Leptin in farm animals: where are we and where can we go?

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Fat affects meat quality, value and production efficiency as well as providing energy reserves for pregnancy and lactation in farm livestock. Leptin, the adipocyte product of the obese (ob) gene, was quickly seen as a predictor of body fat content in animals approaching slaughter and an aid to assessing reproductive readiness in females. Its participation in inflammation and immune responses that help animals survive infection and trauma has clear additional relevance to meat and milk production. Furthermore, almost a decade of discoveries of nucleotide polymorphisms in the leptin and leptin receptor genes has suggested useful applications relating to feed intake regulation, the efficiency of feed use, the composition of growth, the timing of puberty, mammmogenesis and mammary gland function and fertility in cattle, pigs and poultry. The current review attempts to summarise where research has taken us in each of these aspects and speculates on where future research might lead.

Keywords: leptin, farm animals, production efficiency, fat, body composition

Implications

Leptin has not yet delivered the initial expectations of animal scientists eager for a robust and reliable marker of body and carcase fat in farm livestock, but its close association with fat and fat deposition continue to cast it as a potential aid for enhancing production efficiency, reproductive success and immune function. Polymorphisms in the leptin and leptin receptor genes provide powerful additional tools for enhanced selection to meet aspirations for increased global animal production within an overarching need to contain and reduce environmental impacts.

Introduction

Fat is important in all livestock production as it affects product quality and production efficiency and provides the energy reserves for pregnancy and lactation. The discovery of the obese (ob) gene and its product leptin (Zhang et al., 1994; Halaas et al., 1995) and their links to body fat (Frederich et al., 1995; Maffei et al., 1995) excited animal scientists, who quickly saw potential for leptin as a predictor of body composition in animals approaching slaughter and of reproductive readiness in females (Hossner, 1998). Its involvement in inflammation and in the immune responses that help animals survive infection and trauma, also has clear relevance and attention turned to using leptin, directly or indirectly, to influence production characteristics with feed intake, body composition, puberty, mammary gland function, immune response and the reproductive pathway all possible targets. Associations of production traits with polymorphisms in the leptin and leptin receptor genes pointed to additional applications.

Among the many reviews of leptin, those by Houseknecht et al. (1998), Baile et al. (2000), Ingvartsen and Boisclair (2001), Chilliard et al. (2005) and Barb et al. (2006) stand out as most relevant to livestock with Williams et al. (2002), Barb and Kraeling (2004), Barb et al. (2005) and Zieba et al. (2005) emphasising its involvement in reproduction. Those by Ahima and Flier (2000) and Harris (2000) are admirably comprehensive while Kershaw and Flier (2004), Margetic et al. (2002a) and Miner (2004) focus on leptin’s actions in mediating adipocyte endocrine activity.

The discovery of leptin

Zhang et al. (1994) positionally cloned the obese (ob) gene from a homozygous (ob-ob) mutant mouse with a thymine (T) for cytosine (C) substitution at nucleotide 105. The mutation changes an arginine codon (CGA) to a ‘stop’ codon (TGA), giving truncated ‘obese protein’ or ‘leptin’ (Halaas et al., 1995) that is destroyed within its source fat cell. Leptin proved to be the ‘satiety factor’ proposed by Coleman (1973) that is absent from ob mice, allowing them to overeat and become hyperobese and it supported a long-held view of adipose tissue involvement in appetite regulation (Kennedy, 1953). Coleman’s satiety factor is present in similarly obese...
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Leptin characteristics

Leptin has been shown in terrestrial and aquatic mammals, birds and cold-blooded vertebrates but not yet in any invertebrate. The gene is on chromosome no. 7 in man (Issé et al., 1995), no. 4 in cattle and sheep (Stone et al., 1996) and no. 18 in pigs (Sasaki et al., 1996). It has two introns and three exons but only exons 2 and 3 are translated, expressing 167 amino acids (aa) from which a 21-aa signal peptide is removed to leave a 16.7 kDa protein of 146-aa (145-aa in chicken) arranged as four anti-parallel helices (Zhang et al., 1999), no. 4 in cattle and sheep (Stone et al., 1998) while bovine and ovine leptins differ by only two aa (Gertler et al., 1998). The pair and three exons but only exons 2 and 3 are translated, expressing 167 amino acids (aa) from which a 21-aa signal peptide is removed to leave a 16.7 kDa protein of 146-aa (145-aa in chicken) arranged as four anti-parallel helices (A, B, C and D) typical of class 1 cytokines (Zhang et al., 1997). A single disulphide bond links the carboxyl ends of the C and D chains (Rock et al., 1996).

Leptin expression

Leptin is expressed mostly by all white adipose tissues (WAT), with lesser expression in brown fat (Cinti et al., 1997), muscle, mammary gland, stomach, pituitary, placenta, ovary and liver. Stomach leptin (Bado et al., 1998; Cinti et al., 2000) may target leptin receptors in the gut (Guilmeau et al., 2004). Liver is the main leptin source in birds and fish (Johnson et al., 2000; Sato et al., 2003), but mammalian liver does not express it. Expression is lower in mature muscle cells than in undifferentiated myoblasts (Solberg et al., 2005) and intramuscular adipocytes may be a source in muscle (Ramsay and Richards, 2005) as leptin stimulates proliferation of skeletal myoblasts (Yu et al., 2008) but inhibits their differentiation. Gardan et al. (2006) found that isolated porcine skeletal muscle adipocytes expressed leptin in amounts proportional to their growth (increasing diameter) in vitro, but always at lower levels than adipocytes isolated from porcine subcutaneous (s.c.) fat. Leptin is present in placental syncytiotrophoblasts that provide the interface between maternal and foetal bloods (Ashworth et al., 2000). The presence of leptin receptors in placenta suggests autocrine or paracrine effects of leptin on processes like angiogenesis which leptin induces (Anagnostoulis et al., 2008) and which is essential to ensure an adequate nutrient supply for foetal growth and development.

Leptin in human and mouse milks (Houseknecht et al., 1997) suggests transfer from maternal blood or de novo synthesis in the mammary gland, or both. Epithelial cells are the source of milk leptin in man (Smith-Kirwin et al., 1998), mice (Aoki et al., 1999) and cows (Smith and Sheffield, 2002) but Bonnet et al. (2002) detected it mostly in mammary adipocytes in early pregnancy. Leptin levels were 56% lower in day 10 bovine milk than in colostrum (Pinotti and Rosi, 2006) but correlated with fat in each (r = 0.90) and leptin-fortified formula milk increased the gut mucosal mitotic index in neonatal pigs (Wolinski et al., 2003). Colostral leptin might speed gut maturation – but sow milk leptin level did not affect litter size or birth weight or weaning weight of pigs (Whitley et al., 2009).

Leptin among animal species

Leptin is well characterised in man, laboratory rodents, farm animals and other placental land mammals as well as marsupials and fish and seals and whales. Its presence in birds is broadly accepted despite disagreement about the identity of the avian gene (see later). Ji et al. (1998) and Kawakita et al. (1999) cloned the bovine leptin gene and found 82% to 88% homology with human and mouse. The pig leptin gene shares 85%, 88% and 92% homology with mouse, man and cow, respectively (Ramsay et al., 1998) while bovine and ovine leptins differ by only two aa (Grtler et al., 1998). The gene sequence reported for chicken (Taouis et al., 1998; Ashwell et al., 1999) was disputed by Friedman-Einat et al. (1999) and by Burt (2004) but Richards et al. (2000) used antibody to 15-aa of pig leptin to identify leptin protein in chicken plasma, fat and liver while Neglia et al. (2008) showed it in chicken gut. However, Ninov et al. (2008) could not amplify any of three primers deduced from Genbank AFO12727 or another from Dridi et al. (2005a). Low nucleotide sequence homology between species need not mean radically different proteins, e.g. pufferfish leptin has less than 25% homology with mammalian leptin but similar tertiary structure (Yacobovitz et al., 2008). On the other hand, recombinant chicken leptin (re-ch.lep) and human leptin (re-h.lep) similarly affected feed intake by chickens while mouse leptin had no effect (Bungo et al., 1999) despite high aa-sequence homology with chicken (Ashwell et al., 1999). Turkey leptin gene was partly sequenced by Ashwell et al. (1998) while Dai et al. (2007) cloned it from duck and showed 96%, 92% and 84% aa-sequence homology with rat, pig and human. Interestingly, leptin has been shown in a migratory bird (dunlin; Kochan et al., 2006) with expression traced to liver and adipose tissue where, in fish, liver was also shown as the major source (Johnson et al., 2000).
Secretory characteristics
Leptin secretion is pulsatile in man (Licinio et al., 1998) and rat (Bagnasco et al., 2002). Wylie et al. (2001) found 8 to 10 pulses per 10 h in heifers while Tokuda et al. (2000) noted almost 5 per day in ewes. Secretion in rams was described as episodic rather than pulsatile (Blache et al., 2000). Fasting reduced pulse frequency in ewes (Daniel et al., 2002) but 12 days of feed restriction (0.4 M v. 2 M) did not alter the pulsatility in beef heifers (Wylie et al., 2001). Barb et al. (2001a) noted pulsatile secretion in pigs, and restriction of gilts reduced pulse frequency from 2.7 to 1.8 in 4 h (Whisnant and Harrell, 2002). Some reports indicate higher nocturnal levels in some species. Expression is stimulated by oestrogen (Shimizu et al., 1997) and inhibited by testosterone (Wabitsch et al., 1997) so that secretion is sexually dimorphic with pulse height and total output higher in women (2 > 1) than men (Licinio et al., 1998) even at equal fat content (Saad et al., 1997). Levels are higher in ewes than wethers or rams (Blache et al., 2000) and in heifers than steers despite lighter carcasses (Brandt et al., 2007). Gonadectomy reduced leptin secretion in both male and female rats (Bagnasco et al., 2002) but did not disturb the sexual dimorphism. Fat distribution does not seem to dictate the dimorphism in leptin concentrations (Rosenbaum et al., 2001) even though leptin is more strongly linked to the s.c. fat that accumulates more in women.

The leptin receptor (ObR)
ObR was first found in mouse choroid plexus by Tartaglia et al. (1995). Six alternatively spliced isoforms (ObRa, ObRb, ObRc, ObRd, ObRe and soluble, blood-borne ObRe) have been identified (see reviews by Tartaglia, 1997; Houseknecht and Portocarrero, 1998; Ahima and Flier, 2000). Full-length ObRb (1118-aa) has 816 extracellular aa and a transmembrane portion common to all isoforms except ObRe. Signalling is achieved via the intracellular 302-aa chain (Bjørbæk et al., 1998) but this is truncated to as few as 34-aa in ObRa, ObRc, ObRd and ObRe. ObRb has full signalling capability. It is highly expressed in the hypothalamic ventromedial, dorsomedial and arcuate nuclei that regulate appetite and reproduction, but at low levels elsewhere (Fei et al., 1997). As db mice express only intracellularly truncated isoforms, they are unresponsive to leptin and, thus, they are hyperobese (like ob mice) but untreatable by exogenous leptin (unlike ob mice).

ObRa is the smallest membrane-bound isoform, with just 34 intracellular aa (Bjørbæk et al., 1997). It helps leptin cross membranes (Hileman et al., 2000), especially those of the blood brain barrier (BBB; Golden et al., 1997). Impaired BBB transit of leptin results in leptin resistance and obesity. ObRa and ObRb both internalise leptin but ObRa is more effective (Uotani et al., 1999). ObRc, ObRd and ObRe have short intracellular chains (<40 aa). Fei et al. (1997) reported extremely low expression of ObRc, ObRd and ObRe in mice, with no clear role for any of them. Pan et al. (2008) found more ObRc, ObRa and ObRe in the brains of neonatal mice than of 2-month old mice but did not detect ObRd.

ObRe is a soluble protein and is part of the ObR extracellular domain, from which it may arise by proteolysis. It is the only isoform that is not membrane-bound and may underlie the maternal leptin resistance in pregnancy. It may also help leptin cross the placenta (Henson and Castracane, 2006). It probably is the leptin binding protein (LBP) found in man (Diamond et al., 1997) and mouse (Houseknecht et al., 1996). Garcia et al. (2002) did not find LBP in heifers, yet Crouch and Smith (2004) immuno-precipitated ObRe from cow, calf and heifer plasma and found more (2 ∙ 3) in heifers than calves (2 ∙ 3) and more again (3 ∙ 3) in late lactation cows. Leptin circulates in free and bound forms (Zhang et al., 1994) with a higher bound proportion in leaner individuals (range ~ 0.25 to 0.75). Binding proteins protect their ligand from degradation in blood and slow renal clearance. Thus, in rats, the half-lives of leptin and LBP-leptin are 3.4 and 71 min (Hill et al., 1998). ObRe antagonises leptin BBB transport in mice (Tu et al., 2008) but this transport differs between neonates and adults and it may be biologically significant that ObRa and ObRe are more strongly expressed in neonates (Pan et al., 2008).

The leptin receptor and intracellular signalling
Many membrane-embedded hormone receptors use kinases in their intra-cellular signalling actions. As a class I cytokine receptor, ObR has no intrinsic kinase but instead uses Janus kinase (JAK) to phosphorylate STAT3, one of the family of Signal Transducers and Activators of Transcription, and an essential molecular component of the leptin signalling pathway (Buettnner et al., 2006). The leptin intracellular signalling pathway intersects with those of other hormones (e.g. insulin) in muscle (Strat et al., 2005) and other tissues (e.g. liver), resulting in interactions between leptin and insulin (the universal anabolic hormone), which may determine how and why individual tissues differently metabolise glucose and fatty acids. Such interactions may have particular significance in ruminants in which fatty acid supply is always generous and in which monogastric-like hypoglycaemic: hyperglycaemic perturbations do not naturally occur.

Leptin receptor in livestock species
Dyer et al. (1997) screened hypothalamus, anterior pituitary and WAT in ovariectomised ewes and found ObR mRNA in each. In situ hybridisation localised it to ventromedial and arcuate nuclei in the hypothalamus, with more in feed-restricted ewes than in well-fed ewes. Cheikani et al. (2003) found it in bovine WAT, liver, abomasum, mammary parenchyma, small intestine and brain with ObRa in the liver and pituitary. In prepubertal heifers, ObRb accounted for 40% of all ObR in the hypothalamus but little (<3%) elsewhere, while ObRa accounted for nearly all ObR in peripheral tissues but only 19% of hypothalamic ObR (Thorn et al., 2007). Ren et al. (2002) found no difference in hypothalamic ObR expression in dairy-type Holstein or beef-type Charolais bulls fed a common diet to slaughter at 18 months but the Holsteins had higher circulating leptin and more omental and renal fat, consistent with their production-type.
Actually, ObRb, ObRa and ObRc are all strongly expressed in bovine intermuscular, renal and s.c. fat (Kawachi et al., 2007) suggesting that these tissues respond to leptin in addition to secreting it while ObRb was found also in lactating ewe mammary gland (Laud et al., 1999). Ogasawara et al. (2008) identified ObRb in the anterior pituitaries of Holstein bull calves, with a similar distribution of somatotrophs (60%) and gonadotrophs (16%) to that seen in mature ewes (Iqbal et al., 2000). Lin et al. (2000) found ObRb mRNA in the hypothalamus, cerebral cortex, pituitary and other tissues of 3-month old pigs suggesting leptin pleiotropy in pigs, as in other mammalian species. The existence of binding sites of different affinities for a hormone is common. Margetic et al. (2002b) used classical (Scatchard) analysis to characterise leptin binding to crude bovine kidney cell membrane preparations over a range of leptin concentrations from physiological (~1 ng/ml) to excessively supra-physiological (~150 ng/ml), and found two binding sites – one of high affinity and low abundance; the other of low affinity but almost ×100 greater abundance – suggesting that the effects of acute changes in leptin concentration (endogenously or exogenously) might be muted by the natural buffering of leptin, even at high doses. It is clear that the manner and extent of leptin binding to receptors and carrier proteins (in blood and at the tissues) determines the degree of intracellular effects. Accordingly, leptin pharmacokinetics are of significant interest to the determination of intracellular effects. Accordingly, leptin pharmacokinetics are of significant interest to the development and treatment of human obesity but also to manipulative applications in farm livestock (Strat et al., 2005).

Mammalian leptins influenced feed intake, reproduction, energy metabolism and immune function in birds only in some studies. Bungo et al. (1999) injected mouse leptin i.c.v. into hens and found no reduction in intake, whereas Dridi et al. (2005b) infused re-ch-lep for 6 h into 3-week old broilers and reduced feed intake and ch-ObR expression. Hen et al. (2008) transfected ch-ObR into human embryo kidney cells and developed a bioassay that could distinguish pre-partum from post-partum (PP) cows. Ironically, the same assay could not distinguish lean from fat birds. Proteins of 1148 and 1147-aa with ~50% homology to mammalian ObR have been shown in hens (Horev et al., 2000) and turkeys (Richards and Poch, 2003) and exon 20 of avian ObR is spliced, giving a short isoform as in mammals (Liu et al., 2007b). Ohkubo et al. (2007) used a monoclonal antibody to part of ch-ObR to map it and found the short isoform in only a few tissues but the long isoform expressed more widely (mostly brain). These avian receptors use JAK-STAT (Adachi et al., 2008) and are expressed in ovary, brain and pituitary (Paczoska-Eliasiewicz et al., 2003).

The biological significance of leptin in animals

Leptin’s primary role is presumed to be to inform the brain of the size of the body’s fat stores (Ahima et al., 1997) allowing assessment of EB in the short term and long term. The brain responds by causing changes in intake, energy use and thermogenesis (perhaps to stabilise EB) and adjusts the
gonadotrophic axis to allow or deny reproductive activity. Immune function may also be affected.

Leptin as an indicator of fat stores

Correlations initially found between leptin and body fat in man and mice (Frederich et al., 1995; Maffei et al., 1995) triggered interest in leptin as a fat marker in farm species and correlations with body condition and fat mass have been widely shown. When EI and EE are equal, leptin concentrations reflect the total amount of triglyceride (Barb et al., 2006). Early studies in cattle and sheep used a radioimmunoassay (Linco multi-species RIA) based on anti-recombinant human leptin but Delavaud et al. (2000), Ehrhardt et al. (2000) and Blache et al. (2000) raised antibodies to recombinant ovine or bovine leptins and developed ruminant-specific RIAs. Tokuda et al. (2003) reported a poor correlation (r = 0.41) between the non-specific and specific assays.

Cattle and sheep. Minton et al. (1998) first associated serum leptin levels (by Linco) with 12th rib backfat depth in beef cattle and Geary et al. (2003) confirmed similar correlations for 12th/13th rib fat depth (r = 0.34 to 0.46) and KPH fat (r = 0.42 to 0.46) in crossbred steers and heifers slaughtered at 14 to 17 months while McFadin et al. (2003) also found leptin correlated with backfat over the 12th rib (r = 0.35) but not with any lean measure (e.g. ribeye area) in steers. Kawakita et al. (2001) probed links to carcass fat in high eating-quality Japanese Black cattle: leptin increased with age and steers on a higher protein diet had more backfat. Leptin levels in these steers were expected to be higher at slaughter but only weakly correlated with fatness. Higashiyama et al. (2003) compared Holstein and Japanese Black steers of 700 kg and found similar masses of visceral and carcass fat in each, with more i.m. fat in the Japanese Blacks. Marbling and i.m. fat are closely related and leptin is a potential indicator of marbling in beef cattle. However, Yonekura et al. (2002) saw no difference in leptin in Japanese Blacks with different degrees of marbling and the association with i.m. fat was reversed in other studies using breeds of high eating quality. Bonnet et al. (2007) compared leptin mRNA in 23 to 28 months old Angus, Limousin and Japanese Black × Angus steers of very different i.m. and carcass fat levels. Limousins had 27% less rib fat thickness but there was no breed difference in leptin mRNA levels in the i.m. fat. Yamada et al. (2003) compared relationships between leptin and carcass quality in Japanese Black × Holstein steers at 11 months and 21 to 28 months. Using pooled data, leptin level correlated strongly with body fat (r = 0.802) but less strongly when data for young and older steers were treated separately. Similar effects were seen in sheep (Wylie, 2004) and cattle (Ehrhardt et al., 2000). Nkrumah et al. (2007) found leptin ~20% higher in Angus-sired steers than in Charolais-sired steers. Leptin levels were moderately heritable (h² = 0.34) and sire differences correlated with body composition but with backfat, 12th rib fat and marbling all lower, and Longissimus dorsi area and lean yield higher in 249
the Charolais. Leptin level was also more strongly correlated with body fatness than feed intake, and with $L.\ dorsi\ area$ ($r = -0.17$) and lean yield ($r = -0.38$) and differed (8.5 ng/ml v. 14.2 ng/ml) between standard and prime beef carcases in a study involving ~1000 steers and 750 heifers (Brandt et al., 2007). Despite greater marbling in heifers, the strongest correlation was between leptin and 12th rib fat depth ($r = 0.37$). Leptin’s associations with fat extend to bulls but while Holstein bulls deposit more fat than Charolais bulls, Bellmann et al. (2004) found useful correlations with leptin only in Charolais bulls (s.c. fat, $r = 0.76$; total fat, $r = 0.68$).

Leptin mRNA expression was twice as high in s.c., omental and perirenal fat of ram lambs from a fat sheep line compared with a lean line but Kumar et al. (1998) regarded this higher expression in fat rams a consequence of their fatness rather than a cause. Leptin levels correlated with backfat thickness in ewes and wethers (Blache et al., 2000) while in non-lactating, non-pregnant, ewes fed 90% or 39% of their maintenance energy requirements (Delavaud et al., 2000), fatness and body condition correlated similarly with plasma leptin ($r = 0.68$ and 0.72). Leptin and fat depot masses correlated ($r = 0.40$ to 0.56) in lambs at 35 and 45 kg (Altmann et al., 2005) with higher values (0.53 to 0.64) for pooled data and estimates of carcass fat by leptin and ultrasound had similar accuracy ($R^2 = 0.34$). Wylie (2004) fed Texel × Cheviot lambs at maintenance (M), 1.7 M or 2.4 M intake from 36 kg to slaughter 17 weeks later, when leptin levels correlated strongly with carcass fat (2.4, 4.8 and 5.3 kg, respectively; $r = 0.809$) and carcass fat gain ($r = 0.818$).

**Pigs and poultry.** Robert et al. (1998) noted higher leptin mRNA in fat pigs than lean pigs while Ramsay et al. (1998) found greater (3 ×) serum leptin in young ‘USDA high backfat’ pigs (an obese breeding line established for research) than in normal crossbred pigs of similar weight (12 to 20 kg), suggesting leptin resistance in the higher fat line. In a performance test of 310 barrows and gilts of popular breeds (Berg et al., 2007), initial leptin levels were similar but, 24-h pre-slaughter at 111 kg, ranged from ~7 ng/ml in Berkshires to 3.49 and 3.96 ng/ml in Durocs and Yorkshires. Levels were twice as high in barrows as in gilts (6.55 v. 3.35 ng/ml). Pre-slaughter leptin 3.96 ng/ml in Durocs and Yorkshires. Levels were twice as high with $L.\ dorsi\ area$ ($r = 0.37$). Leptin’s associations with fat extend to bulls but while Holstein bulls deposit more fat than Charolais bulls, Bellmann et al. (2004) found useful correlations with leptin only in Charolais bulls (s.c. fat, $r = 0.76$; total fat, $r = 0.68$).

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In lambs fed at 0.7, 1 or 2.4 M from 36 kg, serum leptin levels at slaughter (0.8, 2.44 and 2.28 ng/ml, respectively) correlated well with omental fat ($r = 0.632$) and renal fat ($r = 0.561$) but only poorly with mesenteric fat (Wylie, 2004). Similarly, Altmann et al. (2006) monitored leptin in ram lambs at 5 kg intervals between 20 kg and slaughter (40 kg) and found dissectible fat correlated with leptin level at each weight, but omental fat was the most strongly and consistently correlated ($r = 0.60$). This may not be just a ruminant phenomenon as Shan et al. (2008) monitored pigs at 1, 20, 40, 60 and 90 kg and found leptin mRNA in omental fat increased by 98% between 1 and 60 kg and correlated more strongly with blood leptin and body fat ($r = 0.98$ and 0.81) than with s.c. fat. Leptin and appetite. Leptin is an anorexigenic agent shown to regulate food intake (Houseknecht and Portocarrero, 1998). Its effects on intake are centrally mediated and rapid (Henry et al. 1999)
injected leptin i.c.v. into ovariectomised ewes and reduced food intake significantly within 24 h). However, leptin’s central actions are rate-limited by BBB transport (Caro et al., 1996) and leptin is just one of several agents affecting fat level. Links between leptin and fatness are influenced by nutrition but, conversely, the impact of nutritional change on leptin is affected by the degree of fatness and also by age (Ishii et al., 2000).

Undomesticated animals mostly do not over-consume food. Food scarcity undoubtedly is a greater threat and the main purpose of leptin may be to ensure adequate fat storage for survival when food is unavailable for whatever reason (e.g. food shortage, hibernation or migration). Cold-water mammals accumulate very large amounts of fat as thermal insulation, an energy source and a substrate for milk production potential but feed shortages occasionally are a concern, even amongst long-lived breeding stock, but poor feed management can allow excessive fat accumulation and providing thermal insulation. Importantly, fat depots and WAT, protecting bones, muscles and organs against injury and providing thermal insulation, an energy source and a substrate for milk synthesis but Ortiz et al. (2003) saw no change in leptin concentration or repletion in adults. Nonetheless, it is clear that fat stores and it would seem important, or desirable, that they ‘know’ the size of their fat reserves as depletion of these reserves eventually leads to loss of body (muscle) protein.

Leptin may be the marker but there is no evidence, yet, to link leptin levels to migratory success in birds (Kochan et al., 2006). Leptin may also induce the hypothermia that develops in Barnacle geese days before migration, reducing energy use and conserving it as fat (Butler and Woakes, 2001). Hibernation also impacts energy budgets, as epitomised by 20% lower energy use in Siberian hamsters during periods of winter torpor of up to 8 h/day (Ruf and Heldmaier, 1992), with hamsters that enter torpor nightly having the lowest leptin level. Leptin infusion prevented torpor in hamsters that most strongly exhibited it (Freeman et al., 2004) and, so, low leptin may be linked to the associated suspension of reproductive activity.

Farm livestock other than breeding stock live short and closely managed lives to slaughter, or longer but equally closely managed lives producing milk or eggs. Obesity is rarely a concern, even amongst long-lived breeding stock, but poor feed management can allow excessive fat accumulation. Livestock are generally fed adequately to sustain their production potential but feed shortages occasionally occur. Amstalden et al. (2000) noted a fall in leptin mRNA and plasma leptin in feed-restricted cattle and sheep although Wylie et al. (2002) found no change in plasma leptin when wethers previously held at maintenance intake for more than 20 days were then acutely fasted. Moreover, levels remained unchanged during a 14-day staged return to M intake and increased significantly only after 7 days at 2 M.

Thomas et al. (2001) noted that leptin levels increased in adult rams within 5 days after increasing their feed allowance. Delavaud et al. (2002) fed fat Charolais cows, fat Holstein cows and lean Holsteins at 1.3 or 0.6 M for 3 weeks and found initial leptin 70% lower in lean Holsteins than in fat Holsteins. Restriction depressed leptin by ~ 25% but leptin levels fell post-prandially in well-fed cows and increased in underfed cows. The PP period in dairy cows is an exceptional time in which energy demand for milk synthesis can vastly outstrip supply resulting in a severely negative energy balance (NEB) and fat mobilisation. The accompanying fall in circulating leptin levels may be important to allowing feed intake to progressively increase (Kadokawa and Martin, 2006).

In neonatal lambs fed to gain at 148 or 337 g/day, leptin increased within 6 days in well-fed lambs and then continued to rise but were unchanged in slower growing lambs despite increasing fatness (Ehrhardt et al., 2003). Variation in leptin in sheep was considered by Delavaud et al. (2000) to be more attributable to body fatness (35%) than to nutritional status (15%) while intake, liveweight and backfat together accounted for 40% (18% + 17% + 5%) of the variation in leptin in dairy heifers (Chelikani et al., 2009). Growth rate variations between pig breeds, and within and between litters, are due mostly to variation in feed intake and leptin’s association with intake regulation may find practical use in improving pig production. Giving leptin i.v. to pigs reduced feed intake (Ajuwon et al., 2003) as anticipated, but leptin levels in pigs are more strongly correlated with fatness than intake (Cameron et al., 2000). Liver leptin expression and plasma leptin were higher in broilers selected for high abdominal fat (Dridi et al., 2005a) but infusion of re-ch.lep into 3-month old birds halved intake. Kuo et al. (2005) infused re-h.lep into hens selected, long-term, for high or low 8-week weight, but reduced intake only in the lighter birds, suggesting a change in their leptin responsiveness and a potential target for genetic improvement. Reports of leptin’s associations with food intake in fish are mixed. Johnson et al. (2000) found 3 × higher leptin in fed than fasted fish but a 6-week fast did not affect expression in carp (Huising et al., 2006) while injection of leptin reduced food intake by goldfish (De Pedro et al., 2006). Clearly, a role for leptin in regulating appetite and energy use in fish could have important implications for profitability and for reducing the environmental impact of aquaculture.

**Leptin and meat quality characteristics**

In the context of this review, the most significant fats are the otherwise mundane triglycerides that constitute most of the i.m. and marbling fat in meat, contributing to cooking quality and eating quality (Hausman et al., 2009). Minton et al. (1998) associated leptin with marbling score in beef cattle and showed a positive relationship with L. dorsi area (r = 0.32), predating reported correlations between L. dorsi area and marbling score and s.c. fat in cattle (Bertrand et al., 2001). Geary et al. (2003) found leptin 1 to 3 days before slaughter similarly correlated with marbling in 14 to 17 months crossbred steers and heifers but only weakly or negatively related to L. dorsi area.
area ($r = 0.12$ in Angus × Charolais; $r = -0.45$, others) while Brandt et al. (2007) found leptin only weakly related to marbling score ($r = 0.28$) in beef heifers and steers. In steers with 0%, 50% and 75% Wagyu genetics, leptin levels were unrelated to s.c. fat depth in all groups but correlated positively with percentage of fat in pars costalis and *Musculus longissimus* in zero-Wagyu steers both at 16 weeks pre-slaughter ($r = 0.69$; 0.59) and at 4 weeks pre-slaughter ($r = 0.52$; 0.51). The correlations disappeared in 50% Wagyu steers and became strongly negative ($r = -0.65$; −0.60) in 75% Wagyu steers. Daix et al. (2008) compared carcase characteristics, blood leptin level and leptin expression in s.c. fat of Belgian Blue, Angus and Limousin bulls. Fat content was 10.2% in Belgian Blue and 23.6% in Angus with Limousin intermediate. The more highly muscled Belgian Blues had the lowest leptin expression but plasma leptin level did not differ from the other breeds until adjusted to common carcase fat, when it became significantly higher. Low marbling in Belgian Blues is in line with the inverse relationship of leptin and marbling seen in cattle with high Wagyu genetics (Wegner et al., 2001). Diets with low vitamin A content also are associated with higher marbling and better carcase grading in cattle (Oka et al., 1998). Intriguingly, plasma vitamin A and leptin levels were inversely related in Wagyu (Yang et al., 2008) yet Tokuda et al. (2001) supplemented Japanese Black steers with vitamin A from birth to 20 months with no effect on circulating leptin concentrations. Gorocica-Buenfilet et al. (2007) restricted vitamin A intake by Holstein steers for 112 days but did not affect intake, gain, carcase weight or backfat depth. However, *i.m.* fat percentage in *L. dorsi* increased by 33% after 243 days of restriction, and *i.m.* fat cell number (but not size) also increased, but effects on marbling were inconsistent (Gorocica-Buenfil et al., 2008).

**Leptin and reproduction**

Sexual activity leading to reproduction begins only after puberty in mammals, birds and reptiles. Puberty is hormonally mediated and occurs only when body energy stores are adequate. Leptin provides a putative mechanism by which the brain is made aware that sufficient energy has been retained.

**Leptin and puberty**

Exogenous leptin restored gonadotropin secretion in aleptinaemic *ob* mice (Chehab et al., 1996), reversing the sterility of both females and males. It also caused vaginal opening and it advanced oestrus in normal, prepubertal females (Ahima et al., 1997; Chehab et al., 1997). Humans with the exceptionally rare condition of congenital aleptinaemia remain prepubertal into adulthood but Farooqui et al. (1999) treated a congenitally aleptinaemic 9-year old female with no overt sexual development with leptin and raised gonadotropin levels, allowing puberty to proceed.

Brody (1945) linked puberty in cows to an inflection of the growth curve coincident with the start of sustained fat gain. Ewes also must attain a fat threshold for puberty to proceed (Pittroff et al., 2008). Changes in leptin level and leptin mRNA expression were associated with puberty onset in cattle (Garcia et al., 2002) and pigs (Barb and Kraeling, 2004). Williams et al. (2002) noted leptin increased in heifers going through puberty but Chelikani et al. (2009) saw no distinct peripubertal leptin rise in heifers fed to gain at 0.5, 0.8 or 1.1 kg/day. Block et al. (2003) compared heifers entering puberty early or late (286 days v. 414 days) and found early heifers lighter, leaner and lower in leptin (2.6 ng/ml v. 4.3 ng/ml) suggesting it is not a pre-requisite for puberty but Zieba et al. (2005) and Zhang et al. (2005) regarded it as at least permissive to puberty in heifers and sheep. Cunningham et al. (1999) and some others have described leptin as a ‘metabolic gate’ to initiating reproductive activity in the hypothalamus, the centre of appetite regulation and location of most mammalian leptin receptors.

Paczoska-Elasierwicz et al. (2006) injected 11-week pre-pubertal pullets daily for several weeks with four doses of re-ch.lep. The highest dose advanced first lay (116.3 days v. 121.3 days). In another trial, the highest dose moved puberty from 124.4 to 118.4 days in fed birds and from 131.5 to 125.7 days in restricted birds and promoted follicle development in fully fed birds. Overall, the data suggests leptin may advance puberty in some farm species while only allowing it to proceed in others when energy reserves (indicated by leptin) are adequate. This is an important distinction.

**Leptin in pregnancy**

ObR mRNA in immature and mature bovine oocytes and embryos suggested a role for leptin in implantation and embryo development (Madeja et al., 2009b) as well as in ovary development and steroidogenesis (Henson and Castracane, 2006). Maternal leptin drives conceptus growth and placental angiogenesis but placental leptin may also be involved. Ruminant placenta was thought to produce little leptin until Ehhrhardt et al. (2001) fed monotocous ewes to allow growth of the gravid uterus but avoid non-gravid growth, and found leptin levels higher than in non-pregnant ewes similarly fed to avoid gain, suggesting the placenta does indeed augment maternal leptin supply.

Leptin given to rabbit does for 1-week before inducing ovulation did not affect offspring number or weight but did increase the proportion of pregnant females and numbers of live offspring (Sirotkin et al., 2009). Malik et al. (2001) injected leptin into female *ob* mice to restore levels to those of sexually mature ‘wild-type’ mice, and then mated leptin-treated *ob* females with either wild-type males or with *ob* males treated with leptin. Pregnant *ob* females carried to term only if leptin injections were maintained to at least day 7 *post coitus* (pc) but, in a similar study (Mounzih et al., 1998), leptin-treated *ob* females carried to term even if treatment was discontinued on day 1 *pc* suggesting that leptin is not essential for implantation or for any stage of gestation. However, the latter study used human leptin at 10 times the dose of Malik et al. (2001). Such differences matter when interpreting leptin’s effects.
Leptin and the hypothalamo–pituitary axis
Nutrition underpins animal reproduction and if leptin is a metabolic gate within the neuroendocrine axes, then low leptin in underfed animals and those in severe NEB (e.g. high-yielding early PP dairy cows) keeps the gate shut, ensuring that an apparently unsupportable pregnancy is prevented. Pubertal onset in cattle is accompanied by higher secretion of gonadotropin releasing hormone (GnRH) that increases the essential LH pulses (Zieba et al., 2005). Fasting suppresses FSH levels and pulsatile LH secretion with a temporary loss of fertility. The effects are linked to a fall in GnRH and are reversed by exogenous GnRH (Kile et al., 1991). Re-feeding also restores normal LH secretion and relieves infertility induced by fasting (Cameron and Nosbich, 1991). Furthermore, exogenous leptin accelerated follicular growth in ob mice treated with a GnRH antagonist to prevent GnRH release (Barkan et al., 2005) suggesting that leptin can induce ovulation independently of LH. Yu et al. (1997) showed it to be almost as effective as the natural secretagogue in stimulating GnRH release from arcuate nuclei, even at low dose. Leptin fell sharply in heifers during a 2-day fast near puberty, alongside lower LH pulse frequency (Amstalden et al., 2000) while pulse frequency was not affected in mature cows in good condition and leptin levels correlated with pulse frequency ($r = 0.731$) and amplitude ($r = 0.528$) in PP Holsteins (Kadokawa et al., 2006), when EB was strongly negative ($-100$ MJ/day).

When administered i.c.v., leptin induced the hypersecretion of LH in fasted cows (Williams et al., 2002) but not in well-fed cows while i.c.v. infusion into ewes restricted for 6 months (23 kg below controls) restored LH (Henry et al., 2001). Yet Maciel et al. (2004) infused ovine leptin into prepubertal heifers for 40 days and raised plasma leptin by 7 times but did not affect LH pulses, or mean LH, or induce puberty. Morrison et al. (2001) found lower LH pulse frequency in restricted lambs than fed lambs and this did not change with i.c.v. leptin infusion. However, Wójcik-Gładysz et al. (2009) infused leptin i.c.v. into acutely undernourished prepubertal lambs and increased LH pulsatility. In pigs, i.c.v. leptin injection (10 to 100 µg) into prepubertal gilts did not affect serum LH (Barb et al., 2005) but pig pituitary cells treated with GnRH and >1 nM leptin (alone or combined) had increased LH secretion suggesting that leptin affects both hypothalamic GnRH release and pituitary LH secretion directly.

Leptin and mammalian fertility
Fertility can be defined so as to reflect the success of parts of the reproductive cycle or to give a holistic view from the development of spermatozoa and oocytes, through ovulation and fertilisation, to established pregnancy and, assuming embryo survival, to parturition. Nutrition supports these processes, directly or indirectly, and fertility is affected by extremes of EB. Obese women often are amenorrhoeic with associated reproductive failure (Gosman et al., 2006) but women with only minimal body fat levels may be similarly affected (De Souza and Metzger, 1991). Leptin is implicated in both scenarios. Obesity may also adversely affect male fertility and while it is not clear if this involves leptin, Rago et al. (2009) recently reported leptin and its receptor in the testis and epididymis of boars.

By all measures (days to first heat, days to first service, services/conception and calving interval) dairy cow fertility has fallen for over 30 years in the United States and United Kingdom, threatening the sustainability and economic viability of herds (Esslemont et al., 2001). EB is invariably negative in high genetic merit (HGM) dairy cows early PP and sometimes strongly so, well into lactation. Doepel et al. (2002) and Ferris et al. (2002) identified an EB nadir within the first 2-week PP in HGM cows but a state of NEB persisted for several months. Cows with more severe NEB, and leaner primiparous cows, show longer delays to first ovulation (Butler, 2005) and follicle growth, oocyte quality and embryo survival are all vulnerable to NEB. Low blood leptin, insulin and IGF-1 may be responsible for the adverse effects (Diskin, 2008). Reist et al. (2002) found lepton only weakly associated with EB ($r = -0.027$) up to week 10 PP in HGM Holstein cows whereas Wylie et al. (2008) found it correlated strongly with mean EB ($r = -0.632$) up to 3 months of lactation in primiparous HGM cows. Liefers et al. (2003) saw no relationship between leptin and first PP luteal activity in HGM cows, but acknowledged a possible association between leptin and oestrus expression.

Mann et al. (2005) examined links between leptin and reproductive problems in dairy cows (delayed luteal activity, DLA; cessation of luteal activity, CLA; and prolonged luteal activity, PLA). Problem cows (as identified by milk progesterone) accounted for ~40% of all cows (DLA, 10.8%; CLA, 18.9%; PLA, 9.5%). Cows with cycle abnormalities had a longer interval to first AI and fewer had conceived by 100-day PP (34.5% v. 66.7% for normal cows). In early lactation, DLA was associated with greater milk yield (MY) and greater loss of weight and condition (implicating NEB in the delayed return of cyclicity) but leptin levels were unrelated to the onset of luteal activity. Between week 6 and week 14 PP, the incidence of abnormal cycles was associated with higher MY, lower leptin and a lower rate of increase in weight and body condition. Königsson et al. (2008) monitored leptin weekly from 1-week pre partum to 7 weeks PP in primiparous Swedish Red and White cows and found no relationship between leptin in cows that did, and those that did not, resume oestrous activity by the end of week 7 PP. However, this study involved only 12 cows.

Body condition and milk yield are related in sows, as in cows (Butler, 2005), and leptin correlates with backfat thickness ($r = 0.342$) in sows at farrowing. Cyclic activity resumed earlier in sows with higher lepton on day 5 PP (Summer et al., 2009) so that lepton may link metabolic status and fertility in sows also. Xu et al. (2009) fed Landrace × Yorkshire gilts at 0.6, 1.2 or 2 M after mating and found more viable embryos and the highest 25-day embryo survival rate (80%) in gilts fed at 1.2 M. Plasma lepton levels reflected the feed intake. Lepton given to rabbit does for 1-week before inducing ovulation did not affect offspring number or weight but did increase the proportion of pregnant females and numbers of live offspring (Sirotnik et al., 2009).
Leptin and mammary development and function

Mammary growth, development and function rely on the coordinated actions of growth factors, transcription factors and hormones (e.g., mammary fat cell leptin expression requires prolactin which then cooperates with leptin to influence mammary activity; Feuermann et al., 2006), all underpinned by nutrition. Mammary glands consist of parenchymal epithelial cells within a matrix of connective tissue and fat (the stroma). A high plane of nutrition increased stromal mass in prepubertal heifers but inhibited par enchymal growth leading Silva et al. (2002) to speculate that non-mammary leptin might mediate the impaired mammary development in heifers fed to grow at >1 kg/day. In an in vitro study, they found that larger leptin doses were needed when the media was supplemented with IGF-1. Silva et al. (2008) infused recombinant ovine leptin and IGF-1 for 7 days into separate quarters of prepubertal heifers fed to gain at 700 g/day, but retaining normal mammary development. The IGF-1 dose (10 μg/quarter per day) had previously raised epithelial cell proliferation by ~50% (Silva et al., 2005) while the leptin dose (100 μg/qtr per day) ensured supra-physiological leptin levels in the parenchyma (higher by ~25 × than with placebo). Epithelial cell proliferation was reduced by ~20% from saline-infused quarters but by ~50% from IGF-1-infused quarters that had shown ~40% more proliferation than controls. While plasma leptin and mammary leptin mRNA are greater (2 × and 3 ×) in well-fed heifers, leptin does not activate STAT-3 in mammary epithelial cells (Thorn et al., 2006), consistent with the absence of full-length leptin receptor in the parenchyma. Similarly, restricting late pregnant ewes lowered maternal leptin without altering mammary expression of leptin or IGF-1 (Norgaard et al., 2008). Pre-pubertal heifers fed high-energy diets had high plasma leptin and low parenchymal DNA but no negative impact on epithelial cell proliferation or total parenchymal mass (Meyer et al., 2006), so that leptin may not directly mediate effects of overnutrition on gland development, leaving effects on nutrient supply as a possibility. Glucose uptake into mammary epithelial cells needs GLUT1 glucose transporter, expression of which is high in early lactation, lower in late lactation (Komatsu et al., 2005) and dormant in the dry gland but leptin does not alter mammary GLUT1 expression (Zhao and Keating, 2007) or mammary glucose use (Accorsi et al., 2005).

Leptin and inflammation and the immune response

Immune responses require energy, and susceptibility to infection is highest when nutrition and energy reserves are low (Matarese, 2000) alongside low leptin levels. Aleptineamic ob mice suffer thymic atrophy and acute starvation lowers leptin and induces thymic atrophy in normal mice. Exogenous leptin reverses these effects (Howard et al., 1999), and the general immune suppression of starvation (Lord et al., 1998). The central nervous system and immune system react to bacterial and pathogen infection (Borghetti et al., 2009), which are often accompanied by fever and hypophagia (Ingvarsen and Boisclair, 2001). Effects on the immune system may reflect the proximity of fat and lymphoid cells.

Leptin response to parasite infection in farm animals

Ruminants suffer recurrent gut parasite infections that cause under-performance (Kulcsár et al., 2005) due to competition for nutrients between the immune system and growth (Colditz, 2002) and anorexia is a common feature of nematode infection. Well-nourished animals are better able to resist or eliminate infection, perhaps via leptin’s effects on T-cells and proinflammatory cytokines IL-6 and TNF-α (Lord et al., 1998). Leptin levels typically rise in infection (Faggioni et al., 2001) and may mediate the parasite-induced anorexia seen in sheep. Amarante et al. (2004) found faster-growing Suffolk lambs more susceptible than slower-growing breeds, while they also suffer more intense and longer anorexia (Sandberg et al., 2006). Liu et al. (2007a) saw a non-significant rise in leptin in restricted Merinos infected with T. colubriformis and Teladorsagia circumcincta. Zaralis et al. (2008) infected Scottish Blackface and Suffolk × Greyface lambs with T. circumcincta for 12 weeks and noted a 13% reduction in intake in Suffolk × Greyface but no loss of intake in Scottish Blackface (a breed relatively resistant to parasite infection; Coop and Holmes, 1996). Zaralis et al. (2008) also found higher leptin in infected lambs than in non-infected lambs restricted to similar intake (but not in those fed ad libitum). Greer et al. (2009) noted a 20% fall in intake but no change in leptin level in lambs infected by T. colubriformis, while calves infected with Ostertagia ostertagi (10 000 larvae/day) had lower leptin but with no change in intake or weight gain (Forbes et al., 2009). Differences in dietary energy and protein may explain some of the anomalies in these studies as protein counteracts some effects of gut parasites in sheep (Forbes et al., 2009) but Zaralis et al. (2009) showed that supplementary protein did not affect leptin levels or the degree of anorexia in ewes infected with T. circumcincta.

Depressed immune function in the periparturient cow makes it vulnerable to infection (Elssasser et al., 2006). The extent of the depression is important and the early PP fall in leptin might play a part (Ingvarsen and Boisclair, 2001). Loiselle et al. (2009) milked Holstein cows once or twice daily in week 1 PP and assessed leucocytic cytokine output: as expected, leptin levels fell after calving, but less so in cows milked only once daily. Higher INF-γ in unmilked cows was consistent with lower immunosuppression, but the study did not prove a causal link to leptin.

Leptin single nucleotide polymorphisms (SNPs)

Useful selection traits in farm animals include feed intake and residual feed intake (RFI), rate of gain, carcass composition (fat v. lean), marbling and meat eating-quality, egg number and weight (poultry) and fertility, MY and milk composition and suckling ability (in sows, ewes and cows). Susceptibility to lameness, dystocia and metabolic and
infectious disease is also important. Some traits (e.g. intake) are difficult to measure while carcase data, by definition, cannot be assessed until slaughter. Markers of traits reduce the need for costly phenotype assessment and could be especially useful, if detectable early in life (e.g. like genes). Genome scans have identified single genes for health-related traits and quantitative trait loci (QTL) for multiple-gene traits like fat deposition (Dekkers, 2004). SNPs in genes are responsible for ~90% of genetic variation in animals. There can be few or many SNPs in a gene with most in non-coding intron and promoter regions (Nkrumah et al., 2005). Two-thirds of all known SNPs are thymidine-cytosine (T-C) mutations. Some SNP (A59V) associated with weight at 210 days and mean performance in livestock (see review by Van der Lende et al., 2005). Applying genome scanning and SNPs in animal agriculture – to beef quality improvement in particular – was explained by Hocquette et al. (2007).

The significance of leptin gene SNPs in livestock

SNPs in exons and introns of bovine and porcine LEP (and the bovine promoter) associate with intake, liveweight (LW) gain, carcase quality and reproductive performance in pigs and beef cattle and with milk yield in cows (Van der Lende et al., 2005). Most associate also with blood leptin levels but the strength of these associations may be different under different physiological states (e.g. growth, gestation and lactation).

Beef cattle

Fitzsimmons et al. (1998) investigated a microsatellite (BM1500) ~3.6 kb downstream of the LEP stop codon in purebred beef bulls (Angus, Charolais, Hereford and Simmental) and showed links between polymorphisms in BM1500 and carcase fat. Buchanan et al. (2002 and 2003) found greater carcase fat and leptin mRNA expression in cattle T-homozygous for C305T (LEP exon 2) that encodes an Arg-Cys change at aa-4 in mature leptin. This suggested higher feed intake by TT genotypes. In Angus, Charolais and Hereford, TT genotypes had more backfat at slaughter, attained slaughter quality at lighter weight and 16 days earlier than CC genotypes and blood leptin was correlated (r = 0.45) with backfat depth (Buchanan et al., 2007). Kononoff et al. (2005) reported comparable data for >1500 crossbred heifers and steers with ~7% more TT than CC or CT genotypes having carcases with high marbling (AAA grade or higher) and 12% to 15% fewer TT than CC or CT genotypes with maximum leanness.

Other LEP SNP in beef cattle include A1457G and C963T (Liefers et al., 2005), C207T and C528T (Nkrumah et al., 2005) and A252T (Lagonigro et al., 2003) while LEP exon-3 SNP (A59V) associated with weight at 210 days and mean gain to 210 days in Limousins, suggesting its usefulness in breeding for heavier Limousin carcases (Kulig and Kmieć, 2009). Schenkel et al. (2005) explored links between four SNP (UASMS1 (C207T), UASMS2 (C528T), E2JW (A252T) and E2FB (C305T)) and fat and lean yield and meat quality characteristics (in fat; L. dorsi and M. semitendinosus tenderness) in >1100 crossbred bulls, heifers and steers (Angus, Simmental, Charolais and Limousin). E2JW and E2FB associated with ‘fat and lean yield’ and interacted in effects on L. dorsi tenderness while UASMS1 promoter polymorphism associated only with fat yield. Haplotypes may be superior predictors of carcase characteristics in cattle (Stone et al., 2005) and TCAC, CCAT and TTAC similarly affected all traits while CCTT associated mostly with ‘fat and lean yield’ and TTTT with L. dorsi tenderness (Schenkel et al., 2005).

Gill et al. (2009) studied eight SNP (three in LEP, UASMS1, UASMS2 and exon 2FB) and their association with meat quality in Aberdeen Angus cattle. These SNPs are already incorporated to varying degrees in commercial genotyping tests (Igenity© and Genestar©) for tenderness, fatness and carcase composition in beef cattle and milk yield and quality in dairy cows. In the study, meat from cattle TT-homozygous for UASMS2 gained higher taste panel scores and this test is one element of the Igenity OptiGRID© test. Exonic LEP SNPs also associated with carcase parameters and meat quality in Korean Hanwoo assessed either visually (Shin and Chung, 2007) or by ultrasound (Kong et al., 2006). In the former study, SNP C1180T associated with backfat and marbling score, with CC genotypes having greater fat depth than TT and higher marbling score than CT or TT. Marbling has reduced in many cattle breeds in recent decades (Dunshea et al., 2005). Current breeding policy seeks to increase marbling without increasing s.c. fat. However, Pannier et al. (2009) investigated four LEP SNP in nine purebred cattle types and found no significant association between any of them (or 16 possible haplotypes) and i.m. fat. QTL for marbling and s.c. fat in beef cattle reside on different genes and may allow selection for higher marbling and lower s.c. fat. Importantly, also, QTL linked to marbling show breed specificity (Jiang et al., 2007) being on chromosomes BTA2, BTA3, BTA16, BTA17, BTA23, BTA27, and BTA29 in US beef breeds but on BTA4, BTA6, BTA7 and BTA13 in Japanese Wagyu and Korean Hanwoo.

Dairy cows

Buchanan et al. (2003) found LEP C305T in six dairy breeds. Cows with one or more T alleles had higher MY with TT cows giving 1.5 kg/day more milk than CC cows across lactation (2.44 kg/day to day 100). A similar effect in beef cows should favour heavier or earlier weaned calves (De Vuyst et al., 2008). Buchanan et al. (2003) also noted higher milk protein yield in TT than CC cows over all of lactation. Liefers et al. (2002a) genotyped >600 Holstein heifers for two restriction fragment length polymorphisms (RFLP) and the BM1500 microsatellite and associated them with intake, EB, LW and MY to 15-week PP, and with time to commencement of luteal activity. RFLP1-AB heifers gave 1.32 kg/day more milk and ate 0.73 kg/day
over RFLP1-AA heifers but time to commencement of luteal activity was unaffected. Selection for RFLP1-AB might allow higher MY without lowering fertility. Banos et al. (2008) associated LEPR and LEPR SNP in Holsteins with intake, MY, feed efficiency, LW and body condition and identified a haplotype (C207T, C528T, A1457G, C963T, A252T and C305T) linked to a MY bias of 3.13 kg/day and a feed intake increment of 4.64 kg/day. Importantly, cows with the haplotype tended to retain more body energy in early lactation, suggesting greater likelihood of superior fertility due to lower loss of fat and condition. They concluded that dairy traits are affected in small ways by many genes. Clearly, genotyping for lactational performance need not be confined to females. Madeja et al. (2009a) explored the LEPR HphI RFLP in Polish Black and White bulls and found TT bulls had almost twice the estimated breeding value for MY and milk fat and protein.

Five other LEPR SNPs were listed by Van der Lende et al. (2005) and associations shown for Tyr7Phe, LEPSau3AI and Arg25Cys with intake, MY, carcase fat and reproductive success. Liefers et al. (2002b) showed traits that were linked with serum leptin level in late gestation but not during lactation, whereas Liefers (2004) screened 14 of 18 LEPR promoter SNPs for links with production and reproduction and found G(-282)T associated with serum leptin in late pregnancy and early lactation while C(-211)T associated with leptin levels only in early lactation. Three others associated with production or reproduction: C(-963)T with intake, EB and calving to 1st oestrus interval; A(-1457)G with days to commencement of luteal activity and early-PP weight loss and C(-578)G with percent milk protein.

Sheep

Boucher et al. (2006) reported three LEPR SNPs in Dorset and Suffolk lambs. A103G associated with thickness and shear force of M. longissimus in Suffolks and metabolic activity in M. longissimus and s.c. fat in Dorsets. Zhou et al. (2009) found three SNPs in exon 3 that result in aa changes and could affect leptin structure and function.

Pigs and poultry

Jiang and Gibson (1999) identified four SNP (A2845T, T3469C, G2728A and T3996C) in porcine LEPR. All were poorly associated with backfat thickness. These same SNP were absent or of low frequency in Durocs, Landrace and Yorkshire breeds (Kennes et al., 2001) while A2845T and T3469C associated with intake and growth rate in Landrace only. In pig LEPR, C867T, A2845T and T3469C associated with production and reproduction traits (Van der Lende et al., 2005) but Amills et al. (2008) genotyped Landrace for exon-3 C3469T and found no associations between TT or TC and plasma leptin level, growth rate or carcass fatness. Associations of C3469T with i.m. fat and body composition of Durocs (Villalba et al., 2009), varied with age (160 to 225 days) and may explain the variable data for this SNP. Promoter region SNP do not associate with leptin expression or fatness in Durocs (Stachowiak et al., 2007) but Peixoto et al. (2006) found some carcass characteristics associated with T2411C and T3266G, making them potential markers of pig carcase composition. Out of 56 947 SNP genotyped in pigs, 500 were shown to be associated with fat deposition, for example, carcass fat, abdominal fat, backfat thickness, i.m. fat and intermuscular fat (Rothschild et al., 2007). Marbling and i.m. fat are correlated (r = 0.57) but are linked to different QTL (Huff-Lonergan et al., 2002) with marbling QTL on SSCc1, 8, 10, 12, 14 and 17 and those for i.m. fat on SSC4 and SSC7 and the X chromosome. Only SSC6 has QTL for both the features.

Despite the sequencing of the entire chicken genome (International Chicken Genome Sequencing Consortium, 2004) and the identification of ~ 2000 SNP (International Chicken Polymorphism Map Consortium, 2004) there are few reports, yet, of polymorphisms in ch-LEP.

Leptin receptor gene SNPs

The 20-exon leptin receptor (LEPR) gene is another candidate gene for production traits in livestock. A missense mutation at position 115 of bovine LEPR exon 20 causes a threonine to methionine change in the leptin receptor in Holstein-Friesian cows (Huff-Lonergan et al., 2004) giving allele frequencies of 0.93 (C) and 0.07 (T) with no TT. Blood leptin levels were influenced by this LEPR SNP in late pregnancy but not in lactation with CC cows having higher levels than CT. It associated also with milk fat and protein percentage (but not with yields of milk, milk fat or milk protein) in Jerseys (Komisarek and Dorynek, 2006). Almeida et al. (2008) investigated it in Angus, Brangus and Charolais cows and found no associations with reproductive characteristics. Schenkel et al. (2006) reported only one LEPR SNP associated with s.c. fat mass, fat yield and fat grade in beef cattle but Guo et al. (2008) genotyped LEPR exon 4 SNP in five Chinese breeds and found strong influences on height, weight, length and weight gain at 6 and 12 months.

Vincent et al. (1997) first reported a polymorphism in porcine LEPR, one of several genes linked to fat deposition in pigs (Ovilo et al., 2005). Amills et al. (2008) found no association between an RFLP in LEPR and growth rate or fatness in >300 Landrace pigs but Sun et al. (2009) associated SNP C155T in LEPR exon 2 with first litter size and percentage of live piglets in Large White sows. Birth weight, weaning weight and neonatal weight gain were unaffected. Muñoz et al. (2009b) associated an SSC6 QTL with growth and backfat thickness in Iberian × Meishan and Iberian × Landrace pigs. A QTL linked to live-weight also was found in each crossbreed while an exon 14 SNP also associated with backfat thickness in each. In chickens, one of two recently found SNP in exon 9 of ch-LEP has been linked to abdominal fat in broilers (Wang et al., 2006).

Genomic identity is established at conception and is a lifelong constant. Genomic breeding value provides a neonatal assessment of production potential (Hayes et al., 2009) so that bulls could be used as early as 2-years of age, saving hugely on progeny-testing costs (Schaeffer, 2006). Data volumes in such work are staggering with 38 259 out of a total of 56 947 SNP genotyped in ~ 800 Holstein-Friesian...
bulls in one Australian programme. In similar US, New Zealand and Dutch work about 38,000, 44,000 and 46,000 SNP were genotyped in ~3500, 4500 and 1500 progeny-tested bulls, respectively (Hayes et al., 2009).

**Leptin SNPs and immune responsiveness**

Associations between leptin SNP and immune responses might help predict susceptibility to disease. Asiama et al. (2009) used antibody response to rabies to explore links between an SNP associated with carcass quality in beef cattle and milk composition in cows. Genotypes did not differ in antibody response so that using this SNP to select for production should not dramatically impair immune function. Chebelet al. (2008) looked at associations of exon-2 R4C with common PP problems in Holstein cows (e.g. retained placenta, displaced abomasum and mastitis). Genotype had little effect on the incidence of any single problem except displaced abomasum for which TT cows had a higher incidence than CT cows (which had the lowest risk of suffering any of the conditions).

**Leptin and leptin receptor SNPs and reproduction**

Kuehn et al. (2009) investigated associations of a LEP SNP and two LEPR SNPs with age, weight, backfat and leptin level at puberty in three gilt lines. Age and leptin level were correlated \((r = -0.63)\) and one of the LEPR SNPs was associated with blood leptin levels, though not necessarily causatively.

**The future**

Obesity has been the principal focus of leptin research since its discovery and expectations remain high. However, obesity-associated conditions (Kelesidis et al., 2010) but there are a number of potential applications in livestock production. They include using endogenous leptin as a marker of body composition and metabolism and using exogenous leptin or its gene (and receptor gene) polymorphisms to influence productive and reproductive performance. Invasive applications require evidence of safe use and, thus, are some way off.

**Endogenous leptin as a marker of body composition**

Blood leptin level broadly reflects fat level in growing animals but the relationship is not robust (Aitmann and von Borell, 2007). Age and nutrition effects (especially fasting) make a one-size-fits-all approach untenable but it may still find use in predicting carcass fat within large groups of genetically similar animals near or at slaughter.

**Endogenous leptin as a marker of reproductive readiness in females**

In livestock farming, EB is probably of most practical significance in the peripartum dairy cow. Reproductive success (conception to weaning) is more assured in fatter animals (Friggens, 2003) so that the association of leptin with body condition and EB in ruminants (Chilliard et al., 2005) suggests leptin levels might mark the readiness of recently calved cows to resume breeding. Milk producers aim for a lactation of ~10 months and a dry period of ~6 or 8 weeks (during which a cow repletes fat reserves for the next lactation) giving an idealised calving interval of close to 1 year. Higher genetically driven MY has been accompanied by longer and deeper PP NEB and a lengthening of the calving interval, as either the depth or duration of the NEB or the number of days to the EB nadir affect the time to resumption of oestrus activity (Beam and Butler, 1999). Leptin is a potential marker of EB and the EB nadir: Block et al. (2001) milked cows 3 daily or left them unmilked up to week 8 PP and found that milked cows experienced severe NEB (−70.3 MJ/day average, 1 to 4 weeks) while unmilked cows remained in positive EB (50.6 MJ/day average) with mean plasma leptin of 2.9 and 5.6 ng/ml, respectively. However, Kadokawa et al. (2000) found ‘days from parturition to leptin nadir’ correlated strongly \((r = 0.83)\) with ‘days to first PP ovulation’ while Liefers et al. (2003) saw no clear leptin nadir in >300 primiparous Holsteins to 80-day PP. Such discrepancies remain unresolved, but using PP leptin levels to assess the readiness of dairy cows for renewed mating remains a credible prospect if milk leptin (assayed by an in-line biosensor) can be reliably related to blood concentrations.

**Endogenous leptin as a marker of meat eating quality**

Wagyu and Korean Hanwoo cattle produce meat of exceptional eating quality. Leptin concentrations are inversely related to i.m. fat in these breeds, and in crossbreeds in which they are dominant, and remain a potential marker of marbling. The fatty acids in beef fat triglycerides influence eating quality and oleic acid and PUFA contents are higher in breeds of higher eating quality (see review by Hausman et al. (2009)) but no association has yet been found between leptin and fatty acid composition in i.m. fat, or in any other body fat depot.

**Exogenous leptin to manipulate performance**

Leptin-induced fat loss, while retaining lean mass, has clear clinical applications in man (e.g. pre-surgical weight loss) but is not an option in farm animals on any grounds (welfare, practicality or cost). However, there are other ways in which leptin might be used to influence production quality and production efficiency:

**Exogenous leptin to advance the onset of puberty**

Studies in man, rodents and farm species all suggest leptin is permissive rather than causal to puberty onset with a threshold level necessary only to allow puberty to proceed.
Advancing the age at which puberty occurs should increase lifetime production and reproductive output but Maciel et al. (2004) increased leptin (7×) in prepubertal heifers without inducing puberty. However, Paczoska-Eliasiewicz et al. (2006) injected re-ch. leptin daily into 11-week pullets and advanced the age of first lay by day 5, showing potential for increasing lifetime productivity from layers.

Exogenous leptin to assure physiological readiness for breeding

Delays in recommencing reproductive activity in PP dairy cows is seen as a reaction to an event (pregnancy) that the cow has judged as unsustained in energy terms. Increasing feed availability early PP might be expected to alleviate the energy deficit, but HGM cows direct more of their EI to milk production (Yan et al., 2006) so that, paradoxically, NEB may even be exacerbated. A delayed return to cyclicity in dairy cows has been linked to low insulin levels but occurs during a period of low leptin also. Garnsworthy et al. (2009) improved oocyte quality and pregnancy rate in cows by feeding for higher insulin until cycling resumed and then changing the diet to promote lower insulin at, and after, insemination. They found no change in plasma leptin level, but exogenous leptin might allow hypothalamicgonad activity ahead of a substantive fall in NEB. The model is the restoration of reproductive axis activity in ob mice by leptin (Chehab et al., 1996) and the strategy is to re-establish blood leptin to a level that convinces integrative centres in the brain that fat stores are sufficient to support a pregnancy.

The possibilities for exogenous leptin use (above) are predicated on increasing leptin concentrations in blood but immunising against LBP might have the same result. Conversely, immunising against leptin might allow increased intake and production. Wuethrich et al. (1998) immunised against leptin might have the same result. Conversely, immunising against LBP might have the same result. Wuethrich et al. (1998) immunised against leptin using aa 1 to 20 of mouse leptin but, despite high titre antibody, they did not change feed intake whereas Shi et al. (2006) immunised hens and pullets against leptin and increased intake and abdominal fat by 66% in pullets and fat by ~ 50% in hens, while also reducing laying performance.

Exploiting leptin in farm species may require cheaper and more robust leptin analogs and novel delivery systems. Grasso et al. (1997) localised leptin’s effects on appetite to aa106 to aa140, but Nonaka et al. (2006) could not replicate LH induction in pig pituitary cells using aa116 to aa130. With regard to delivery, Chen et al. (1996) used recombinant adenovirus to transfer the leptin gene into rats, increasing leptin expression and causing fat loss and this may be a viable way to promote leptin expression in pigs also (Barb et al., 2001b).

Applying LEP or LEPR gene polymorphisms to improving performance

The use of DNA-based marker assisted selection has been slower to develop in animal agriculture than in crop agriculture due to the many gene loci involved in complex traits like milk production (Hayes et al., 2009) making extensive genotyping necessary. This has been made easier by the sequencing of the bovine genome (Bovine Genome Sequencing and Analysis Consortium, 2009) and chicken genome (International Chicken Genome Sequencing Consortium, 2004) and by high genotyping capacity and falling genotyping costs. The leptin gene is a candidate gene for fat deposition and meat quality. With marbling a major contributor to meat quality, the challenge is to increase i.m. fat while avoiding increases in s.c. fat and other fats. LEP gene SNPs (and other gene SNPs) may enable early detection of a genotype that favours i.m. fat deposition, allowing nutrition to be customised to produce more marbling (Hocquette et al., 2007 and 2010). Commercial SNP tests can already identify cattle with higher potential for leaniness or milk yield. And while associations of LEP SNP with milk yield and milk quality have proven value, the recent association of C305T with weaning weight and maternal longevity (Mitchell et al., 2009) might also enhance profitable calf rearing. Improving cow health while sustaining milk production is also desirable and any links between LEP SNP and disease susceptibility will be valuable. These qualities are related through interaction of a cow’s genotype for MY and its energy status, i.e. cows with adequate fat will more easily satisfy the energy requirement for genetically driven yield and suffer less severe NEB than those of similar genetic potential, but lesser fat, with a likelihood of earlier ovulation and a lower risk of metabolic or infectious disease.

Early bovine leptin SNP studies mostly targeted Bos taurus, but associations with production are now evident in Bos indicus breeds. Corva et al. (2009) investigated two leptin gene SNPs (one in exon 2; one in the promoter) in Brangus (5/8 Angus, 3/8 Brahman) steers and found associations with backfat thickness, rib-eye muscle area and carcass yield. Lower marbling in B. indicus is important, but Fortes et al. (2009) failed to find associations between leptin SNPs and backfat, rib-eye area or marbling score in pure-breds or B. indicus × B. taurus crossbreeds, and there was no association with shear force (tenderness) which is lower in B. indicus. The validity of applying B. taurus gene markers to B. indicus is not yet clear and new markers may need to be developed for B. indicus.

Feed intake, conversion efficiency and carcass quality are equally important in pig production. Associations of LEP and LEPR SNP with age and weight at puberty in gilts (Kuehn et al., 2009) suggests possible QTL to select for earlier puberty in pigs but many breeds are used worldwide with different strengths of association between LEP SNP and production and reproduction traits. D’Andrea et al. (2008) identified 18 LEP SNPs (and other gene SNPs) may enable early detection of a genotype that favours i.m. fat deposition, allowing nutrition to be customised to produce more marbling (Hocquette et al., 2007 and 2010). Commercial SNP tests can already identify cattle with higher potential for leaniness or milk yield. And while associations of LEP SNP with milk yield and milk quality have proven value, the recent association of C305T with weaning weight and maternal longevity (Mitchell et al., 2009) might also enhance profitable calf rearing. Improving cow health while sustaining milk production is also desirable and any links between LEP SNP and disease susceptibility will be valuable. These qualities are related through interaction of a cow’s genotype for MY and its energy status, i.e. cows with adequate fat will more easily satisfy the energy requirement for genetically driven yield and suffer less severe NEB than those of similar genetic potential, but lesser fat, with a likelihood of earlier ovulation and a lower risk of metabolic or infectious disease.

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Few associations have yet been made between LEPR SNP and production traits in Western dairy and beef cattle but recently established links with body measures in Chinese cattle may speed productivity increases in these demographically
important breeds. Associations of a LEPR exon-2 SNP (C155T) in Large Whites with first litter size and percentage of liveborn might help selection for reproductive output in pigs. The balance between hunger and satiety has changed in modern broilers (Richards, 2003) with selection for rapid growth accompanied by resistance to anorexigenic signals from the periphery (Cassy et al., 2004), giving hyperphagic birds that readily accumulate fat (Bokkers and Koene, 2003). Reducing fat without risking the established genetic gains in growth is challenging, but necessary, and associations of fat with recently found ch-LEPR SNP might help in broiler breeder selection.

Other considerations

Neonatal livestock performance

Leptin's involvement in immune responses makes it a potential candidate for monitoring and/or intervention to ensure satisfactory progress in neonates and peripartum females in which disease is a serious welfare concern. In pigs, decades of intensive genetic selection has brought larger litters but more very low birth weight pigs (<1 kg) and higher within-litter birth weight variation (Morise et al., 2008). Very low birth weight pigs (runts) account for 10% to 15% of littersmates with mortality statistics typically between 9% and 12% of all neonates (Ramsay et al., 2010), but as high as 50%. Leptin and its receptor mRNAs are detectable in s.c. fat in neonatal pigs (Chen and Heiman, 2000) suggesting that piglet survival and performance might be influenced by leptin and it may be significant that Litten et al. (2008) gave leptin i.v. to 6-day-old pigs and altered their ability to thermoregulate. Attig et al. (2008) showed that runt piglets have altered leptin receptor distribution in hypothalamic centres involved in metabolic regulation. Leptin supplementation partially corrected the runt phenotype (normalising food intake and post-natal growth and reducing the increased adiposity) and normalised the development of organs involved in metabolic regulation. Ramsay et al. (2010) reported differences between early neonatal runt piglets and control piglets in respect of leptin mRNA levels in s.c. adipose tissue on day 1 (runts < controls) and in perirenal adipose tissue on day 7 (runts > controls) but levels of many other adipokine mRNAs were also differentially affected. Lambs born to ewes restricted in early or mid-pregnancy developed higher leptin levels and males deposited more fat as adults (Muñoz et al., 2009a). Similar data in rats (Vickers et al., 2005) point to 'developmental programming' or 'foetal programming' with lifetime health and performance implications.

Welfare implications

The public often perceive thin dairy cows as undernourished and a welfare concern. Paradoxically, overfat cows can be a potential predictor for carcass composition and daily gain. Meat Science 74, 600–604.

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offer a tool to improve the management of periparturient cows, ewes and sows and reduce the incidence of metabolic and infectious PP diseases. The possibility that low early-PP leptin concentrations might increase disease susceptibility needs investigation and could lead to novel therapeutic or prophylactic applications for leptin.

Environmental impact (carbon footprint, etc.)

Energy efficiency is fundamental to profitable animal production and is central to all initiatives to lower animal contributions to climate change. Feed evaluation and EE have been research focuses for most of the past century (Johnson et al., 2003) while variation in energetic efficiency of individuals has received rather less attention, but may be a lucrative route to future improvements. RFI (the difference between the actual and expected feed intake for a given level of performance) is one useful measure of efficiency. Leptin levels were positively associated with RFI in beef steers (Nkrumah et al., 2007) but not in beef heifers (Kelly et al., 2010). Associations of leptin with RFI could be extremely valuable for genetic improvement of Bos indicus breeds and LEP SNPs in B. indicus breeds might enable selection for more meat and milk output from indigenous cattle in regions in which man’s consumption of animal protein is growing, but from a low base.

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