New strategies on targeting host response to inflammation by novel dietary means for the purpose of reducing growth promoting antibiotics

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Introduction Healthy animals fed antibiotics often have increased growth, feed efficiency, and reduced intestinal weight. Recent finding suggest that antibiotic-associated improved animal growth and feed efficiency is due to reduce gastrointestinal (GIT) microbial activation of immune/inflammatory processes. Reduced plasma cytokines and immune cell infiltration in the GIT mucosa of antibiotic fed animals support decreased immune/inflammatory activation. However, the direct regulators of growth and feed efficiency during an immune/inflammatory response are mediators released during immune activation. Immune mediators (e.g., pro-inflammatory cytokines, gut peptides, and select enzymes) can induce skeletal muscle degradation and decreased appetite that slows animal growth (see Cook, 2004; 2010). Inhibition of inflammatory mediators has now become standard therapy in inflammatory diseases such as arthritis. Over the last two decades we have investigated the regulation of select inflammatory mediators that are released in the GIT lumen as a strategy to improve animal growth and feed efficiency. The method used to inhibit these host mediators was oral egg antibodies produced against the mediator of interest (Cook and Trott, 2010).

Material and methods Single Combs White Leghorn laying hens were used to produce eggs containing desired antibody. Three host inflammatory mediators were selected and consisted of the gut peptides cholecystokinin-8 (CCK), neuropeptide Y (NPY), and secretory phospholipase A_2 ($sPLA_2$). Selection of the host targets were based on scientific literature of host products of inflammation secreted into the lumen of the gastrointestinal tract (GIT) and mediators known to have adverse effects on animal growth and feed efficiency (Cook, 2004; Cook 2010). Peptides (CCK and NPY) were synthesized according to published sequence, and conjugated (1mg/1mg) to bovine gamma globulin. Whole enzyme $sPLA_2$ was obtained from porcine pancreas (90% pure). Vaccination procedures are as previously described (Cook and Trott 2010). Antibody detection was by way of enzyme-linked immusorbent assay as described (Trott *et al*., 2008). Broilers were fed dried egg antibody yolk powder (0.25 to 1 g/Kg diet) for the first 3 weeks of life in battery brooders with raised wire floors, and growth rate and feed efficiency were assessed (n=4, 5, and 16 trials for Anti-CCK, Anti-NPY and anti-$sPLA_2$, respectively). Additional studies were conducted using anti-$sPLA_2$ to determine broiler or poult susceptibility to coccidosis or salmonellosis (Scanes *et al*., 2008). Summaries of data were published (Cook, 2004, and Cook 2010).

Results Broilers fed egg antibodies to CCK, NPY, or $sPLA_2$ (but not other peptides studied) had an average improvements in feed conversion of 8%, 5%, 4% , respectively. Feeding anti-CCK did not stimulate growth, but broilers fed anti-NPY or anti-$sPLA_2$ had a 9% and 5% improvement in body weight gain (see Cook *et al*., 1998; Cook, 2004). Broilers fed anti-$sPLA_2$ had no increase in disease in a coccidiosis model (unpublished), but decreased clinical signs of disease during salmonellosis (Scanes *et al*., 2008).

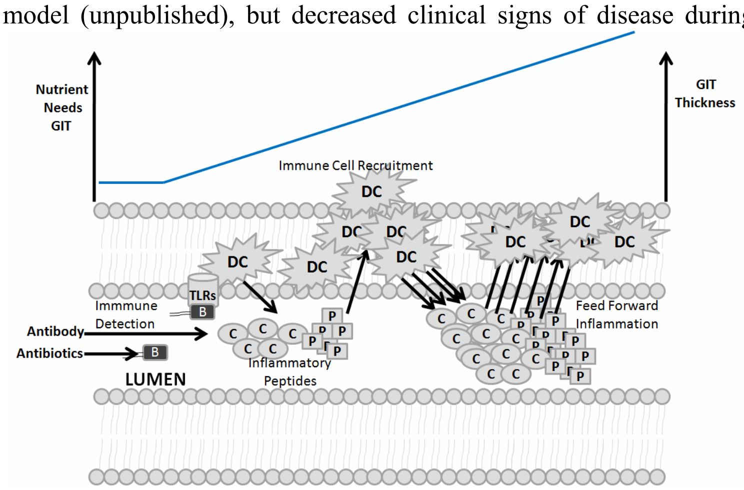

Figure 1 Inflammatory response creates a feed forward loop that increases GIT thickness. When the immune system detects microbes in the GIT, inflammatory mediators are released. This release recruits more inflammatory cells and more inflammatory mediators. There are two ways to inhibit this feed-forward loop, either by inhibiting the microbes with antibiotics or inhibiting the inflammatory mediators with an antibody to the mediators. C-CCK, P-$sPLA_2$, DC-dendritic cell

Conclusions Figure 1 presents our current hypothesis of how antibiotics stimulate growth and why feeding antibodies to key host inflammatory mediators also improve growth. Dietary antibiotics reduce inflammatory stimuli associated with the mucosal immune system. Decreased stimulation of inflammation in turn decreases host mediators of inflammation which can act as a feed forward loop to increase mucosal immune defence and the need for nutrients to maintain this defence and repair collateral damage associated with the inflammatory process. When key host inflammatory mediators released into the GIT lumen are targeted with antibody, the unwarranted inflammatory response to normal microbes in the GIT can be prevented. Since the antibodies are not absorbed, systemic defences are intact and during infection, clinical sign of disease are not exacerbated.

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Endocrines and host-pathogen interactions

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Background The health and welfare of animals are strongly influenced by their environment. Food animals, particularly those farmed intensively can suffer from poor welfare. Production systems of broiler chickens will be used as an example of the public health implications of poor animal health and welfare.
Broiler chickens, grow rapidly and are often housed at high stocking density. One consequence of this system is that chickens can suffer from production-related conditions such as hock marks and pododermatitis, which are contact dermatitis of the lower leg and feet. Broiler houses may also have high dust and ammonia concentrations in the air, both of which compromise the health of the animals. Broiler chickens are also frequently infected with endemic disease agents like avian pathogenic *Escherichia coli* (APEC) and can also suffer from Necrotic Enteritis caused by *Clostridium perfringens,* both of which markedly affect the health and welfare of the birds. Chronic poor welfare can lead to immunosuppression, which affects the ability of the animal to control not only endemic diseases but zoonotic ones such as *Campylobacter* and *Salmonella*.

Chickens also experience acutely stressful events during catching and transport and this leads to the enhanced excretion of neurotransmitters such as noradrenaline. This neurotransmitter markedly affects the behaviour of the animal and the bacteria it carries, particularly those found in the intestinal tract.

***Campylobacter* as zoonotic pathogens** Poor welfare in food production animals is a concern in its own right but recent work has demonstrated that it can have direct and important implications for public health and food safety. This is best illustrated by *Campylobacter* spp. This is a major international zoonotic pathogen and in the UK in 2010 it is estimated that ~700000 people were infected. Other estimates suggest that up to 80% of human cases are chicken-associated. Around 70% of chicken carcasses are *Campylobacter*-positive when sampled at UK retail outlets. Contaminated chicken poses two health threats. Carcass surface levels can exceed 10^9/bird, posing a cross-contamination risk. Perhaps more importantly, *Campylobacter* can be isolated from edible tissues such as liver and muscle. The UK has seen a marked upsurge in chicken liver- and pate-associated outbreaks in the last few years. Undercooked chicken meat is the most important vehicle for human infection worldwide.

Poor animal welfare and the *in vivo* behaviour of *Campylobacter* It is a common observation that *Campylobacter* levels, and those of other zoonotic pathogens, are higher in the faeces of chickens after transport to the slaughterhouse than on the farm. This can be explained by the fact that during catching and transport, chickens release high levels of the neurotransmitter, noradrenaline in response to the fear induced by the processes. Much of the noradrenaline is released into the gut and one consequence of this is that the hormone acts as an iron-capture mechanism for *Campylobacter*, increasing intracellular iron levels in the bacteria. Many genes in *Campylobacter* are iron-regulated and if intracellular levels of this metal increase, the bacteria grow much more rapidly, colonise chickens more effectively and are capable of *in vivo* extra-intestinal spread to edible tissues such as the liver.

Chronic poor welfare leads to the enhanced production of steroids. These can have a marked effect on the ability of chickens to control *Campylobacter*. Although these bacteria have been regarded as 'harmless commensals' in chicken it is becoming clearer that this belief only holds true if the chickens are well and are reared under conditions of very high welfare. Chickens mount both innate and adaptive immune responses to *Campylobacter*. These responses do not result in clearance of the bacteria and seem to serve to confine them to the gut of the animal. If chronic stress is simulated by giving chickens corticosterone for two days in the drinking water, very marked changes in the *in vivo* behaviour of *Campylobacter* jejuni are seen. The bacteria become invasive, being found in high numbers in deep liver tissues, and cause acute and potentially fatal diarrhoea.

Food security, animal welfare and food safety Food security is a major issue in the UK and the country needs to make sure that it has a secure supply of wholesome and safe food at an affordable price. The current low retail price for chicken in the UK is because intensive systems of production are used. It is likely that there will be an increase in the volumes of chicken meat produced using such production systems. It is important that a better understanding is gained of the public health impacts of intensive animal production so that industry can identify ways by which they can be mitigated.

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EU legislation: what can we change?

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There is a wide-range of EU derived animal feed legislation in place. This covers marketing (including labelling of feeds), feed additives, contaminants, feed hygiene, dietetic feeds and GM feed. Much of this legislation has been revised and strengthened over the last ten years. The legislation covers both feeds for food producing animals and non-food producing animals (e.g. pet foods).

Most of the legislation is in the form of Regulations adopted by the Council of Ministers and European Parliament which are directly applicable in Member States. Most of these major measures have been reviewed and revised over the last ten years.

Certain of the requirements can only be varied by amending the principal articles of Council/European Parliament Regulations. More commonly amendments are made by qualified majority votes by Member States in the European Commission's Standing Committee on the Feed Chain and Animal Health (Animal Nutrition section). Requirements that can be changed include the following.

Feed additives (e.g. vitamins, trace elements, preservatives, micro-organisms) – applications can be made for new or revised authorisations on the basis of a dossier covering safety, efficacy and quality. The EU is currently carrying out a large programme to re-assess and re-authorise existing feed additives, including against enhanced environmental criteria. Improved management and use of feed additives can provide an effective way of improving digestibility, reducing pollution, prolonging or preserving feeds and therefore reducing waste.

Contaminants (undesirable substances) The legislation sets maximum permitted levels (MPLs) in feeds for a range of contaminants such as dioxins, heavy metals and aflatoxins. These can be amended on the basis of emerging data on risk and an assessment by EFSA. It is expected that the controls will be extended to include non-PCB like dioxins. Control of persistent organic pollutants or heavy metals offers environmental benefits.

Marketing and Use of Feed If a valid case can be made and accepted by the Commission and Member States it is possible to change some requirements as set out in the Annexes of this Regulation. This includes declarations of analytical constituents (protein, fibre etc), and of additives contained in feeds, and a list of materials prohibited for use in feed. The Regulation permits Codes of Good Labelling practice to be drawn up by the feed industry. There is also a Catalogue of the names and descriptions of commonly use feed materials that can be extended as appropriate – other materials marketed must be included in a Register of feed materials. New nutritional purposes for dietetic feeds can be introduced – this includes products such as boluses, pastes and drenches that contain high levels of additives.

GM Feed New varieties of GM feed can be authorised on the basis of a risk assessment by EFSA. There are currently discussions on the presence of trace amounts of unauthorised GMOs in imported commodities.

Feed Hygiene It is possible to vary the operational requirements that apply to feed business establishments e.g. standards relating to facilities, equipment, storage and transport as well as the establishments that are subject to approval. The Commission may bring forward proposals for the control of *Salmonella* in feeds.

In addition, the Commission plans to replace the existing Medicated Feedingstuffs Directive (90/167/EEC) with a new Council/European Parliament Regulation.

Use of new feed materials The industry may consider the use of new or alternative types of feed materials (e.g. microbial fermentation co-products). Although under EU feed law there is no pre-authorisation procedure, all materials must be safe and not have a direct adverse effect on the environment or animal welfare. Enforcement authorities may identify the use of new materials via the aforementioned Catalogue/ Register but it is feed business operators' responsibility to comply with the legislation. The feed industry already takes advantage of co-products from the food industry (e.g. the sugar and milling industries), but there may be also scope for more recycling of human foods for feed use from retailers. Any use of animal by-products would be subject to a change to TSE legislation in relation to existing prohibited materials. The Advisory Committee on Animal Feedingstuffs is keeping a watching brief on co-products used in feed from the growing biofuel sector and other new materials and products.

In summary, there have recently been major revisions of EU feed legislation. However, it is possible to change certain details of the legislation, in particular in response to technological developments and risk assessments. In taking advantage of the use of new sources of feed materials, industry must make sure it complies with feed safety requirements.

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Continuous housing of dairy cows: challenges and opportunities

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Plans to establish very large dairy units in the UK have stimulated media attention and opposition from some lobby groups. Specific challenges include environmental pollution, disease and welfare risks, the behavioural needs of the animals, the disposal of manure and the carbon footprint of the operation. Continuous housing of dairy cows is common practice where land is in short supply and where the climate is unsuitable for growing grass for grazing. In the UK, the majority of cows are housed and grazed in periods of around six months each. The percentage of herds kept in continuous housing in the UK is quite low, probably only around 5%. Elsewhere in Western Europe, however, the percentage of dairy herds kept in continuous housing ranges from zero in Sweden (where grazing is mandatory in animal welfare legislation) to more than 50% in the Alpine region and Italy. Opinions differ on the value of grazing for cows, so the aim of this paper is to discuss some of the issues raised by continuous housing of dairy cattle, focussing on annual milk yield per cow, animal behaviour, welfare and health.

In pasture-based systems annual milk yields are restricted to 4 to 5 thousand litres per cow. Yields in mixed housing/grazing systems average 6 to 8 thousand litres, rising to 10 to 12 thousand litres per cow in some continuously housed systems. High-quality grazed pasture alone does not provide a diet balanced for milk production. Specifically, the concentrations of total and rumen-degradable nitrogenous components in grazed pasture are too high for optimal utilisation by the rumen microflora. Thus efficiency of N use by the grazing dairy cow is typically less than 0.2. Further, there is a limit to the quantity of DM which can be consumed daily by the grazing animal, which can fall to as low as 11 kg per head in autumn. These challenges present opportunities to both scientists and dairy farmers to develop novel nutritional strategies for the supplementation of grazed pasture to increase total feed intake economically and to optimise efficiency of N use.

Continuous housing of cows presents opportunities for skilled management and for greater biosecurity. As a result it is usually practiced with larger herds where economies of scale justify greater investment in labour and specialist advice, and higher capital expenditure on buildings and equipment. However, there is concern that the Fourth Freedom (to express normal behaviour) may be compromised in continuously housed cows. Normal behaviour is the most prevalent type of behaviour for the individual or herd over a defined period of time in a relatively stable environment. But normal behaviour does not equate to "ideal" behaviour which varies according to human perceptions. Solving problems with one aspect of animal behaviour may create problems with another. For example, grazing may be more "natural", but may deprive the animal of shade and create periods of hunger and heat stress in times of drought, thereby compromising the first two freedoms (from hunger and discomfort). Given the choice in summer, cows prefer to be fed indoors than to graze (Charlton *et al*., 2010). The preference may be for shade since Legrand *et al*. (2009) found a preference by cows for indoors in daytime when temperature and humidity are at their peaks but a strong preference for access to pasture at night.

Many dairy buildings are relatively old and cow size has increased progressively over recent decades. Consequently, cubicles, feeding passages, and ventilation in older buildings may have become incapable of maintaining good cow health and welfare. There is a need for detailed specifications of the recommended environment for the continuous housing of cows. There is also a need to determine the welfare implications of providing grazing for specific periods, e.g. the dry period.

There has been a decline in the health and welfare of dairy cattle over recent decades and both type and duration of housing are contributory factors. Lameness and mastitis are of greatest concern. On average, 37% of UK dairy cattle are identifiable lame on any single day of assessment (Barker *et al*. 2010) and the incidence of mastitis is 71 cases per 100 cows per year (Bradley and Green, 2007). Risk factors associated with housing have been identified for both conditions (Barker *et al*. 2010). Housing designed to solve one problem may inadvertently predispose to others. For example, mats and mattresses in cubicles significantly increased lying times but were a risk factor for hock lesions (Fulwider *et al*. 2007).

Welfare standards are underpinned by the skills of the stockperson and the production system itself does not relate directly to the health and welfare status of the cow. Better advice is needed to allow the industry to understand the health and welfare consequences of different housing practices and to manage the conflicting issues they create. This is especially true if the dairy industry is to meet the recommendation of the Farm Animal Welfare Council that every animal should have "a life worth living" (FAWC 2009).

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The risk of exotic disease and the threat to the equine industry

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An exotic disease is one which we do not normally consider to occur in the UK. The incidence of outbreaks of exotic disease is increasing and affects all animal species including man. The reason for this increase is a combination of factors but primarily linked to increased mobility of populations, poor enforcement of controls and climate change.

The UK is free of Foot and Mouth Disease, therefore it is classed as an exotic disease and if you want an example of the impact of an exotic disease look no further than the 2001 outbreak when the countryside was effectively closed for months with footpaths and bridleways barricaded and a shutdown of any form of equestrian activity. More recent examples are the outbreak of Equine Infectious Anaemia in Ireland in 2006 and the equine influenza outbreak in Australia in 2009. In both instances the racing industry and movement of horses in these countries was halted for several months. The equine diseases generally considered as exotic to the UK are African horse sickness (AHS), equine infectious anaemia (EIA) and West Nile fever. All of these diseases are transmitted by insects and climate change has a big effect on the viability of the insects and their ability to harbour and transmit specific viruses. We already have sporadic outbreaks of EIA in the UK and West Nile virus is widespread in parts of Europe. Because of its potential to decimate the UK's 1.3 million horse population, recent attention has been focussed on African horse sickness. Although the risk is officially classed as low, we now know AHS could thrive in the UK as evidenced by the recent spread of its very close relative, Bluetongue disease of cattle and sheep. There are new regulations coming into force in 2011 on the control of an outbreak of AHS. There would be an immediate short term ban on all horse movements throughout the UK followed by movement controls extending 150kilometers around an outbreak and lasting indefinitely. The government has the power to slaughter horses in a bid to control the initial spread of the disease. There would be compensation for animals subsequently proven to be infected and a cap of £2500 compensation for slaughtered non-infected animals. Insurance would not cover compulsorily slaughtered animals although horses dying or sick animals destroyed on humane grounds would be covered until existing policies came up for renewal.

The economic impact of an outbreak of AHS and the associated control measures was investigated by the Epidemiology and Economics Research Unit at Reading University in a study commissioned by the AHS Government/Industry Working Party. The report identified a UK horse industry worth £7billion annually million people ride annually; 250,000 people attend Badminton 3 day event; the leisure riding sector (non-racing) spends £3.2 billion annually on keeping its horses and £409 million pounds indirectly on items like riding clothes and books; £11billion is bet annually on racing.

Describing the potential impact of exotic disease as devastating is therefore not an exaggeration and we must strive to use all the tools available to combat these diseases. Proper control of animal movements is important but the development and availability of safe, effective vaccines, in accordance with the EU strategy for disease control, offers the best prospect for averting disaster.

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West Nile Virus

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This paper reviews aspects of West Nile Virus biology with emphasis on those which relate to epidemiology, diagnosis and control in the equid species.

Introduction West Nile Virus (WNV) originated in Africa and was first described in 1937. There are many reviews and reports describing the origin, epidemiology, pathology, clinical features and diagnosis of WNV encephalitis. Several of these derive from the outbreak of encephalitis in birds, humans and horses (and other animals) first recognised in the eastern United States of America as being caused by WNV, a virus previously thought to be exotic (Long *et al* 2002, Castillo-Olivares and Wood 2004). However, there are many publications describing the risks and detection of arboviruses including WNV in humans over the preceding 20 years.

WNV is a member of the *Flaviviridae* family and belongs to the Japanese encephalitis (JE) serogroup of the genus *Flavivirus* and its natural hosts are mosquitoes and birds (usually corvids, e.g. rooks, crows, blackbirds, jays, magpies). It is endemic in Africa, the middle east and large parts of Asia. The most significant epidemiological feature is that virus spread is related directly to bird migrations and mosquito activity. Since the 1970s there has been occasional seasonal spread of WNV to eastern Europe (particularly France and Italy) but incursions have been more pronounced (perhaps because of improved diagnostics and increased awareness) in Europe since 2000. It is important to note that WNV replicates to high titres in competent birds and mosquitoes and that on occasions, mosquitoes may "bridge" to mammalian species including horses and humans. Nevertheless, although these mammals are susceptible to infection, they are incidental "dead end hosts". This is because WNV does not develop to high enough titre in the bloodstream of affected animals to constitute a significant risk of uptake and transfer of infection via further mosquito feeds. It is axiomatic that mammals do not spread WNV to new areas.

Diagnosis Clinical diagnosis in horses is difficult because of the non-specific nature of signs associated with central nervous system disease and encephalitis. However, detailed clinical decision-trees have been constructed (J Cardwell, pers.comm.) to aid in this venture and examples will be shown during the presentation. Laboratory diagnosis is based on serological tests for WNV-specific antibodies (with differential detection of infection versus vaccination responses) and PCR-based tests for direct detection of WNV genetic material (Long *et al* 2002). While it is important that diagnostic tests in birds, humans and horses are consistent, there is some evidence that PCR-based assays developed by Centres for Disease Control and Prevention in USA do not detect virus in ~60% of WNV-infected equine brain tissue samples (News and Reports 2003). Similar findings were made for birds, which highlights the importance of appropriate tests for the species under investigation.

Surveillance Some surveillance for WNV in mosquitoes has been undertaken since 2003 and syndromic-based wildlife monitoring has been carried out by the Veterinary Laboratory Agencies (VLA) using PCR and virus isolation. Over the last 8 years there have been no human cases originating in the UK identified by The Health Protection Agency at Porton. Two cases were identified in humans, but they related to international travel from endemic areas. A network is in place at VLA for surveillance using live and dead bird material, but to date no evidence for WNV has been reported. In contrast to this, there were reports of antibodies to flaviviruses including WNV in wild birds in the UK and development of these antibodies in sentinel chickens (Buckley *et al* 2006). The proposition has been made that the presence of virus-neutralising antibodies in the UK bird population may relate to the lack of evidence for clinical signs and deaths compared with USA. No cases of WNV have been found in horses in the UK, although native breeds are susceptible to infection with viruses of equine (USA) and bird (Madagascar) origin (Castillo-Olivares *et al* 2011). However, there is a large archive of post-mortem material within UK research institutes from cases of neurological diseases which have previously been shown to be negative for rabies and EHV-1. This material would benefit from some investigation, in the light of the findings in UK birds.

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African horse sickness

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African horse sickness (AHS) is a lethal viral disease of equids, caused by an orbivirus that is closely related to bluetongue virus (BTV). The African horse sickness virus (AHSV) is transmitted by biting midges of the genus *Culicoides*, which become infected after taking a blood meal from an infected and viraemic host (Mellor and Hamblin, 2004). The disease is endemic to Sub-Saharan Africa but sporadic outbreaks have had devastating effects in Northern Africa, Europe, Middle East and India (Mellor and Hamblin, 2004; Coetzer, 2004).

The AHSV genome is composed of ten linear segments of dsRNA, encoding seven structural proteins VP1 to VP7 and four non-structural proteins NS1, NS2, NS3 and NS3a (Roy, 1994). The AHSV particle is organised as three concentric layers of proteins. The outer capsid, which is composed of two proteins, VP2 and VP5, interacts with neutralizing antibodies that are generated during infection of the mammalian host. There are nine distinct serotypes of AHSV, which can be distinguished in virus or serum neutralisation tests (VNT or SNT). The identity of each serotype is controlled primarily by the amino acid sequence of VP2, which contains the majority of neutralising epitopes and is the principal serotype-specific antigen of AHSV (Burrage *et al*., 1993). Animals that survive infection by a single AHSV serotype are subsequently protected against the homologous type, although they can still be infected by the other serotypes. The AHSV core consists of two major proteins that form distinct capsid layers: VP7 forms the core surface layer; while VP3 forms the innermost 'subcore' shell. The subcore also contains three minor proteins, VP1, VP4 and VP6 that form core associated transcriptase complexes, and surrounds the 10 segments of the viral genome (numbered segment 1 to segment 10 [Seg-1 to Seg-10] in order of decreasing molecular weight) (Roy, 1994).

One of the most effective intervention strategies to combat AHS is vaccination, allowing horses to survive in endemic regions. Live attenuated strains of AHSV that were developed as vaccines have been available for more than 60 years and are still routinely used in South Africa and other endemic countries (Coetzer, 2004). However this type of vaccines causes viraemia in the host and therefore has the potential to be transmitted in the field. Recent experience with similar 'live' BTV vaccines in Europe shows that they can also exchange genome segments (reassort) with field strains, potentially resulting in reversion to virulence. Since these live vaccines work by causing 'infection' in the host, it is also difficult, or impossible, to design serological assays that will reliably distinguish (naturally) infected and vaccinated animals ('DIVA'), making surveillance more difficult or more expensive. These drawbacks are considered to make the live vaccines unsuitable for use in the naïve host populations in non-endemic geographic regions such as Europe. For these reasons a number of recombinant vaccination strategies have been pursued over the years, as an alternative to attenuated vaccines. Baculovirus expressed AHSV capsid proteins VP2, VP5, VP7 and VP3, either individually or combined to form virus-like particles (VLP), have shown promising results (Roy, 1994). The use of recombinant pox-virus vectors expressing these AHSV proteins have also shown potential as vaccines for AHS (Chiam *et al*., 2009; Guthrie *et al*., 2009).

Although more work is needed to improve vaccines against this disease, progress is hampered by the need to perform vaccine efficacy studies in horses. Work with AHSV infected horses in high biosecurity installations represents a logistical and financial burden. For these reasons we have initiated a programme of research based on developing an experimental mouse model for AHS, based on recent successes with a similar model for bluetongue.

In this presentation, a summary of the current knowledge on African horse sickness will be given, focusing primarily on aspects related to the control of the disease by vaccination. In addition, an overview will be given of the research that is currently being done at the Institute for Animal Health on the development of novel vaccination approaches for AHS. This includes the development of a small animal model for AHS, based on interferon-α receptor knock-out (IFNAR - / -) mice, the generation of recombinant poxvirus vectors encoding AHSV genes as potential vaccine candidates for AHS.

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Welfare issues for horses competing in international equestrian competitions

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At international level, sports horses, defined here as those competing in the sports[1] controlled at international level by the Fédération Equestre Internationale (FEI), frequently travel large distances to compete in strenuous major equestrian competitions. A number of welfare issues must be addressed in such use of horses and this presentation will highlight some of these. Transportation of horses carries certain risks, whilst equestrian events may take place in hot and humid conditions that present a significant thermal challenge to the horse. Sometimes horses require veterinary treatment for illness and injury during or close to competition. It is important that the use of medications is regulated so that horses are not used for competition when they are unfit, as well as so that competitors cannot gain an unfair advantage by the use of drugs.

The transportation of horses is not a wholly modern phenomenon and the first flight involving horse transport appears to have been a journey between Paris and London in 1924. The potential adverse effects of horse transportation include respiratory disease ('shipping fever'), colic, diarrhoea, weight loss, laminitis, and possibly gastric ulceration. Shipping fever is a bacterial infection of variable severity. Clinical signs can include fever, dullness, anorexia, coughing, nasal discharge and dyspnoea. Whilst the definitive causes remain uncertain, factors that may be involved include environment contamination by allergens and infectious agents, poor ventilation (especially in stationary vehicles), increased respiratory rate and depth associated with thermoregulation, sweating (causing dehydration), altered immune function and head posture; the latter may be a particularly important factor.

Even in temperate climates horses performing strenuous or extended exercise can suffer from the adverse effects of heat and humidity. In the build up to the Olympic Games in Atlanta in 1996, a considerable amount of research effort went into the investigation of thermoregulation in the horse, adaptation during acclimatisation to hot conditions and methods of treatment of heat stress. Since that time there have been greatly improved methods of dealing with thermoregulatory challenges in the horse with respect to the need to adapt cross country courses according to climatic circumstances, as well as the recognition and treatment of the overheated horse.

Most drugs are prohibited in horses if detectable at the time of competition. The FEI now publishes an Equine Prohibited Substances List in which drugs are divided into 'Banned Substances', deemed to have no place in the treatment of sports horses and the detection of which constitutes a 'doping offence', and 'Controlled Medication Substances', agents that are commonly used in equine medicine but are prohibited in competition. Medications not appearing on the Equine Prohibited Substances List are permitted during competition and these include: antibiotics (with the exception of procaine penicillin), anthelmintics (with the exception of levamisole and tetramisole), licensed vaccines, certain anti-gastric ulcer drugs (e.g. omeprazole, ranitidine, cimetidine, sucralfate) altrenogest (in mares only and following declaration of its use), rehydration fluids (in volumes of not less than 10 litres and not on the day of cross country competition prior to starting), vitamin and electrolyte supplements and preventative or restorative joint therapies (e.g. chondroitin sulphate or glucosamine). Substances can be given by injection during FEI competitions only after completion of an appropriate form seeking permission for treatment and such therapy must be administered in a designated treatment area. Intra-articular medication may not be given during FEI competition. It should be noted that the Rules of Racing for Thoroughbred horses in the UK differ at times from those of the FEI, for example, racehorses are not permitted to compete on anti-ulcer drugs or altrenogest.

The use of horses for equestrian competition presents a number of challenges and it is our responsibility, as the guardians of equine welfare, to maintain a high standard of care in these animals as in all equids.

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[1] Dressage, show jumping, eventing, vaulting, driving, reining, endurance and paraequestrianism.

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Food security – challenges and opportunities for animal science

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Introduction Meeting the food needs of a growing and increasingly affluent global population without harming the environment will be the grand scientific challenge of the next half century. The notion of food security has different implications for developed and developing countries. For developed nations, these include the politics of self-sufficiency and opportunities to participate in global food markets. However, since 98% of the almost one billion people classified as undernourished live in the developing world, the implications for these countries are more immediate and dire. This distinction is especially sharp for the livestock industries in that animal-derived foods account for a much greater fraction of energy and nutrient supply in affluent countries yet many of the world's poorest people depend on livestock for their sustenance and livelihoods. At the same time, the demand for animal products in many developing countries is accelerating with their growing prosperity, exacerbating existing pressures on the environment and raising questions about consequences of changes in diet and livestock management systems for human health. This paper will identify some important challenges facing the livestock sector as it seeks to address issues of human nutrition, animal and human health, and environmental stewardship, and will offer examples of opportunities for animal scientists to meet these challenges.

Challenges (1) Because of increasing urbanisation and concerns about the environmental impact of agriculture, necessary future increases in food production must come from a static or shrinking land and resource base, with an imperative to dramatically increase productivity. For the livestock sector, this will require increases in the efficiency of production of both animals and the feeds on which they depend. (2) The livestock industries contribute significantly to the environmental impact of food production systems. For example, enteric production of methane by ruminant livestock accounts for about 30% of the total anthropogenic emissions of this potent greenhouse gas. Although the amount of water used to produce meat and other livestock products has been disputed, there is no doubt that these industries place considerable pressure on water supplies, especially where animal feeds are produced by irrigation. The impact of livestock production systems on biodiversity also must be addressed. (3) The competition between livestock and humans for grains and other high quality plant foods, whether real or perceived, is recognised as a major challenge to the sustainability of intensively managed livestock industries including poultry, pigs and lot-fed beef. (4) The intensification of livestock production systems and globalisation of product distribution have increased threats to human health via actual or potential emergence of new zoonotic diseases and heightened risk of infection with food-borne pathogens. (5) Increasingly, public concerns about the ethics of livestock management and food production are influencing consumer choice and behaviour. While this phenomenon is mostly confined to developed nations, it will undoubtedly spread to other countries as they become more affluent, better educated and increasingly sophisticated. (6) Among the challenges listed above there are several contradictions and dilemmas. For example, there is compelling evidence that intensification of animal production can be part of the solution to the problem of reducing the environmental footprint of the livestock industries, yet many in developed countries resist this notion. Similar concerns exist about the potential for genetically modified crops and animals to contribute to meeting the huge productivity challenge facing the agricultural industries.

Opportunities for animal scientists (1) There is considerable opportunity for genetic and non-genetic improvement in productive efficiency of animals and their feeds. The combination of genome-wide association approaches to genetic selection and development of technologies to enable novel phenotyping offers the opportunity to increase the speed of genetic progress in hard-to-measure complex traits such as reproductive and feed efficiency, and disease resistance. Proven non-genetic genomic applications, such as use of recombinantly-derived growth hormone to enhance milk yield and lean tissue growth, offer opportunities for refinement and specificity of impact on efficiency of nutrient utilisation. These technologies also offer win-win solutions to the challenge of reducing environmental impact at the same time as enhancing productivity of livestock. (2) Rapid, specific diagnosis and control of transboundary infectious diseases, including actual (e.g. SARES) or potential (e.g. avian influenza) zoonoses also are amenable to genetic and non-genetic applications of genomic knowledge through novel approaches to enhancement of disease resistance (e.g. transgenesis) and development of new diagnostics and therapeutics. Genomic and metagenomic detection of viral and bacterial pathogens in the animal product food chain also will reduce risks to human health. (3) The relative advantages of ruminant livestock in being able to convert human-inedible plant feeds and by-products into nutritious human foods, coupled with heightened concerns about use of high quality plant foods to feed livestock, will increase the priority for optimising the production and utilisation of forages in intensive temperate (e.g. New Zealand, Western Europe) and extensive tropical (e.g. Northern Australia, Brazil) pastoral systems, as well as use of by-products (e.g. Indonesian feedlots). (4) Introduction of even small quantities of meat and milk into the diets of undernourished children in East Africa has caused significant improvement in their physical and cognitive development. This supports the case for further research on management of livestock in small-holder production systems in developing countries in Africa and Asia. (5) Examples of enhanced nutritional and nutraceutical properties of animal products include enrichment of ω3 and conjugated linoleic fatty acids, and of micronutrients such as selenium in animal products. Further opportunities undoubtedly exist.

Conclusion Despite daunting challenges, the application of contemporary animal sciences combined with appropriate social policies can underpin the future of sustainable livestock production systems to enhance the nutrition and food security of humans world-wide.

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The new food security challenge: balancing agriculture, health and environmental sustainability

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This paper examines the rapid (re-)emergence of food security as a UK policy issue, considering in particular the implications for policies for agriculture, health and nutrition, and agri-environmental sustainability. I argue that a global prioritisation of food production does not necessarily translate into a new national productivism in either practice or political rhetoric in the UK. A simple return to 'dig for victory' is compromised by other pressures on land use, notably energy; by thirty years of a ‘consumer countryside'; and by public concerns over biodiversity, climate change, and agro-food sustainability. 'Obvious' technological solutions to the challenges of food security, such as genetic modification, remain contested. However, it is clearly the case that the agri-environmental consensus forged in the 1980s has broken down as a result of the new politics of food security and that no new settled agro-food politics has yet emerged. In that context, the UK is potentially entering into a new period of agro-food policy debate to match the intensity of agri-environmental debate of the 1970s and 1980s.

That earlier debate largely centred around the use of land, and conflicting demands made upon the land. It resulted in the emergence of the notion of a multi-functional landscape, with farmers and land managers providing both food and a wider set of public goods (recreation, wildlife conservation etc). The challenges of maintaining multi-functionality remain but the new emphasis on food security alters the way in which these issues are framed. This occurs in two ways particular. First, now that food security is recognised as a global rather than a local issue, it is no longer possible to ignore the implications of domestic land use for global markets and social justice. Second, the importance of diet to health and nutrition, again in a global context, has equally important potential implications for domestic agricultural systems.

The role of livestock is totemic in these debates, and highly contested. The paper therefore provides an overview of the animal debate in the context of food security and multi-functionality by contrasting positive and negative images of animals in agricultural systems, depending on policy objectives (biodiversity, human health, sustainable farming systems, energy and water efficiency, protein availability etc).

The paper concludes with an assessment of whether or not the ecosystems services approach provides a creative way to resolve the tensions identified and the basis for a new policy settlement around sustainable food security.

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Human metagenomics in the gut

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Human beings are superorganisms and the phenotypes we observe and measure are not solely due to the expression of the karyome, but a more complex interaction between the later and the host's microbiome. In recent years we have started to realise that one of the largest repositories of host associated genetic information resides in the intestinal tract of every human being. The microbes which colonize us from birth, present a unique challenge if we are to understand what contributions they make to host development and physiology. Past forays into this ecosystem, using traditional microbiology, i.e. growing organisms aerobically and anaerobically on laboratory agars, provide a very biased view of the species present. Furthermore the functions that these cultured organisms provided to the host were not necessarily those that were abundant in the gut. This bias was observed in the late 1970s, however the technology to overcome it did not become available until the late 1990's. Two significant developments made investigating the gut microbiota possible; the first was the application of DNA biomarkers as surrogates for the absence or presence of an organism in a system. In particular using the small subunit rRNA genes (16S and 18S rRNA genes) couple with the PCR, this method allowed microbiologists to create inventories of the species present in a gut without the need to grow them in the laboratory. The second milestone was the development of metagenomics first published in 1998 from Stephen Giovannoni's laboratory (Vergin *et al*., Appl Environ Microbiol. 1998 64(8):3075-8) and further developed by Jo Handelsmann group in 2000 (Appl Environ Microbiol. 2000 66(6):2541-7). Metagenomic approaches have allowed us to start to determine the functions which the bacteria bring to an ecosystem, again without the need grow any of the indigenous bacteria. Metagenomics can be implemented in one of two ways functionally or sequence based.

In functional metagenomics DNA from the ecosystem is cloned into a surrogate host, usually *Escherichia coli* and the library screened for novel phenotypes. While sequence based metagenomics takes the same DNA and proceeds to sequence it either using the 1st generation Sanger based chemistry or more frequently 2nd generation high throughput platforms such as the Roche 454 or Illumina GAIIx. Using both these metagenomic approaches we can now gain an un-precedented view of the complexity of the functions associated with the gut, but the actual simplicity and resilience of the system. To this end gut microbiologists have started to explore a whole range of "omic" approaches or culture-independent methods, mainly developed by environmental microbiologists, to create inventories of the genes, proteins, metabolites and try and link these to the organisms that inhabit the gut. They have begun to show how events which impact the gut microbiota are not localised to that site, but have an impact which can be measure throughout the whole body.

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Rumen microbial ecology and its role in cattle feed efficiency

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Improvement of cattle feed utilization is a key research priority for beef/dairy industry because feed typically accounts for 60 – 65% of the total fixed costs of production. Ruminal microbial fermentation of ingested feed contributes fundamental components for cattle's daily nutrition, production, and maintenance of health. The rumen microbial ecosystem comprises a diverse population of microorganisms, and its diversity is highly responsive to changes in diet, age, antibiotic use, and the health of the host animal.

The influence of rumen microbial structure and functions on host physiology remains poorly understood. This study aimed to investigate the interaction between the ruminal microflora and the host by correlating bacterial diversity to fermentation measurements and feed efficiency traits including dry matter intake, feed conversion ratio, average daily gain, and residual feed intake using culture-independent methods. Residual feed intake (RFI) is one of the measurements that has recently become more widely accepted by the industry as a useful measure of feed efficiency. RFI is the variation in feed intake that remains after the requirements for maintenance and growth have been met. Efficient animals eat less than expected while inefficient animals eat more than expected.

Rumen fluid, digesta and tissue samples were collected from more than 300 steers with different residual feed intake. Total DNA was then extracted from all the samples and used for analysis. This report is mainly focused on a comprehensive analysis that was performed for rumen fluid, digesta and tissue for 58 steers under high and low energy diets.

Universal bacterial partial 16S rRNA gene products were amplified from ruminal fluid, rumen digesta, and rumen tissues collected from these 58 steers and were subjected to PCR-denaturing gradient gel electrophoresis (DGGE) analysis. In total, 86 PCR-DGGE bands were identified from all animals and 76 of the bands were successfully sequenced. Using multivariate statistical analysis developed in house, specific PCR-DGGE bands (bacterial species) were related to various feed efficiency traits and metabolites. Our results suggest that particular bacteria and their metabolism in the rumen may contribute to differences in host feed efficiency under growing and feedlot diets. This study identified probable associations between ruminal ecology and activities with cattle feed efficiency by defining a statistical method to link the PCR-DGGE profile, microbial fermentation parameters and feed efficiency paramaters.

This is the first attempt to categorise bacterial PCR-DGGE band patterns in the rumen and to link them to phenotypic characteristics of the host, specifically to feed efficiency. Improved understanding of the contribution of rumen microbes to feed efficiency will provide insight into the mechanisms that cause variation of feed utilization between animals and will contribute to the development of a rapid economic technologies to select for more efficient animals and/or to improve their management.

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Non-ruminant - host microbial interaction in the gut

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Development of the commensal microbiota The mammalian gastrointestinal tract contains an immense number of microorganisms, collectively known as the microbiota. Prior to birth, the gastrointestinal tract is sterile. Mammals are quickly colonized in early life by a highly complex, diverse and dynamic microbiota. First exposure to bacteria occurs during passage through the birth canal during delivery. Commensal bacteria are subsequently ingested from both maternal and environmental sources. The immediate microbiota is heterogeneous and dynamic, reflecting the mixture of microbial populations associated with the birthing environment. Recent studies have shown that the first year of life is characterized by fluctuating diversity of microbial composition until convergence towards a stable, adult microbiota.

Successful microbial colonization and maintenance of a 'healthy' microbiota is affected by a number of factors such as nutrition, mode of delivery, gestational age, rearing environment and antibiotic exposure. Recently, a direct link has been shown between specific gut microbiota compositions in early life and subsequent predisposition to disease and allergy.

Effects of commensal microbiota on the host Commensal bacteria are involved in complex mechanisms regulating host development, lipid metabolism, pathogen response, tissue repair and immune homeostasis. Bacteria can access nutrients from the diet, such as plant polysaccharides, that would otherwise be indigestible for the host. Specific bacterial species such as *Bacteroides thetaiotaomicron* play an important role in epithelial cell maturation and maintenance, while *Clostridia* bacteria from the *Firmicutes* phylum are able to produce short chain fatty acids such as butyrate, which is important for colon health. The microbiota can also prevent pathogen colonization by competing for niches and by promoting the production of antimicrobial factors. Acquisition and development of a stable 'normal' gut microbiota can have life-long consequences for the individual, and the factors that control the composition of the gut microbial ecosystem are important determinants of overall health.

Importance of commensal microbiota for immune development The early-life expansion of the gut microbiota corresponds to a key immune developmental window. Gut maturation is directly influenced by the presence of commensal bacteria, and the mucosal immune system of germfree animals remains underdeveloped.

Bacterial colonization activates the innate immune system by influencing expression and localization of epithelial pattern recognition receptors and increases expression of defensins and other antimicrobial peptides. Bacterial colonization also increases production of secretory intestinal IgA by B cells. Activation of innate mechanisms and B cell development seems not to be dependent on specific bacterial species, and there is great redundancy between species.

Not all commensal bacterial species are equally able to induce CD4+ T cell maturation. Rather, defined members of the microbiota, such as *Bacteroides fragilis* and SFB, have evolved the unique ability to direct specific aspects of immune system maturation. Colonization by commensal microbiota *per se* might therefore not be sufficient for maturation of the different T cell subsets; the microbiota must contain certain specific bacterial species.

In a healthy animal, a delicate balance is maintained between beneficial and potentially harmful bacteria in the gastrointestinal tract. However, during times of stress, such as during weaning in the case of piglets, this balance may be affected and can cause a rapid growth of harmful microorganisms, leading to poor performance or disease. Imbalance between the commensal microbiota and the host appears to drive a wide range of mucosal and systemic immune-mediated disorders, and changes in human gut microbiota have been associated with autoimmune diseases such as diabetes, rheumatoid arthritis and obesity.

Manipulation of the microbiota for benefit of the host – practical applications There is increasing pressure for livestock producers to minimize the use of antibiotics as growth promoters in food animals. Supplementing beneficial microorganisms in the gastrointestinal tract to improve intestinal function and a healthy gastrointestinal tract is one potential alternative. Prebiotics are food additives that selectively stimulate the growth of so-called 'good' bacteria to improve intestinal function and a healthy gastrointestinal tract. Commonly investigated prebiotics include inulin, inulin-type fructans, and butyrate. Probiotics are defined as 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host' by improving the microbial balance in the gut. Most current probiotic products contain *Lactobacillus*, *Bifidobacterium* or the yeast *Saccharomyces cerevisiae*. Synbiotics are the combination of a prebiotic and probiotic; they can improve survival of the probiotic bacteria in the gastrointestinal tract.

A large number of studies have failed to consistently demonstrate beneficial effects of current pre-, pro- or synbiotics. However, the bacterial species currently favoured as possible health-promoting candidates have not been selected using robust scientific evidence or biological efficacy in the gastrointestinal tract. These species might therefore not be the best strains to promote immune function and disease resistance. A new generation of probiotics based on proven ability to prevent or attenuate inflammation and infections in the gut will provide new opportunities for successful implementation of probiotic products as additives to pig diets.

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Management of obesity in dogs and cats – maximising success

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Causes of obesity Obesity can arise secondary to a number of diseases including endocrinopathies, drugs, and rare genetic disorders (in humans), although most cases are primary i.e. the result of an imbalance in the 'energy balance equation'. Numerous factors may influence the relative ease with which weight is gained, and these include genetics, age, neuter status, concurrent diseases, amount of physical activity, and energy content of the diet.

Treatment of obesity in dogs and cats Drug therapy. Two drugs are currently approved to assist in the management of obesity in dogs. *These drugs are neither licensed nor safe in cats*. Both are microsomal triglyceride transfer protein inhibitors that block the assembly and release of lipoprotein particles into the bloodstream. Efficacy for weight loss is good but, for long-term success, it is ESSENTIAL tomodify owner and animal behaviour. Unless steps are taken to change feeding habits and exercise patterns, weight regain will occur. This rebound effect is a well-known phenomenon of any weight loss program.

Conventional options for weight management include dietary therapy and behavioural modifications; such strategies are likely to remain for dogs and, given that no pharmaceutical agents have yet been approved for cats, this approach will remain the mainstay of therapy in this species. It is preferable to use purpose-formulated diets, and most formulated rations are restricted in fat and calories, whilst being supplemented in protein and micronutrients. Protein supplementation is important since, although weight loss is not more rapid, the amount of lean tissue lost is minimised.

Developing strategies to improve satiety would greatly assist in case management. The most recent work has demonstrated that supplementing diets with both protein and fibre has the greatest satiating effect in dogs, and such diets are known to improve outcome of weight loss regimes. However, given that protein content is a key determinant of voluntary food intake in cats, the best effect on satiety occurs with fibre supplementation, whilst only modestly increasing protein content.

Monitoring weight loss In addition to the above strategies, it is essential that the whole weight reduction regime be closely supervised. This is labour-intensive, requires some degree or expertise and training in owner counselling, and often requires a dedicated member of staff. In the author's opinion, correct monitoring is the single most important component to the weight loss strategy. It is essential to continue to monitor body weight, after ideal weight has been achieved, to ensure that weight that was lost is not regained; as with humans, a rebound effect has been demonstrated after weight loss in dogs. This has been seen in ~50% of dogs that successfully lose weight.

Review of conventional weight loss programs Conventional weight loss regimes, involving dietary caloric energy restriction, are highly successful in obese colony dogs, with rates of weight loss of 1.3-2.6%/week using a caloric allocations of 50-87%. However, weight loss in client own dogs is slower (average 0.85% body weight/week), and requires a greater degree of energy restriction i.e. mean 52% of mean energy requirement at target weight. For obese pet cats, the average rate of weight loss is 0.8% body weight/week; for this, mean energy intake during a weight loss regime is 32 Kcal/kg target weight is required.

Preventing weight regain In obese humans who successfully lose weight, subsequent 'regain' is very common. Experimental studies in dogs have demonstrated a similar tendency for weight regain, with maintenance energy requirements decreasing significantly after weight loss. A recent study has examined long-term follow-up in obese pet dogs that had successfully reached target weight. 42% of dogs maintained weight, 9% further weight, and 48% regained weight. Dogs fed a purpose-formulated weight management diet, during the weight maintenance phase, regained less weight than those switched onto a standard maintenance diet.

Summary Successful weight loss in dogs and cats requires dedication and commitment. Conventional weight loss strategies involving diet and exercise can be highly successful in both dogs and cats, whilst pharmaceutical agents provide another means to achieve target weight. Successful weight loss involves not only achieving an ideal body weight, but also maintaining it subsequently. Preventing the known and predictable rebound effect is a key indictor of true success during weight loss.

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