Thorngren and Hansson, 1977). However in ruminants, the situation appears more complex.

Plasma prolactin, daylength, feed intake and nutrient utilisation

Increasing daylength causes increases in plasma prolactin in ruminants (e.g. cattle, Peters and Tucker, 1978; sheep, Lincoln *et al.*, 1982; red deer, Suttie and Kay, 1985), as does an increase in ambient temperature (Wetteman *et al.*, 1982) or an increase in food intake (Peticlerc *et al.*, 1983; Forbes *et al.*, 1975). However, there is also evidence of positive effects of daylength *per se* on intake (Simpson *et al.*, 1983/84). The question is whether such interrelationships between daylength, temperature, food intake and prolactin can be resolved in terms of cause and effect in relation to prolactin.

In the red deer, with its pronounced annual cycle, the correlation between plasma prolactin and feed intake is especially striking (Suttie and Kay, 1985); in this work, peaks of prolactin tended to occur slightly before peaks of food intake, pointing to the fact that prolactin could perhaps be involved in stimulating the increasing intake in spring. In this respect Milne (1980) suggested that the increasing prolactin in the spring may be involved in the increase in size of the reticulo-rumen. Similarly Mainoya (1978) proposed that prolactin was involved in the hypertrophy of the gastrointestinal tract observed at the onset of lactation in rats. It is also pertinent that Forbes (1982) concluded that a significant proportion of the liveweight gain response to long daylength observed in his experiments was due to change in gut fill.

There is some evidence that changes in nutrient utilisation are associated with changes in day length. Increases due to long days have been recorded for growth rate in cattle and sheep (Peters *et al.*, 1978, 1980; Forbes *et al.*, 1979; Schanbacher 1979; Schanbacher and Crouse 1981; Peticlerc *et al.*, 1983) and milk yield in cows (Peters *et al.*, 1978, 1981). However when differences in feed intake are allowed for, the effects are much less convincing, although in a controlled pair-feeding experiment, Forbes *et al.* (1981) found that the long day lambs had larger, leaner carcasses pointing to a diversion of nutrients away from fat to protein deposition. However such studies are not convincing in supporting a role for prolactin and indeed in one recent study, the increased growth rate of heifers was not related to plasma prolactin concentrations (Peters *et al.*, 1980).

Manipulation

In order to define the role of prolactin, initial experiments in which the level of prolactin is manipulated are vital. Such manipulations include:

increasing prolactin	(i)	infusion or injection
	(ii)	pituitary implants
	(iii)	pharmacological methods, e.g. TRH
	(iv)	increased daylength (16 hours per day or greater)
reducing prolactin	(i)	immunisation
	(ii)	reduced daylength (8 hours per day or less)
	(iii)	bromocryptine (dopamine agonist) treatment

All of these manipulations have been used in animal experiments, although to date pituitary implants apparently have not been used in studies with ruminants. Combinations of the treatments listed above involving a reduction in prolactin followed by prolactin replacement would provide a rigorous experimental approach.

Growth

There have apparently been only two experiments reported, albeit both so far only in abstracts, in which the influence of prolactin provided by infusion or injection has been ascertained in non-lactating ruminants. Brinklow and Forbes (1983) placed sheep in continuous darkness in order to depress plasma prolactin. When prolactin was infused to produce levels which mimicked a 16 hour day, nitrogen (N) retention was increased significantly due mainly to a reduction in urinary N output. However the very small number of sheep used (2 per group) and the absence of a normal light control means that the results must be treated with caution. The second experiment is one where light:dark regimes were combined with bromocryptine and prolactin injection treatments (Eisemann *et al.*, 1981). Although prolactin concentrations responded in the expected manner, and both intake and weight gain were higher in lambs on the longer day length and higher in control than in bromocryptine treated lambs, the group receiving daily injections of prolactin grew only at the same rate as the controls over the 9 week period.

Chronic treatment with thyrotropin releasing hormone (TRH) of lambs and heifers promoted increases in plasma prolactin; increases were recorded in N retention in one experiment (Davis *et al.*, 1976, 1977) but reduced gains were recorded in the other (Muir and Wien, 1983). However such treatment is essentially pharmacological in relation to prolactin, while the possible involvement of the thyroid hormones in these responses cannot be overlooked.

Active immunisation of rams against prolactin dramatically reduced plasma prolactin concentrations and slightly reduced growth rate (Ohlson *et al.*, 1981). The rams were fed *ad libitum*, but the group sizes were very small and feed intake was apparently not recorded. Bromocryptine treatment has been used to suppress prolactin concentrations; however long term treatment had no effect on body weight gain in two experiments in sheep (Brown *et al.*, 1976; Ravault *et al.*, 1977). Where the objective is to investigate effects on growth, then the use of young animals with a high growth potential and fed a high quality diet is extremely important.

Therefore although there is some evidence for a role for prolactin in altering nutrient utilisation and growth in non-ruminants, any such evidence is tenuous in the growing ruminant. Convincing experiments await ready supplies of large amounts of prolactin.

Lactation

In rats, mice and rabbits prolactin is essential for both the initiation and maintenance of lactation (Schar and Clemens 1972; Tindal, 1978). Studies in ruminants convincingly

show that prolactin is essential for normal initiation of lactation (sheep — Fulkerson *et al.*, 1975, Gow *et al.*, 1983; goats — Hart and Morant 1980; dairy cows — Karg and Schams 1974, Akers *et al.*, 1981) although the long term effects of an early lack of prolactin are not clear (Gow *et al.*, 1983). In contrast, prolactin is not necessary for maintenance of lactation in either cows (Karg *et al.*, 1972) or goats (Hart, 1975). In the latter work, although prolactin release was maintained during autumn by daylength manipulation milk yield declined in the usual way.

The approach used by Akers (1981) and his colleagues provides a sound experimental method for investigating the role of prolactin. In this work, suppression of the periparturient rise in prolactin with bromocryptine treatment for 10 days pre-parturition reduced milk yield to very low levels and although yield did increase over the first 10 days of lactation, the bromocryptine treated cows had much lower yields than the controls. The low yields were associated with low rates of fatty acid and lactose synthesis, coupled with low levels of critical enzymes. In this study it is the third group which provides the convincing evidence for the role of prolactin : cows were treated with bromocryptine but were also given a continuous infusion of prolactin over 6 days pre-parturition, and in this group milk yields were similar to controls.

Prolactin and other hormones

The possibility that it may be the interaction of prolactin with other hormones which is of real importance, must be considered. This was suggested by Nicoll and Bern (1972) who proposed that prolactin conditions the response of the target organ to the effects of other hormones. In this respect, the possible relationships with the pineal hormone, melatonin are of interest. Melatonin is involved in interpreting the daylight pattern for the animal (Lincoln, 1983). Therefore it is interesting that pinealectomy of sheep prevented both the rise in prolactin due to long days and the effect of long days on growth rate (Forbes, 1982).

In conclusion there is little convincing evidence, that prolactin has any cause/effect consequences on the net synthesis of fat or protein in the milk or carcass of ruminants, the only exception being in the initiation of lactation. The one conclusion is that long days, intake and temperature all tend to increase prolactin concentrations in ruminants. Although such changes are frequently correlated with changes in food intake, body growth and milk yield any impact of prolactin itself or net fat and protein synthesis is not yet proven. Therefore critical, well-designed experiments are required.

ANDROGENS

Androgens are substances which produce and/or maintain male sexual characteristics; they are characteristically higher in males than females. They are used widely in the animal industries to improve food conversion efficiency and growth rate in ruminants (Heitzman, 1978), muscle to fat ratios in pigs (Fowler *et al.*, 1978), to increase muscle mass in horses (Beroza, 1981) and are also used by athletes to improve performance (O'Shea, 1978). In this section we will concentrate on the effects of the principal endogenous androgen, testosterone (and its metabolites), referring only to the exogenous anabolic agents where it may be helpful in elucidating mechanisms of action. Studies with the ruminant species, sheep, cattle and deer will be cited where available; otherwise data from non-ruminant species will be used to illustrate points.

Metabolism

The principal circulating androgens are testosterone, 5 α dihydrotestosterone (5 α DHT), androstanediol, androstenedione, dehydroepiandrosterone (DHEA) and its sulphate and androstenediol. By far the most potent are testosterone and 5 α DHT. Testosterone may also be converted to oestradiol-17 b via the aromatase pathway. Androgens are secreted by the testis, ovary and adrenal cortex of most mammals. The testis is the major source in males with testosterone being secreted by the Leydig cells in response to the release of luteinising hormone (LH) from the pituitary. LH release is in turn controlled by the hypothalamic peptide, LH-releasing hormone (LH-RH). The actual overall regulation is complex with negative feedback from the testis to the hypothalamus and pituitary. Although the testis is the principal source of testosterone, both testosterone and 5 α DHT are also formed from weakly active precursors in peripheral tissues, there being good evidence that conversion occurs in both fat and muscle (Vermeulen, 1979).

Manipulation

Any study of the influence of androgens on nutrient utilisation requires manipulation of the androgen levels. Manipulations include:

(i) castration
(ii) supplementation with androgens
(iii) immunisation

The most common manipulation of androgens in animals is that of castration; the practise has an ancient and somewhat colourful history whether it be to maintain a soprano voice or to care for a harem. In recent times it has fallen out of favour among the human population and it is pleasing to note that now some of these principles are being recognised in our management of farm animals.

It is very well known that compared with entire bulls and rams, castrates have a lower voluntary energy intake, a lower rate of liveweight gain and an increased rate of fat deposition (see Galbraith and Topps, 1981; Rhodes, 1969). Although there are theoretical reasons to believe that the timing of castration, or alternatively the extent of androgen priming, may influence the rate of liveweight gain and the propensity towards fat deposition, convincing evidence is lacking (Ford and Gregory, 1983). However female rats treated during the neonatal period with androgens subsequently grew faster than controls (Perry and McCracken, 1978). Testosterone supplementation (via implants) have indicated that the principal cause of the castration effect is a loss of testosterone. This is apparent when considering the effects of testosterone replacement on carcass composition but perhaps less so on growth rate (Table 1; Schanbacher *et al.*, 1980). However, the actual effects may still be mediated through the metabolites of testosterone assuming of course that the enzyme systems are still active (or potentially active) in the castrate.

Immunisation against LH-RH effectively provides a method of chemical castration in that it reduces dramatically the level of LH-RH; consequently LH is not released from the pituitary and testosterone is not released from the testis (Jeffcoate *et al.*, 1982). Studies have now shown that such animals have a faster rate of growth than steers and are substantially leaner although no comparisons with entire animals have been reported (Robertson *et al.*, 1982). The apparent reduction in fatness in the immunised animals raises the question as to what other hormones (or factors) may be involved in the high rate of lean relative to fat deposition in the entire animal compared with the castrate. That is, if testosterone were the sole cause of the faster rate of growth and the lower fat content in entires (as suggested by the Schanbacher study) then animals immunised against LH-RH

Table 1. Influence of testosterone replacement on rate of gain and backfat thickness in castrated sheep (after Schanbacher et al., 1980).

Treatment group	Plasma testosterone (ng/ml)	Gain (kg/d)	Backfat (mm)
Ram	3.1	0.34	4.8
Wether	0.2	0.21	6.9
Wether — Testo 1[1]	1.2	0.28	6.3
Testo 2	2.8	0.32	5.1
Testo 3	6.6	0.26	4.6

1. 3 levels of testosterone were used in the implants.

Table 2. Influence of castration and zeranol treatment on rate of liveweight gain (LWG) of rising 2 year old stags in spring.

	Experiment 1[1]		Experiment 2[2]	
	Castrate	Entire	Entire	Zeranol
LWG (g/d)	201	266	330	394
s.e.m.	23		31	
n	5	6	8	9
Relative LWG	76	100	100	119

1. Drew *et al.*, 1978 — stags castrated at *c.* 3 months of age.
2. P.F. Fennessy and G.H. Moore (1977 unpublished) — 12 mg Ralgro (Cooper Wellcome) subcutaneously.

would be expected to be similar to castrates in respect to growth and composition. The apparent fact that this is not the case raises numerous questions.

Immunisation against testosterone itself provides a very different approach. The actual effects of such immunisation on circulating testosterone levels may depend on a variety of factors. For example, in stags immunised against testosterone, the actual total concentration of testosterone in plasma was substantially higher in immunised than in control animals (Suttie *et al.*, unpublished). However it appears that immunisation against testosterone may have some effect on nutrient utilisation in that Schanbacher (1982) found that immunised ram lambs had a slower rate of growth than entires but were leaner at the same carcass weight. However the interpretation of such immunisation experiments is not usually straightforward.

Androgens, oestrogens and metabolites

The differences in growth rate and composition between males, females and castrated males point to the fact that the male sex hormones influence growth. However somewhat ironically it is the female hormones, oestradiol-17 b and the synthetic oestrogens which have been used to stimulate growth in castrated males (Galbraith and Topps, 1981). Conversely synthetic androgens will stimulate the growth rate of females (Sinnett-Smith *et al.*, 1983).

Studies in the highly seasonal red deer raise many questions as to the role of testosterone and/or its metabolites in various aspects of nutrient utilisation. For example, in the spring, the oestrogen-like growth promoter, zeranol, greatly increases the rate of

liveweight gain in entire red stags (Table 2). This is perhaps not surprising in that in many ways the entire red stag at this time of the year is a functional castrate in that virtually no testosterone is detectable in the plasma and the testis does not release testosterone in response to an induced pulse of luteinising hormone (Fennessy and Suttie 1985). However despite the "castrate" nature of the stag at this time, the entire stag still grows at a substantially faster rate than the castrate (Table 2). These results raise the important question as to just how much testosterone is required to obtain maximal growth rates and just how important are the metabolites of testosterone.

Since entire animals have a higher voluntary feed intake than castrates, then clearly some level of circulating testosterone (or a metabolite) is necessary to maximise intake. However very high levels are associated with a depressed voluntary intake, as in the red deer stag during the rut. The precise involvement of testosterone *per se* versus the influence of testosterone on behaviour is not clear although the intake depression does occur in penned deer in the absence of females. Therefore the effects on voluntary intake would appear to involve a two-phase mechanism where low levels of testosterone are stimulating but high levels are inhibitory. The actual site of the intake effect is unknown but it may well be directly at the level of the brain. Certainly receptors for testosterone and/or some of its metabolites are present in the brain notably in the hyothalamus, although receptors are also present in the pituitary (Jeffcoate 1978; Clark *et al.*, 1982; Kniewald and Kniewald 1981). Such a two-phase mechanism could involve different receptor sites and different metabolites but further speculation is not warranted.

The androgen response

Androgens can be considered to exert both qualitative and quantitative effects on the organism. In terms of understanding the mode of action considerable progress is being made in defining the roles of androgens in differentiation and development of the sex organs (Griffin 1981; Barden and Catterall, 1981). However, such effects are essentially qualitative whereas in this paper, it is the quantitative effects, namely those on voluntary intake, nutrient partitioning, and net fat and protein synthesis that we are particularly interested in. Unfortunately this is a relatively neglected area and any speculation as to the actual mode of action of the androgens must rely mainly on studies on the organs of sexual system such as the prostate (Michel and Baulieu, 1976) and seminiferous tubule (Fritz, 1978).

Although overall, the net rate of growth is apparently greater for all muscles in entires compared with castrates, some muscles are relatively more androgen-responsive than others as shown for red deer in Table 3. In this study, certain neck muscles were greatly increased in size in the entires compared with the castrates, relative to the total

Table 3. Effect of castration on total side weight and selected muscle weights in male red deer (after Tan and Fennessy, 1981).

	Castrate	Entire	Difference %
Side weight (kg)	30	38	+27
Muscle weights (g)			
Rhomboideus (neck)	145	229	+58
Splenius (neck)	55	120	+118
Biceps femoris (hindleg)	1372	1638	+19
Semitendinosus (hindleg)	398	502	+26

muscle mass. The increased size was due mainly to an increase in the amount of protein although the water to protein ratio was also higher in the entire stags. Testosterone receptors have been found in skeletal muscle with higher concentrations in muscles which are androgen-responsive such as the *levator ani* than in relatively less responsive muscles, such as the thigh muscles of the rat (Jung and Baulieu 1972; Michel and Baulieu 1976). The essential difference between the muscle and prostate androgen receptors appears to be that the active hormone in the prostate is probably 5 α DHT whereas in the muscle it is testosterone itself (Michel and Baulieu 1976).

Mechanisms

It is recognised that the site of action of steroid hormones is in the nucleus of the target cell where, in the current standard model, the steroid is thought to bind to its receptor in the cytoplasm of the target cell, with the steroid-receptor complex then being translocated into the nucleus (Jensen and De Sombre, 1972). However, recent evidence from both reproductive tissues (King and Greene, 1984) and cell lines (Welshons *et al.*, 1984) strongly suggests that this model is in error and that the receptor actually resides in the nucleus of the target cell. Once at its site of action in the nucleus, the steroid exerts its action by modifying DNA transcription, thus influencing the amount and/or species of messenger RNA and perhaps the amount of transfer or ribosomal RNA. In fact there is evidence that the synthesis of all types of RNA is stimulated (Davies and Griffith, 1973). Such a modification of gene transcription is the generally accepted mechanism of steroid action. Certainly Dube *et al.* (1976) showed that testosterone stimulated RNA synthesis in skeletal muscle via an effect on nuclear chromatin. However Liao *et al.* (1975) proposed that androgens may operate independently at both nuclear and extranuclear sites such that they exert a dual role in both transcription and translation.

Two major hypotheses have been advanced to explain the actual mechanism of the androgenic stimulation of muscle protein accretion, namely a direct effect of androgen and an indirect effect via the inhibition of glucocorticoid action. The presence of specific receptors for testosterone on skeletal muscle tends to support the former hypothesis. The alternative hypothesis moots the possibility that androgens bind to the muscle glucocorticoid receptor thus inhibiting the effect of glucocorticoids on muscle protein degradation. While the weight of evidence is tending to support the former hypothesis, the observation that different synthetic androgens have grossly different effects leaves the question of the actual mechanisms very open. For example, while nandrolone phenylpropionate appears to increase net protein accretion by increasing protein synthesis, trenbolone acetate actually reduces both the rates of synthesis and degradation but the net rate of accretion is increased (Buttery, 1983). However it is likely that the androgenic effect on muscle is mediated at various sites including stimulation of glucose uptake (Max and Toop, 1983) and amino acid uptake by muscle, increasing blood flow through the muscle and directly modifying the effects of other hormones. In addition, the effects of the androgens on lipid metabolism *per se* have apparently received little attention, although the reported effects of androgens on fat deposition suggest that some effort would be very worthwhile.

Therefore, in conclusion, while it is abundantly clear that the androgens have very large effects on nutrient utilisation, only very broad principles regarding the actual mechanism of action have been elucidated.

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