

Executive summary

During the accession negotiations with the European Union, Sweden was granted a derogation from community legislation to maintain national legislation within the area of feed additives of the groups antibiotics, chemotherapeutics, coccidiostats and growth promoters. In Sweden, the use of such substances is restricted to the purpose of curing or preventing diseases, i.e. used as veterinary medicines according to the Feedingstuffs Act of 1985. The Swedish government has appointed a commission to evaluate the hazards and risks associated with use of antimicrobial feed additives in animal production.

Our conclusions can be summarised as follows:

- Antibacterial feed additives have favourable economic effects on livestock production, but from a long term perspective these are questionable, especially regarding animal welfare and animal health. Antibacterial feed additives can, at levels permitted in feedingstuffs, be used for treatment or prevention of animal diseases, which is in violation of directive 70/524/EEC.
- The quinoxalines and the nitroimidazoles are potentially genotoxic and may be regarded as an occupational hazard.
- Halofuginone and the quinoxalines are toxic for target species. This is deleterious for animal well being.
- The risk of increased resistance associated with the general use of antibacterials as feed additives are far from negligible and the potential consequences are serious for both animal and human health. Antibacterials that are presently not used as therapeutics in human or veterinary medicine are valuable templates for future drugs. As emergence of resistance is considered to be a threat to animal and human health, all AFA should be restricted to medical and veterinary purposes.

It is the conclusion of this commission that the benefits of antibacterial feed additives do not outweigh the risks.

With regard to coccidiostats and other medicinal substances, they are specifically used to prevent disease and should therefore be treated as pharmaceutical specialities, i.e. as medicated feed.

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1 Introduction

1.1 The Swedish derogation

In 1986, the Swedish Parliament imposed a ban on antibacterial growth promoters. During the accession negotiations with the European Union Sweden was granted a derogation from Community legislation concerning the use of antibiotics, chemotherapeutics, coccidiostats and growth promoters as feed additives. Before December 31, 1998 the Commission shall decide on the applications for adjustment to be submitted by Sweden. These applications are to be accompanied by a detailed scientific report on motives for the applications. The decision shall be taken in accordance with the procedures laid down in article 7 of the directive 70/524/EEC concerning additives in feedingstuffs.

In 1995 the Ministry of Agriculture appointed a Commission (Commission on Antimicrobial Feed Additives) to collect and review scientific data which are relevant for a decision on the above mentioned feed additives. Professor Lars-Erik Edqvist was appointed chairman of the commission and Laboratory Veterinary Officer Christina Greko was appointed scientific secretary. Laboratory Veterinary Officer Susanna Sternberg was appointed assisting scientific secretary. The following experts have been appointed to deal with particular areas of the work of the commission: Professors Sigvard Thomke and Klas Elwinger to conduct a scientific review for Chapter 3; Laboratory Veterinary Officer Desirée Jansson to undertake a similar review for Chapter 7 and Associate Professor Ivar Vågsholm to undertake the economic analysis in Chapter 3.

The appointed Commission has gathered the scientific data which it has found appropriate in two reports, one in English (SOU 1997:132) and one in Swedish (SOU 1997:133). The Swedish report is an abstract the English report.

Among the feed additives dealt with, the coccidiostats hold a specific position since they are used to prevent specific diseases and not as growth promoters. They are according to 705/524/EEC classified as medicinal substances. As the indications for use of these substances are specifically to prevent disease, they are treated separately.

Chapters 3-7 of the report provides scientific documentation on hazards and risks of using antibiotics, chemotherapeutics, coccidiostats and growth promoters as feed additives. In Annexes A-F is a case by case review of substances which are used as feed additives within the European Union. In chapter 8 the assessment of identified hazards is undertaken.

2 Background

2.1 Introduction

Antimicrobials are probably the single most important discovery in the history of medicine. The antimicrobial era began in the 1930s when the first sulphonamides made their way into clinical use in human medicine. Penicillin, the first antibiotic, was discovered in 1929 but was not introduced for therapy until 1940. Shortly after its introduction into human medicine penicillin was used in animals for the treatment of different bacterial diseases. Since these early discoveries, a large number of antimicrobial substances have been discovered or developed. Although some of these drugs have been developed specifically for animal use, the substantial costs for development are often not covered by profits from the veterinary market alone.

Table 2.I. Some terms used and corresponding definitions

Tabell 2.I. Några använda termer och dessas definition

Term	Abbreviation	Definition
Antibiotic		Substance produced by a microorganism, with an inhibitory effect on other microorganisms
Chemotherapeutical		Chemically synthesised substance with an inhibitory effect on microorganisms
Antibacterial		Antibiotic or chemically synthesised substance with an inhibitory or lethal effect on bacteria
Antiprotozoal		Antibiotic or chemically synthesised substance with an inhibitory or lethal effect on protozoa
Antimicrobial		Substance with an antibacterial and/or antiprotozoal effect
Anticoccidial		Antiprotozoal with inhibitory or lethal effect on coccidia
Coccidiostat		Anticoccidial used specifically for prevention of coccidiosis
Antihistomonal		Antiprotozoal with inhibitory or lethal effect on histomonads
Antibacterial feed additives	AFA	Antibacterials used as feed additives for the purpose of performance enhancement (corresponds to antibiotics and growth promoters in Directive 70/524/EEC)
Medicinal feed additives	MFA	Antimicrobials used as feed additive for the purpose of preventing a specific disease (corresponding to medicinal feed additives according to Directive 70/524/EEC)

An antibiotic is defined as a substance produced by living micro-organisms that inhibits or kills other micro-organisms. Synthetic antimicrobial substances, such as the sulphonamides and the quinolones, are referred to as chemotherapeutics. The word "antimicrobial" (as a noun) is often used to encompass any substance of natural, semi-synthetic or synthetic origin that kills or inhibits the growth of a microorganism. With respect to spectrum of activity, antimicrobial substances may be divided into antibacterial, antiprotozoal, antifungal and antiviral drugs. In the following, the term antibacterial will be used for antibiotics and chemically synthesised substances that have an inhibitory or lethal effect on bacteria. Terms used in the text are defined as shown in table 2.I.

2.2 Role of antibacterials in animal husbandry

A better understanding of many aspects of animal husbandry have resulted in a development towards improved productivity. Such factors include the combat of epizootic diseases, control of parasitism, improvements in animal nutrition, genetic improvements and the use of antimicrobials.

Antimicrobial agents are used for three major purposes in domestic animals:

- 1 Therapy, to treat an identified illness
- 2 Prophylaxis, to prevent illness in advance
- 3 Performance enhancement, to increase feed conversion, growth rate or yield

2.2.1 Therapeutic and prophylactic use of antibacterials

Therapy usually involves an individual animal or a defined group of diseased animals while prophylactic treatment involves the treatment of a herd or group of animals. The aim of the latter is to prevent diseases that might otherwise occur. A special form of prevention, also called metaphylaxis, is when all animals in a herd or group of animals are medicated, in situations when the proportion of animals diseased during a defined time period reaches a threshold value. In such situations, the probability of most, or all, of the animals getting infected is high. In both therapy and prophylaxis, the drug is administered over a defined, preferably short, period of time and in both instances the drug is used upon prescription by a veterinarian. The dosages used must be high enough so that concentrations that are inhibitory for the infectious agent are reached at the site of infection (e.g. the lung).

The animal diseases currently requiring the most extensive use of therapeutic or prophylactic drugs are respiratory and enteric diseases of pigs

and calves, and mastitis in dairy cattle. Prophylactic treatment is particularly common during periods when stress is imposed on the animal, e.g.. changes in diet and loss of maternal interaction at weaning, after transport and commingling. Although in recent years much emphasis has been placed on disease prevention through improved management and environmental conditions, intensive animal production systems still depend on antimicrobials, as shown by the continuously growing market for antibacterials.

2.2.2 Chemoprophylaxis of protozoal diseases

In the case of certain protozoal diseases, the probability of clinical outbreaks or production losses due to subclinical disease is so high that chemoprevention has become standard practice. The main diseases in question are coccidiosis of poultry and rabbits and histomoniasis of turkeys and pheasants. The appropriate drugs for prophylaxis are referred to as anticoccidials (coccidiostats) and antihistomonals.

Anticoccidials and antihistomonals are medicinal substances preventing specific diseases with a high probability of occurrence. In that respect, they are by definition veterinary medicines. On the other hand, in most parts of the world they are used as standard feed additives and are, from a regulatory point of view, treated as such.

2.2.3 Antibacterials as performance enhancers

The growth-promoting properties of antimicrobials for farm animals were discovered in the late 1940s. Trials where fermentation waste from tetracycline production were fed to chicken as a source of vitamin B₁₂ revealed that the chickens fed the fermentation waste grew more rapidly than did the controls. It was soon found that this effect was not due to the vitamin content of the feed but to residual tetracycline (Stokestad and Jukes, 1949; Stokestad and Jukes, 1950). This growth promoting effect of tetracyclines was soon confirmed for other antibacterials and other animal species.

Growth enhancement in children

The findings of improved growth as an effect of administration of subtherapeutic doses of antibiotics led to investigations on possible similar effects in humans. In 1952, Snelling and Johnson reported lowered morbidity, increased growth rate and shortened hospital stay of premature infants following daily administrations of 50 mg chlortetracycline. Similar results, from a controlled trial in twins and triplets, were also reported by

Robinson (1952). MacDougall (1957) found that low doses of chlortetracycline given to hospitalised malnourished children resulted in, among other effects, improved weight gain. Positive effects on the weight gain of undernourished school children have also been reported (Mackay *et al.*, 1956; Guzman *et al.*, 1958). MacDougall (1957) concluded that chlortetracyclines could prove a valuable adjunct to dietary therapy in clinical practice under the conditions described. In contrast, both Mackay and co-workers (1956) and Guzmán and co-workers (1958) concluded that there was no justification for considering continued administration of antibacterials to children in underdeveloped areas. Subsequent research with focus on the relative impact of improved nutrition and control of infectious diseases on the growth of children demonstrated, among other things, the absolute necessity of maintaining adequate nutrition during weaning (Taylor, 1967; Behar *et al.*, 1968).

Growth enhancers in animal husbandry

In animal husbandry, the early findings initiated an intensive area of research. The practice of feeding subtherapeutic doses of antibiotics was readily adopted and AFA soon became an integrated part of the systems developed in the animal industry. Apart from increased growth rate and/or increased feed conversion, examples of other observed effects of antimicrobials at low doses are improved egg production in laying hens, increased litter size in sows and increased milk yield of dairy cows. When antimicrobials are used for the latter purposes the term yield promoters has been used. Alternative terms proposed for growth promoters such as "growth permitters" or "digestive enhancers" appear misleading. "Growth permitters" would imply that growth of animals is not permitted without these additives. The term "digestive enhancers" indicates that the effect of AFA is primarily on the digestion of feed. This is not in line with the, admittedly scarce, knowledge on the mode of action of AFA (see chapter 3).

The various claimed benefits of antibacterial performance enhancers have been listed by the European Animal Health Federation (FEDESA) as:

- To improve feed utilisation
- To improve growth rate
- To improve carcass quality
- To improve rate of throughput of livestock
- To improve farm economics
- To improve building utilisation
- To reduce labour
- To permit economical redeployment of resources
- To help indebtedness of farmers
- To permit controlled production from fewer stock to achieve quotas
- To reduce energy consumption
- To conserve natural fuels

- To reduce waste
- To reduce environmental contamination
- To reduce livestock numbers and slurry disposal problems
- To improve the environment

(Source: FEDESA 1994 cit. by Colegrave and Wesley, 1995)

The exhaustive nature of this list is due partly to the fact that some effects have been duplicated using different phrasings, and partly to the listing of various secondary and tertiary effects. If feed utilisation and/or growth rate of the animals is improved, the throughput of animals on a specific premise will increase. The latter is equal to improved building utilisation. From this follows an economic benefit, which, for indebted farmers certainly will mean an improvement. Improved use of buildings and feed would, in certain situations, reduce energy consumption which normally is the same as conservation of natural fuels. Reduced waste, reduced slurry disposal problems, reduced environmental contamination and improvement of the environment also appear synonymous and are sequels to improved feed utilisation. The statement "improved carcass quality" is doubtful. In some cases, a higher fat content of the carcass has been noted (Fiems *et al.*, 1995).

A list encompassing only the primary effects attributed to of AFA would read:

- Prevention of disease
- Improved feed utilisation
- Improved growth rate

2.3 Community legislation

2.3.1 Antibiotics and growth promoters

In the EU, feed additives (AFA and MFA) are regulated separately from veterinary medicines (including medicated feed).

Approval of feed additives is co-ordinated through Directorate General VI of the European Commission. The basic Council Directive concerning AFA is 70/524/EEC, with amendments (especially 84/587/EEC and 96/51/EC). Certain provisions of the latest amendment will be in force on April 1 1998 and the remaining provisions by October 1 1999.

When the "5th amendment" (Council Directive 96/51/EC) takes force, some important changes will follow. Approvals of high technology additives will be brand-specific. The responsibilities of all involved parties have been clearly defined. Further, possibilities of periodical renewal of authorisations are provided.

In article 3 of this Directive (formerly Article 7 of Directive 84/587/EEC) important conditions that must be fulfilled are listed.

It should be noted that, although the phrasing of some of the conditions has been altered (cf. Article 7, 2A of 84/587/EEC), the spirit is essentially the same as in the directive presently in force.

Directive 70/524/EEC as amended in 96/51/EC, Article 3a

Community authorisation of an additive shall be given only if:

- a) *when used in animal nutrition it has one of the effects referred to in Article 2 (a)¹;*
- b) *taking into account the conditions of use, it does not adversely affect human or animal health or the environment, nor harm the consumer by altering the characteristics of livestock products;*
- c) *its presence can be monitored:*
 - *as an additive per se*
 - *in premixtures*
 - *in feedingstuffs or, where appropriate, in feed materials;*
- d) *at the level permitted, treatment or prevention of animal disease is excluded; this condition does not apply to additives belonging to the group of coccidiostats and other medicinal substances;*
- e) *for serious reasons concerning human or animal health its use must not be restricted to medical or veterinary purposes.*

¹ *In article 2 of this directive, favourable effects of additives are specified*

According to Council Directive 95/69/EC, establishments where AFA are produced or mixed need specific approval.

Council Directive 94/40/EEC (amending 87/153/EEC), presently under revision, fixes standard data requirements for dossiers used to support approval of additives, so called guidelines. Specified data with relevance for the conditions cited above are to be provided.

After consulting the Member States in the Standing Committee, and evaluation by the Scientific Committee of Animal Nutrition (SCAN) the Commission may, if the substance is found to meet the requirements of the directives, be authorised for use.

If, as the result of new information or a reassessment of existing information, a Member State has specific grounds for establishing that the use of one of the authorised additives (or its use in specified conditions) constitutes a danger to animal or human health or the environment, the Member State may temporarily suspend or restrict the application (so called "safe guard clause", Article 11 of Council Directive 70/524/EEC). The other Member States and the Commission shall immediately be notified and informed of the reasons for the decision. The Commission shall examine the grounds cited by the member state and consult the Member States. The

Commission shall then deliver its opinion and take appropriate measures. If any of the conditions laid down in the article cited above are no longer met, a regulation shall be adopted to withdraw the additive.

Presently authorised AFA (annex I or II of 70/524/EEC) are bacitracin, flavomycin, avoparcin, spiramycin, tylosin, virginiamycin, carbadox, olaquinox, monensin, salinomycin and avilamycin (table 2.II). Ardacin has been approved under Annex II but the authorisation is not expected to be prolonged.

A number of additives have, since the original list in 1970, been withdrawn from the list of authorised additives because of a decision to restrict certain antibiotics to therapeutic use (e.g. penicillin, streptomycin and tetracyclines) .

2.3.2 Coccidiostats and other medicinal feed additives

The anticoccidial agents authorised for use in poultry within the EU (Council Directive 70/524/EC) are listed in Table 2.III. Apart from being used as anticoccidials in poultry some coccidiostats are also approved for the prevention of coccidiosis in rabbits. Some ionophores are also approved for growth promotion (see table 2.II). The other medicinal substances include the antihistomonals which are mainly used in turkeys.

Table 2.II. Antibacterial feed additives authorised for growth promotion, including most of the applications (Council Directive 70/524/EEC)

Tabell 2.II. Antibakteriella fodertillsatser godkända som tillväxtbefrämjande medel, samt flertalet godkända applikationer (rådsdirektiv 70/524/EEG)

Substance	Animal species or category	Maximum age	Min-max content, mg/kg	Examples of other provisions
ANTIBIOTICS				
Avilamycin	piglets	4 months	20-40	
	slaughter pigs	4-6 months	10-20	
	chickens		2.5-10	
Bacitracin (zinc bacitracin)	layers		15-100	
	turkeys	4 weeks	5-50	
	turkeys	26 weeks	5-20	
	other poultry	16 weeks	5-20	certain species excepted
	piglets	4 months	5-50	
	calves	16 weeks	5-50	
	calves	6 months	5-20	
Flavo-phospholipol (flavomycin, bambermycins)	layers		2-5	
	turkeys	26 weeks	1-20	
	other poultry	16 weeks	1-20	certain species excepted
	piglets	3 months	10-25	milkreplacements only
	slaughter pigs	6 months	1-20	
	calves	6 months	6-16	
	beef		2-10	
Spiramycin	turkeys	26 weeks	5-20	
	other poultry	16 weeks	5-20	certain species excepted
	calves	16 weeks	5-50	
		6 months	5-80	milkreplacements only
	piglets	4 months	5-50	
	slaughter pigs	6 months	5-20	
Tylosin (tylosin-phosphate)	piglets	4 months	10-40	
		6 months	5-20	
Virginiamycin	layers		20	
	turkeys	26 weeks	5-20	
	other poultry	16 weeks	5-20	certain species excepted
	piglets	4 months	5-50	
	slaughter pigs	6 months	5-20	
	calves	16 weeks	5-50	
	beef		15-40	
Monensin	beef		10-40	
Salinomycin	piglets	4 months	30-60	
	slaughter pigs	6 months	15-30	
GROWTH PROMOTERS				
Carbadox	piglets	4 months	20-50	withdrawal time 28 days
Olaquinox	piglets	4 months	15-50	withdrawal time 28 days
	piglets	4 months	50-100	milkreplacements only

Table 2.III. Coccidiostats and other medicinal substances authorised as feed-additives within the European Union (Council directive 70/524/EEC)

Tabell 2.III. Koccidiostatika och andra medicinska substanser godkända som fodertillsatser inom den Europeiska Unionen (rådsdirektiv 70/524)

Generic name	Species/category	Maximum age	Content in feed (mg/kg)	Withdrawal, days
Amprolium	poultry	from laying onwards	62.5-125	3
Amprolium + ethopabate	chickens for laying, turkeys, guinea fowls	from laying onwards	66.5-133	3
Arprinocid	chickens		60	5
	chickens for laying	16 weeks	60	5
Decoquate	chickens for fattening		20-40	3
Diclazuril	chickens for fattening		1	5
Dinitolmide (DOT)	poultry	from laying onwards	62.5-125	3
Halofuginone	chickens for fattening		2-3	5
	turkeys	12 weeks	2-3	5
Lasalocid	chickens for fattening		75-125	5
	chickens for laying	16 weeks	75-125	
	turkeys	12 weeks	90-125	5
Maduramicin	chickens for fattening		5	5
Meticlorpindol	chickens for fattening	laying and onwards	125	5
	guinea fowl		125	5
	rabbits		125-200	5
Meticloprindol+ methylbenzoquat	chickens for fattening		110	5
	chickens for laying	16 weeks	110	-
	turkeys	12 weeks	110	5
Monensin	chickens for fattening		100-125	3
	chickens for laying	16 weeks	100-120	
	turkeys	16 weeks	90-100	3
Narasin	chickens for fattening		60-70	5
Narasin + nicarbazin	chickens for fattening		80-100	7
Nicarbazin	chickens for fattening	4 weeks	100-125	9
Robenidine	chickens for fattening		30-36	5
	turkeys		30-36	5
	rabbits		50-66	5
Salinomycin	chickens for fattening		50-70	5
Semduramicin	chickens for fattening		25	5
OTHER MEDICINAL SUBSTANCES				
Dimetridazol	turkeys	from laying on	100-200	6
	guinea fowl	from laying on	125-150	6
Ipronidazol	turkeys	from laying on	50-85	6
Ronidazol	turkeys	from laying on	60-90	6
Nifursol	turkeys		50-75	5

2.4 Current Swedish legislation

2.4.1 Antibiotics and growth promoters

The basic Swedish legislation with relevance for AFA and medicinal feed additives is the Feedingstuffs Act (SFS 1985:295), that came into force in 1986. According to this act, antibiotics and chemotherapeutics may only be incorporated in animal feed for the purpose of preventing, alleviating or curing disease, i.e. not for growth or yield promoting purposes.

2.4.2 Coccidiostats and other antiprotozoals

In Sweden, as in other countries, the coccidiosis control in broilers relies primarily on coccidiostats. The drugs approved in Sweden for this purpose are listed in table 2.IV.

In accordance with the Feedingstuffs Act, coccidiostats are available as pharmaceutical specialities (veterinary medicinal products), on veterinary prescription only. As a result of the legislative changes in 1986, some coccidiostats earlier approved as feed additives are today instead authorised for use as medicated feed.

Table 2.IV. Coccidiostats approved as pharmaceutical specialities in Sweden

Tabell 2.IV. Koccidiostatika godkända som läkemedel i Sverige

Generic name (ATCvet code)	Species/ category	Maximum age	Content in feed (mg/kg)	With- drawal time, days
Amprolium + ethopabate (QP51A X59)	chickens for laying	14 weeks	125+8 in starter feed, 75+4.8 in grower feed	3
Halofuginone (QP51A X08)	poultry		3	3
Lasalocid (QP51 A H02)	chickens for fattening		75-125	3
Maduramicin (QP51A X10)	chickens for fattening		5	3
Monensin (QP51A H03)	chickens for fattening		100-120	3
Narasin (QP51A H04)	chickens for fattening		70	3

Ronidazole (Ridzola[®] vet.) and dimetridazole (Emtryl[®] vet.) were approved as pharmaceutical specialities for use against swine dysentery and, for dimetridazole, also against histomoniasis in poultry, until 1995. Ronidazole and dimetridazole were then withdrawn from the market in accordance with the provisions of the Committee of Veterinary Medicinal Products (CVMP). Ipronidazole has never been approved in Sweden.

2.4.3 Antibacterials for use in therapy or prophylaxis

Antibacterials for use in animals are authorised by the Medical Products Agency. Withdrawal times, based on the maximum residue limits (MRL) fixed by the CVMP, are assigned by the National Food Administration. All antibacterials, including dermatological and other formulations, for use in animals are available on prescription only.

Veterinary prescriptions are subject to the regulations of the Medical Products Agency. All veterinarians are under the supervision of the Swedish Board of Agriculture, that also issue regulations pertinent to antibacterials. The general veterinary regulations of the Board of Agriculture (LSFS 1982:43) state that "the usage of medicinal products shall be well motivated. When choosing type and dosage of a medicinal product the risk of residues in food of animal origin shall be taken into account". Further, in the regulations for veterinarians with special reference to prescriptions of medicinal products (LSFS 1979:8), it is stated that "prescribing and handing out medicinal products shall be done with great restrictivity and only when the need is apparent...". According to the same regulations, medicinal products may, on each occasion, be prescribed or handed out only after careful examination of the diseased animal or herd, on the premises, except in the case of general prophylactic measures other than antibiotics and chemotherapeutics, or if other special circumstances are at hand.

Regarding medicated feed, the provisions of Directive 90/167/EEC are implemented.

Of the antibacterials authorised as feed additives in the EU, tylosin and virginiamycin are presently available in Sweden for use in medicated feed. Olaquinox was withdrawn from the market in 1997. Spiramycin is authorised for use in food animals for injections only.

2.4.4 Distribution of medicinal products

All prescriptions and deliveries of drugs are administered by the pharmacies which all belong to Apoteksbolaget (The National Corporation of Swedish Pharmacies). This means that all prescriptions of drugs for therapeutic use,

including those mixed by the feed mills, are handled by the Swedish pharmacies. From the above follows that veterinarians are not allowed to sell or dispense medicinal products but may, for practical reasons, hand out the products to the end-user. In such cases, the quantity delivered may only satisfy the need for treatment of the animal or group of animals in question.

2.4.5 Control

The manufacturing of animal feed is supervised by the Board of Agriculture. As part of the supervision, annual statistics on quantities of feed delivered and on specifics such as medicated feed are gathered.

All veterinarians are, as mentioned, subject to supervision by the Board of Agriculture. On a regional level, the County veterinarian (an official employee) is in charge of supervision of the work of the veterinarians, animal welfare and disease control. Traditionally, most of the veterinarians in food animal practice are employed by the Board of Agriculture. However, private practice in food animals is becoming more common.

Sales statistics of antibacterials for use in animals is an indirect way of control. As mentioned above, the Board of Agriculture gathers annual statistics on sales of medicated feed. The National Veterinary Institute has, based on data provided by Apoteksbolaget, calculated the total sales of antibacterials for veterinary use in Sweden (see 2.6). Recently, Apoteksbolaget has initiated yearly surveys of all veterinary prescriptions of medicated feed. By combining these three sources of statistics, the surveillance is maximised. Discrepancies or undesired trends may be detected and their causes found and corrected (see 2.6.5).

2.5. The Swedish ban

2.5.1 The preambles

Similar to the situation in other countries, some Swedish scientists viewed the practice of routine addition of antibacterials to animal feeds with scepticism. The knowledge of the transmissibility of resistance between bacteria through plasmids led to exhortations for a restrictive use of antibacterials in animals (Rutqvist, 1970). Following the recommendations of the Swann Committee in the UK, a broader debate was initiated, which eventually led to a reassessment of the use of antibacterials as feed additives (LBS, 1977).

A working group of the Board of Agriculture concluded, among other things, that "the use of AFA entails a risk of increased resistance in bacteria but as the substances in use are mainly active against gram-positive bacteria

from which resistance is not transferred, the impact of such development is negligible". On the other hand, a negative attitude to all kinds of additives among consumers was noted by the group. The benefits, in terms of increased production and prevention of certain diseases, were also acknowledged (LBS, 1977). Legislative changes, especially in the requirements for approval, were proposed in order to mitigate possible risks.

At the same time, the farmers were growing increasingly sceptical towards feed antibiotics. They were concerned that the continued use of antibiotics might harm consumer confidence. The Federation of Swedish Farmers (LRF) made a policy statement, declaring that Swedish agriculture aimed towards a more restricted and controlled use of antibiotics. In a letter to the Ministry of Agriculture in 1984, the LRF requested a ban on the use of antibacterials as feed additives.

In response to the above, the Ministry of Agriculture drafted a new Feedingstuffs Act (Government Bill 1984/85:146, Swedish Government). Among other things, the draft proposed that the use of antibacterials in feed should be restricted to treatment, prevention or cure of diseases, i.e. their use for growth promotion should not be allowed. The grounds cited for this amendment was the risk for increased resistance, especially the risk for cross-resistance to other substances and the risk of increased susceptibility of animals to salmonella and other enteric pathogens.

The Feedingstuffs Act was accepted by the parliament in November 1985 and came into force in January 1986 (SFS 1985:295).

2.5.2 Effects on animal health

The effects of the ban on AFA on animal health has recently been reviewed by Wierup (1996). In essence, the following is based on his review.

Calves

In the 60s and 70s different AFA (bacitracin, flavomycin, oleandomycin, spiramycin) were used in concentrate and milk replacers for calves. However, the effect was considered doubtful and documented improvements in animal growth was not always seen in repeated experiments (e.g. Jonson and Jacobsson, 1973; Wierup *et al.*, 1975). Due to this scepticism, the use of AFA in calf rearing had more or less come to an end before the ban in 1986. Negative clinical or other effects as a consequence of the ban have not been reported.

Chickens

The antibacterial feed additive nitrovin came into use in the 70s and through this, existing problems with necrotic enteritis in broiler chickens were almost completely eliminated. Later, nitrovin was gradually replaced by avoparcin. Reports on an effect of avoparcin on salmonella colonisation and excretion (e.g. Smith and Tucker, 1978; Smith and Tucker, 1980) gave rise to concern, as the broiler production put considerable efforts into the salmonellosis control programme. This prompted a change from avoparcin to virginiamycin in the early 80s. In the autumn of 1985 (before the ban), outbreaks of necrotic enteritis were reported with increasing frequency. This was dealt with by increasing the dosage of virginiamycin from 10 to 20 ppm.

At the time of the ban, the chicken producers identified the occurrence of clinical or subclinical necrotic enteritis as the main problem to tackle. A committee with representatives of the producers, the feed industry and veterinarians from animal health areas as well as from the meat inspection services was put together. The aim of the committee was to initiate studies and to suggest appropriate solutions to the problem. It was agreed that a transition period would be necessary and that virginiamycin would be prescribed during this period, in the dose of 20 ppm.

Field trials where no medicated feed was used indicated that a number of factors needed correction. It was concluded that the construction and climate of stables, hygiene, management and feed composition all contribute to the occurrence of necrotic enteritis in broiler production. Further, it was found that coccidiostats of the ionophore type also prevent necrotic enteritis. Strong emphasis was placed on improving animal environment because many diseases, including necrotic enteritis, have a multifactorial background. A special bonus was given for good animal management and care which also led to improvements in the total level of quality of the production.

In 1988, all prophylactic medications were abandoned and, in case of outbreaks, a two day treatment with phenoxymethyl penicillin in the drinking water was applied. The amount of active substance of antibiotics which was used for treatment decreased from about 2 000 kg of virginiamycin in 1987 to 100 kg of phenoxymethyl penicillin in 1988 (expressed as active substance, Wierup, 1989). Today the need for such treatment of necrotic enteritis is more or less completely eliminated.

More formal, scientific studies were also initiated to find alternative ways to control necrotic enteritis. Studies on animal feed included effects of enzymes and certain probiotics, the structure of the feed, different levels of protein and different sources of proteins and coccidiostats (Elwinger and Teglöf, 1991; Elwinger *et al.*, 1992; Elwinger *et al.*, 1994; Elwinger *et al.*, 1996).

The results indicated that additions of enzymes acting on carbohydrates and the addition of certain probiotics to develop an adequate intestinal

bacterial flora reduced the incidence of necrotic enteritis. Dietary levels of protein were reduced and amino acids were added which also resulted in an improved hygiene and health.

In retrospect the most important changes were related to feed, involving a reduction of protein content, a higher fibre content and supplementation with enzymes together with the utilisation of ionophores for prevention of coccidiosis. The feeding strategies employed were developed through close collaboration between the feed industry and the farmers.

As mentioned, the coccidiostats of the ionophore type that are now used do have antibacterial effects and act prophylactically against necrotic enteritis. However, the sanitary situation of broiler chicken rearing in Sweden today would not have been reached without the above mentioned enforcements.

After the ban, coccidiostats were prescribed as medicated feed. Close monitoring of the efficiency of coccidiostats was introduced in Sweden in 1986. Since 1991, the efficiency of the drugs is studied by lesion scoring (according to methods described by Johnson and Reid, 1970) in approximately 5% of the Swedish broiler flocks, representative of all individual feed mills. The results are used for surveillance of the coccidiosis situation and to adapt the coccidiostatic regimen accordingly. In addition, all broiler flocks are under direct veterinary supervision as part of the salmonella control programme.

Egg production

AFA have never been authorised for use in laying hens in Sweden. Consequently, the ban in 1986 had no impact on the egg production. Following the ban, coccidiostats were prescribed as medicated feed to replacement birds. This practice is now being gradually replaced by the use of vaccines.

Turkeys

The situation for turkey producing units was similar to that for production of broiler chickens. Before 1986, AFA were used for prevention of necrotic enteritis. The ban did not result in noticeable clinical problems or reduced growth rate.

Weaner pigs

Before 1986, practically all piglets were given AFA (olaquinox or carbadox), from weaning until delivery to the finishing units at the age of 10-12 weeks. Slaughter pigs were, to a lesser extent, given AFA (avoparcin or virginiamycin) until slaughter.

Production averages from 220 piglet producing herds from the years 1985 and 1986 have been assessed statistically (Robertsson and Lundeheim, 1994). For piglet production, problems emerged as a consequence of the removal of olaquinox which was used for weaning piglets. However, the pre-weaning piglet mortality, and the number of piglets produced per sow and year, did not show significant differences between the two years. In contrast, post-weaning mortality was significantly higher ($p < 0.001$), about 1.5 percentage units, during 1986 than in 1985. The time to reach 25 kg increased by 5-6 days. Total efforts to prevent post-weaning diarrhoea, including advisory and preventive measures and treatments, increased four-fold. Data from units which were used for the above evaluation has now also been evaluated for the 10-year period 1986-1995. A comparison of the average values for 1994-1995 and 1986-1987 reveals post-weaning mortality to have decreased with 0.9 percentage units and the age at 25 kg to have been reduced by 1-2 days (Wierup, 1996).

In connection with the ban of AFA it became obvious that clear guidelines for veterinarians on how to prescribe antibiotics as medicated feed were lacking. Such guidelines were adopted by the Swedish Veterinary Association in 1990 (SVS). The guidelines emphasise that the prescription of feed antibiotics should always be based on a diagnosis together with a thorough evaluation of contributory factors and accompanied by recommendations on hygienic and other prophylactic measures.

In a study from 1994, Holmgren and Lundeheim analysed the results from 55 piglet producing units in the western part of Sweden. They concluded that the medicated feed prescribed to weaning pigs was clinically motivated and in accordance with the guidelines formulated by the SVS. The need for medicated feed differed markedly between herds and this could be ascribed to weaning and management systems. The production results were highly correlated to the rearing systems and to the level of hygiene. In herds rearing post weaning pigs on deep litter bedding the use of medicated feeds was three to four times lower than in herds with pigs in traditional post weaning pens. The study also underlines the problem with preventing weaning diarrhoea in units with limited facilities to arrange satisfactory sectioning and hygiene.

Efforts have also been undertaken to make adjustments in the composition of pig feed. The most prominent changes have been a lowering of the protein content, use of water soluble fibres and supplementation with amino acids (Göransson *et al.*, 1995). An antisecretory factor, preventing liquid penetration to the gut induced by enterotoxin, has also been explored as a preventive measure (Göransson *et al.*, 1993).

A notable effect on weaning diarrhoea through addition of high levels of zinc oxide has been reported (Holm, 1989; Poulsen, 1995). Zinc oxide has a preventive effect on weaning diarrhoea equal to the effect which is reached

when using olaquinox (Holmgren, 1994b). Since 1992, zinc oxide is approved for incorporation into piglet feed, at 2000 ppm of zinc. Not all piglet feed contains high levels of zinc, as many producers do not experience problems with weaning diarrhoea. The intention was that the use of zinc oxide would only be temporary, until other measures to prevent weaning diarrhoea had been developed. Presently, the long term impact of the use of zinc oxide is under debate, and the Board of Agriculture is currently considering a phase-out and subsequent ban on the use of zinc oxide in high doses.

After the ban of AFA numerous measures have been, and are continuously, undertaken to optimise rearing and production systems and to employ available techniques (e.g. sectioning of buildings, age segregation, planned production). The ban also stimulated a development towards new rearing systems. The weaning of piglets on deep litter in large groups is one example and the so called birth-to-slaughter system which is based on production in the same pen from birth to slaughter is another. The adjustment of old buildings and pens to the new production system is expensive and until such adjustments can be done antibiotics are used to combat weaning diarrhoea in some units.

The experiences from the clinical problems which emerged after the ban indicate that single environmental or rearing measures are seldom enough to correct the situation. A combination of efforts aiming towards a health-orientated production system is often necessary. Many problems have been solved while others await solution.

Slaughter pigs

The ban of AFA did not create obvious clinical problems for growing or finishing pigs. The production results from this sector are comparable to those from, for example, the Danish production (source: Advisory Service Optima).

2.6 Sales of antibacterials for veterinary use in Sweden

2.6.1 Materials and methods

Data for 1980-1993 have been compiled from earlier publications (Wierup *et al.*, 1987; Björnerot *et al.*, 1996). For the years 1994-1996, data have been calculated from the same sources as these authors have used (see table 2.V).

All data on pharmaceutical specialities are based on the sales statistics in the Central Statistics System of Apoteksbolaget (National Corporation of Swedish Pharmacies). This system contains registers of all sales from the

wholesalers to the local pharmacies (all belonging to Apoteksbolaget), and to the feed companies. As all pharmaceutical specialities are distributed from these wholesalers to local pharmacies or to authorised feed companies, these figures represent the total consumption in Sweden.

The local pharmacies normally have short storage periods for these products, so it can be assumed that the products sold were also consumed during the respective periods.

With regard to antibacterials used as feed additives before 1986, data has been gathered from the Board of Agriculture. Additionally, for 1980-1993 data on sales of unlicensed pharmaceutical specialities, sold with special permission from the Medical Products Agency were gathered from the respective pharmaceutical companies.

The antibacterials included are pharmaceutical specialities approved for general or local use in veterinary medicine (ATCvet¹ code QJ01, QJ51, QA07A, QG01A, QP51AA and QP51AG). For 1980-1993, preparations for dermatologic use (ATCvet code QD06, QD07C, QS02C) are also included. For 1980-1984, feed additives not approved as pharmaceutical specialities and thus not delivered through pharmacies were also included. Thus, the statistics show the total amount of antibacterials sold by pharmacies or delivered by feed mills during the specified time period. It was assumed that the whole amount sold was used for animals. The figures include antibacterials for all animal species (food animals, fish, pets and horses).

All data are expressed as kilogram of active substance. In formerly published data, the weight of the procaine part of procaine penicillin was included in the presentations. As the procaine part of the molecule is not active in an antibiotic sense, procaine penicillin has been calculated to benzyl penicillin as 0.6 times the weight of procaine penicillin. Further, some corrections have been made of erroneous macrolide figures.

2.6.2 Total consumption

The total usage of antibacterials is presented in table 2.Va. For clarity, table 2.Vb lists examples of pharmaceutical specialities included in the different groups in table 2.Va.

Since 1986, the total usage has decreased from a mean on 45 tonnes/year during 1980-1984 to 20 tonnes in 1996 (a decrease by 55%). The total sales of formulations intended for treatment of groups of animals, i.e. through feed or water, was about 6 tonnes in 1996 (sum of figures in table 2.VI). This means that more than two thirds of the total consumption in 1996 was in the

¹ ATC = Anatomical Therapeutic Chemical classification system

form of formulations for treatment of individual animals (e.g. parenteral drugs, intramammaries or tablets).

As the different substances in question are not equal in their biological activity per weight unit, total figures might be misleading (i.e. if a substance requiring high dosages for full efficacy is replaced by a more active substance, a false impression of a reduction could be given). Therefore, each substance group should be assessed separately for trends.

Of the pharmaceutical specialities that were authorised at the time of the ban, the sales of G- and V-penicillins, macrolides and trimethoprim-sulphonamides have increased over time. Among the penicillins, only formulations suitable for treatment of single animals (parenteral drugs, tablets) are presently approved. The group consists mainly of formulations for injection and are therefore assumed to be used largely for the treatment of mastitis in dairy cattle (Nilsson and Greko, 1997).

Similarly, the trimethoprim-sulphonamides are only available for treatment of individual animals. About 15 to 20 years ago, the main indication for trimethoprim-sulphonamides in pigs, namely neonatal piglet diarrhoea, was very common. However, due to improved management and housing conditions in combination with efficient vaccines, neonatal piglet diarrhoea is no longer a major clinical problem. The observed increase in consumption is therefore likely to be derived from an increased usage of trimethoprim-sulphonamides in dairy cows and lately, following the introduction of formulations for oral use, in horses.

Although the amount of macrolides sold as formulations for injection increased in the beginning of the 90s (Nilsson and Greko, 1997), the major part of this increase derives from macrolides for oral use (mixed in feed or water). This increase, and that of the pleuromutilins, is believed to reflect an increase in the incidence of swine dysentery (the major indication for those drugs). The increase during 1995 and 1996 is likely to be explained by the fact that the nitroimidazoles, the third category of drugs (formerly) available for this indication, were withdrawn from the market in 1995.

The usage of tetracyclines and aminoglycosides has decreased. The changes in the latter group is partly explained by a decrease in usage of the combination of penicillin and dihydrostreptomycin. The usage of tetracyclines will be further commented on below.

The consumption of substances formerly approved as feed additives and subsequent to the ban approved as therapeutics (quinoxalines and streptogramins) has decreased substantially in spite of higher doses being given for therapy than for growth promotion.

Table 2.Va. Total animal usage of antibacterials in animals Sweden during 1980-1996 expressed as kg active substance (Data for 1980-1993 from Wierup *et al.*, 1987; Björnerot *et al.*, 1996 and for 1994-1996 Greko, unpublished)

Tabell 2.Va. Total förbrukning av antibiotika för användning till djur uttryckt som kg aktiv substans 1980-1996 (Data från 1980-1993 från Wierup *et al.*, 1987; Björnerot *et al.*, 1996 och från 1994-1996 Greko, opublicerat)

Active substance	Year (kg active substance)												
	1980	1982	1984	1986	1988	1989	1990	1991	1992	1993	1994	1995	1996
G and V penicillins ¹	3232	4153	4788	5934	7144	7007	7414	7423	7446	8301	10374	9082	8555
Aminopenicillins	60	248	714	540	655	681	738	769	837	859	941	928	829
Aminoglycosides	5274	4776	5608	2885	3194	2823	2539	2255	2139	1938	1696	1342	1066
Tetracyclines	9819	10765	12955	6585	4691	4624	4572	5414	8023	8815	7730	4968	2733
Macrolides	603	616	887	1144	1205	1156	1399	1478	1701	1562	1701	1803	1486
Fluoroquinolones						1	84	123	147	173	246	200	173
Sulphonamides	6600	4931	4325	3093	3072	2988	2510	2372	2362	2045	2323	2135	2198
Trimethoprim incl. derivatives	134	142	186	197	250	282	272	257	284	303	352	331	339
Pleuromutilins					124	140	229	236	268	384	465	889	1142
Quinoxalines ²	6250	7700	9900	1300	7164	7202	5778	5128	4917	3523	1904	1191	1098
Streptogramins ²	0	0	8800	1610	1088	2388	2413	1350	1275	550	600	575	525
Feed additives ³	8380	9370	700	870									
Other substances ⁴	918	869	1688	1616	1603	1871	2326	2666	1644	1627	1915	1125	163
TOTAL	41270	43570	50551	25774	30190	31164	30274	29274	31043	30080	30247	24569	20307

¹ Procaine penicillin has been calculated to and expressed as benzylpenicillin as 0.6 times total weight of procaine penicillin

² For 1980-1984 used as feed additives, 1986-1996 on veterinary prescription only at therapeutic dosages

³ Including avoparcin, bacitracin, nitrovin, oleandomycin and spiramycin

⁴ For 1980-1995 mainly nitroimidazoles (withdrawn in 1995)

Table 2.Vb. Explanations of groups in main table (table 2.Va)

Tabell 2.Vb. Förklaringar till de grupper som ingår i tabell 2.Va

Active substance	Examples of substances included
G and V penicillins	procaine penicillin, benzyl penicillin, phenoxymethyl penicillin
Aminopenicillins	ampicillin, amoxicillin
Aminoglycosides	dihydrostreptomycin, gentamicin, neomycin
Tetracyclines	oxitetracycline, chlortetracycline
Macrolides	spiramycin, tylosin
Fluoroquinolones	enrofloxacin
Sulphonamides	sulphadiazine, sulphadoxine, formolsulphathiazole
Trimethoprim incl. derivatives	trimethoprim, baquiloprim
Pleuromutilins	tiamulin
Quinoxalines	olaquinox, carbadox
Streptogramins	virginiamycin
Feed additives	avoparcin, bacitracin, nitrovin, oleandomycin and spiramycin as feed additive for growth promotion
Other substances	nitroimidazoles, clindamycin, cephalosporins

2.6.3 Flock treatment

Of special interest is the consumption of antibacterials intended for group or flock medication. When considering the risk for development of resistance, it is unimportant whether these medications take place by feed or water.

Sales data on formulations of antibacterials intended for such use have therefore been extracted from the data on total consumption shown in table 2.Va for the years 1993-1996. In table VI, some trends can be observed. The consumption of tetracyclines and quinoxalines has decreased sharply during the period. The usage of macrolides and streptogramins show only minor fluctuations. The pleuromutilins seem to have replaced the nitroimidazoles following the withdrawal from the market of the latter substances in 1995.

Olaquinox

Olaquinox is exclusively used in pigs. The only indication is diarrhoea around weaning but some off label use against swine dysentery is likely to occur. The usage of olaquinox was reduced by 82% (total quantity of active substance per number of pigs produced) from 1985 to 1987. Thereafter, however, more antibiotics were prescribed and the total amount used during the subsequent years 1988 and 1989 was 5 and 6%, respectively, higher than

in 1985 (Björnerot *et al.*, 1996). Considering that the dosage prescribed in from 1986 and onwards was about three times higher than the AFA dosage employed before, a smaller fraction of the weaning pigs was treated with olaquinox. Calculated this way, the proportion of pigs that were treated was 12% in 1986 and 76% in 1989 and 12% in 1995 (Wierup, 1996). As mentioned, olaquinox is no longer available in Sweden.

Table 2.VI. Sales of formulations of antibacterials intended for treatment of animals through feed or water (flock or group medications) during 1993-1996 expressed as kg active substance

Tabell 2.VI. Försäljning av beredningsformer av antibakteriella medel avsedda för behandling via foder eller vatten (flock- eller gruppmedicinering) under 1993-1996 uttryckt som kg aktiv substans

ATCvet code	Active substance	Year			
		1993	1994	1995	1996
QJ01AA	Tetracyclines	8106	7036	4243	2088
QJ01FA	Macrolides	959	791	1029	975
QJ01XX91	Streptogramins	550	600	575	525
QJ01XX92	Pleuromutilins	344	422	815	1069
QJ01MA	Fluoroquinolones	13	30	31	27
QJ01MB	Quinoxalines	3523	1904	1191	1098
QP51AA	Nitroimidazoles	1490	1764	953	0

2.6.4 Coccidiostats

The combination of amprolium and ethopabate has been the drug of choice for prevention of coccidiosis in chickens intended for laying in Sweden. During the last few years, this product has been largely replaced by vaccination of replacement breeders.

For broilers, a limited number of ionophores have been available to the during the past ten years. Narasin is, by far, the most widely applied product.

The total usage of anticoccidials in feed has been calculated from the same sources as the antibacterials (see 2.6.1). Presently, approximately 13 500 kg active substance of coccidiostats is used annually in Sweden. The quantities in kg active substance sold from 1980-1996 have then been calculated to tonnes medicated feed on basis of recommended dosages. For the year 1986, an apparent error in the reports to the Board of Agriculture was discovered. Therefore, all figures for that year have been excluded. In figure 2, the results are compared with the number of broiler chickens produced in Sweden are shown in figure 2.I.

2.6.5 Some comments on the Swedish consumption statistics

The consumption statistics from Sweden show that the usage of antibacterials in animals can, and has been, reduced substantially. Little data are available on consumption statistics other than from the Nordic countries. Comparisons between countries are difficult, as no generally accepted method of correcting for differing numbers of animals of different species is available. The total numbers of animals in Sweden during 1988-1995 are presented in table 2.VII.

As mentioned, figures on the total usage of antibacterial drugs should be interpreted with caution. Each substance group should be assessed separately, and knowledge on common indications is necessary in order to interpret the figures. In attempts to control resistance to antibacterials and to minimise the environmental impact of these drugs, monitoring systems for both usage of antibacterial drugs per animal species and for resistance to antibacterials in animal microbiota are crucial. Whenever undesirable trends are observed, activities to disclose underlying causes and, if possible, corrected.

During the years 1980-1984, the consumption of tetracyclines in animals in Sweden increased markedly. Thereafter, the usage of these products decreased until 1988. From 1988-1993, an increase was again noted. The latter could not be connected to an altered disease situation, and therefore no obvious explanation was at hand. Further investigations into the precise origins of this were initiated in 1994 by the Board of Agriculture. It was revealed that the increase could almost entirely be explained by the prescriptions of one veterinarian to one herd. The veterinarian was reported, the cause corrected, and the tetracycline consumption is now 50% lower than before 1986.

The comparison of statistics from different sources can also be a valuable asset. Results from the recently introduced system for statistics on prescriptions of medicated feed (Odensvik, 1997), were compared to data on sales from wholesalers and to data on sales of medicated feed reported by feed mills to the Board of Agriculture. An excellent agreement between the different systems for statistics was found in question of antibacterial substances. However, for coccidiostats a major discrepancy was noted. The quantity covered by prescriptions in 1995 was about 65% of the quantities registered in the two other systems. The amounts used, according to the latter, match well the number of chickens produced in that year (figure 2.I). Therefore, it can be assumed that one or more feed-mills have delivered coccidiostats without veterinary prescriptions. One such case has been identified and legal action has been taken.

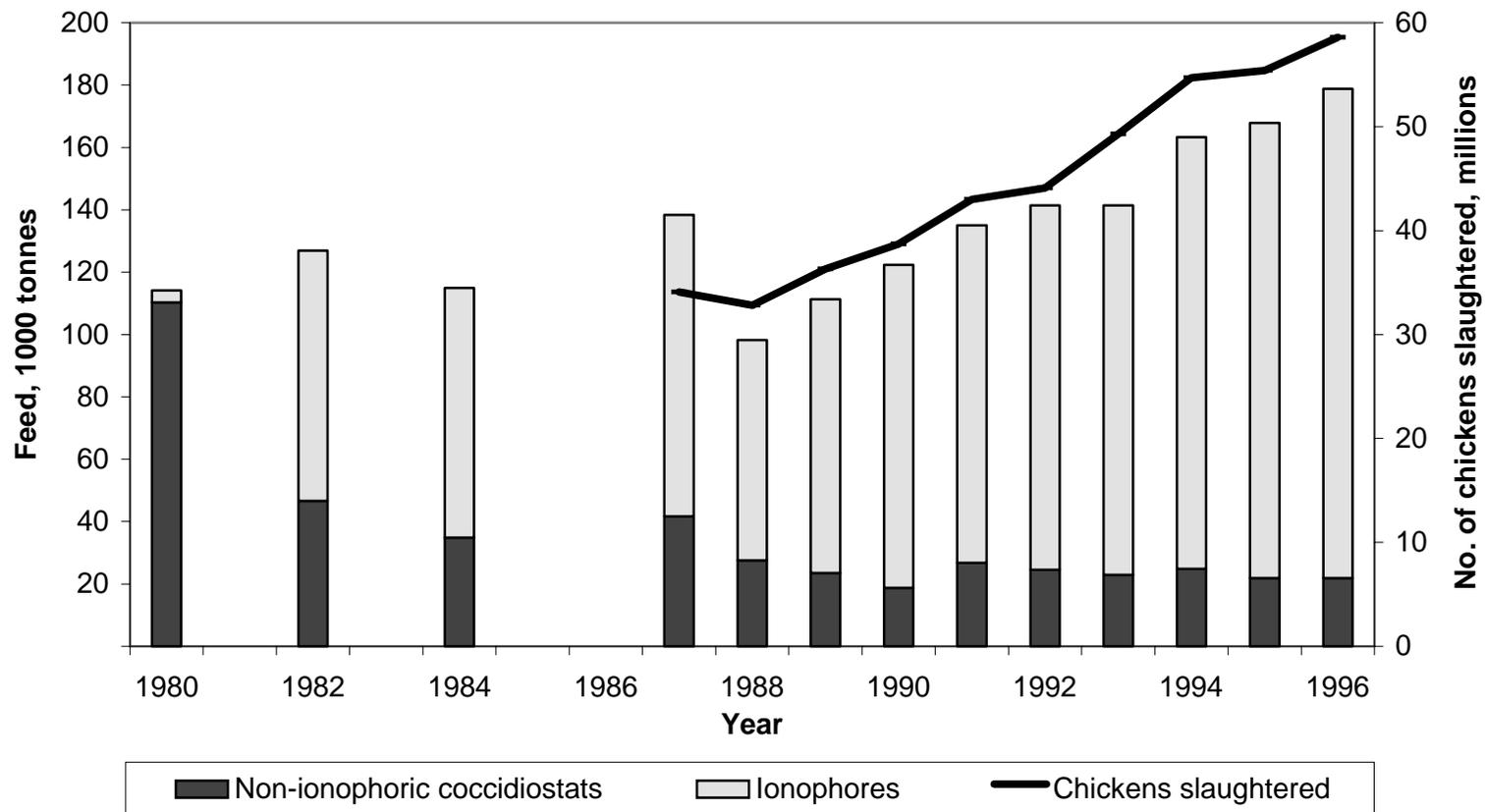


Figure 2.I. Usage of coccidiostats in Sweden expressed as quantity of medicated feed expressed as 1000 tonnes (left axis), and number of broiler chickens slaughtered in millions (right axis)

Figur 2.I. Användning av koccidiostatika i Sverige uttryckt som mängd foder uttryckt som 1000-tal ton med tillsatt läkemedel (vänster axel), och antalet slaktade slaktkycklingar i millioner (höger axel)

Table 2.VII. The number of food- and fur-producing animals and horses in Sweden during 1988 to 1995 (from Björnerot, 1996)

Tabell 2.VII Antal livsmedelsproducerande djur, pälsdjur och hästar i Sverige under 1988-1995 (från Björnerot, 1996)

	Year							
	1988	1989	1990	1991	1992	1993	1994	1995
Dairy cows ¹	564 550	568 857	576 409	528 212	525 948	524 520	509 431	482 118
Beef cows ¹	56 412	64 199	74 544	97 653	135 791	153 555	164 578	157 128
Heifers, bulls, bullocks ¹	527 522	533 296	543 458	543 418	565 463	548 620	560 911	595 521
Calves ¹	513 044	521 298	524 032	537 495	548 100	580 671	591 569	542 328
Ewes and rams ¹	159 107	160 647	161 974	168 178	180 067	188 944	195 736	195 439
Lambs ¹	235 606	239 859	243 621	250 605	267 394	281 743	287 692	266 410
Sows and boars ¹	242 872	239 482	229 683	226 839	233 133	249 314	249 171	244 950
Pigs slaughtered ² (fattening)	3 551 360	3 642 213	3 471 482	3 224 570	3 297 326	3 483 439	3 657 067	3 634 837
Layers ¹	6 411 821	6 366 499	6 391 943	6 145 174	6 063 357	5 764 401	5 918 015	6 100 270
Pullets ¹	2 274 343	2 267 732	2 175 676	2 579 920	2 166 105	1 908 371	2 175 337	1 811 509
Broilers slaughtered ³	32.8 x 10 ⁶	36.4 x 10 ⁶	38.7 x 10 ⁶	43.0 x 10 ⁶	44.1 x 10 ⁶	49.3 x 10 ⁶	55.8 x 10 ⁶	60.3 x 10 ⁶
Turkeys slaughtered ⁴	790 387	673 011	660 396	590 733	610 700	719 950	685 727	458 516
Domestic geese slaughtered ⁴	22 290	18 512	22 186	20 813	15 771	20 823	22 140	29 962
Ducks slaughtered ⁴	97 142	83 516	49 003	30 207	61 508	52 410	32 796	78 839
Horses ² (estimated figures)	~180 000	~180 000	~180 000	~180 000	~180 000	~180 000	~180 000	~180 000
Minks ⁵ (breeding females)	490 324	439 271	285 589	255 037	260 919	184 741	~212 450	~244 320
Foxes ⁵ (breeding females)	19 463	13 028	9 052	4 554	5 239	3 627	~ 3 600	~3 600

Sources: ¹Yearbook of Agricultural Statistics, 1989-1994. The numbers of individuals of different animal species are counted at a certain time (in June) every year, which means that the total numbers are not always included, for example calves born in the autumn that year are missing. Note, only animals on farms with more than 2.1 hectare land or in herds with at least 50 dairy cows or 250 cattle (bovines) or 250 pigs or 500 sheep (including lambs) or 1000 poultry (including chickens) are counted. ²Swedish Board of Agriculture. ³Swedish Poultry Meat Association, ⁴National Food Administration, ⁵Swedish Fur-breeding Association - estimated figures for 1994 and 1995.

2.7 Prevalence of bacterial resistance

Acquired resistance in bacteria to antibacterials is the results of reflects the exposure to the substances in question. Therefore statistics on prevalence of resistance in various bacterial species is an indirect way of assessing the consumption of antibacterials. Trends over time in prevalence of resistance among bacterial populations are likely to reflect quantities and patterns of usage.

2.7.1 Zoonotic pathogens

Salmonella

In accordance with the recommendations of WHO, antibiotic resistance in *Salmonella* spp. isolated from animals in Sweden has been monitored since 1976. Salmonellosis in animals is a notifiable disease in Sweden. All strains from animals isolated at or sent to the National Veterinary Institute are included. This surveillance is mainly motivated by the implications of resistant zoonotic bacteria for human health .

Table 2.VIII. Antibacterial resistance in *Salmonella* Typhimurium from animals in Sweden (Data from Franklin, 1997 unpublished and Franklin and Wierup, 1987; Franklin *et al.*, 1994; Franklin, 1995)

Tabell 2.VIII. Resistens mot antibakteriella medel hos *Salmonella* Typhimurium isolerade från djur i Sverige (Data from Franklin, 1997 unpublished and Franklin and Wierup, 1987; Franklin *et al.*, 1994; Franklin, 1995)

Substance	Breakpoint ¹ (µg/ml)	Resistance (%)		
		Year		
		(No. of isolates investigated)		
		1978-86 (n=117)	1988-91 (n=62)	1992-96 (n=108)
Ampicillin	>8	2	2	3
Streptomycin	>32	78	32	25
Tetracycline	>8	14	6	8
Trimethoprim-Sulphonamides	>0.5	0	0	2 ³
Enrofloxacin	>0.5	ND ²	0	1 ⁴

¹Breakpoint = value above which the isolate has been classified as resistant; ²ND = not determined; ³ Isolated from a pigeon; ⁴ Isolated from a horse

¹Brytpunkt = värde över vilken isolatet betecknats som resistent; ²ND = ej undersökt; ³Isoleraed från en duva; ⁴Isoleraed från en häst

The antibiotic resistance in *S. Typhimurium* in different time periods is shown in table 2.VIII. In the period 1978-1986, most strains were isolated from cattle. In 1988-1991 and 1992-1996, 43 and 36%, respectively, of the isolates were of bovine origin. In the last period, 45% were isolated from other sources than production animals, mostly wild birds. As apparent from the table, the number of resistant *S. Typhimurium* has decreased since the surveillance programme was initiated. All investigated *S. Typhimurium* strains and *S. Dublin* strains isolated in 1988-1996 were sensitive to modern quinolones, trimethoprim-sulphonamides, neomycin and gentamicin, except one isolate of *S. Typhimurium* from cattle which was resistant to trimethoprim-sulphonamides and one isolate of *S. Typhimurium* from a horse that was resistant to modern quinolones. The low prevalence of resistant salmonellae can probably be ascribed to the fact that antibacterials are not used to eliminate salmonella infections in animals.

Campylobacter

The frequency of resistance in *Campylobacter jejuni* isolated from chicken was investigated by Berndtsson (1996). Data on obtained minimum inhibitory concentrations (MIC) values are given in table 2.IX. Antibacterials are rarely used in broiler production in Sweden and the low proportion of resistant isolates seems to confirm the absence of a selective pressure. From other countries, quinolone resistance has been reported following the introduction of this drug (Endtz *et al.*, 1991).

Table 2.IX. Minimum inhibitory concentration (MIC) of antibacterials against 200 strains of *Campylobacter jejuni* isolated from chickens in Sweden

Tabell 2.IX Minsta hämmande koncentration av antibakteriella medel för *Campylobacter jejuni* isolerade från kycklingflockar i Sverige

Substance	Minimum inhibitory concentration (µg/ml)													
	< 0.06	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	> 128
Ampicillin						9	28	82	60	14	5	2	0	
Cephalotin										5	11	59	111	14
Doxycycline	15	18	99	40	23	3	1		1					
Enrofloxacin	4	71	91	18	7					9				
Erythromycin				7	39	92	39	21	2					

2.7.2 Animal pathogens

Escherichia coli

Escherichia coli strains with ability to cause piglet diarrhoea and edema disease are among the most important intestinal pathogens in piglets. Weaning diarrhoea in pigs has also been associated with *E. coli* but the role of *E. coli* in that disease is not clear. In order to assess the development of resistance over time, three different investigations regarding antibiotic resistance in porcine *E. coli* were compared. The majority of the included strains originated from pig herds with diarrhoea problems.

From table 2.X, it is apparent that the frequency of antibiotic resistant strains has not changed dramatically over the last ten years. The number of strains resistant to trimethoprim was unexpectedly high in 1981 to 1982 (9%), taking into account the fact that trimethoprim had only been used for 6 to 7 years in Sweden at that time. A further increase was expected but, as shown, did not take place. This might be explained by the improved health situation in piglets which presumably has been accompanied by a similar decrease in the usage of this particular combination of drugs.

Resistance to streptomycin and tetracycline is still high, but compared to results from other countries (e.g. Pohl *et al.*, 1991; Morvan and Moisan, 1994) the overall figures indicate a favourable situation.

Table 2.X. Antibiotic resistance in porcine *E. coli* strains isolated in Sweden during different time periods (%). (From Franklin unpublished data and Franklin, 1984; Melin *et al.*, 1996)

Tabell 2.X. Antibiotikaresistens hos *E. coli* från svin, isolerade i Sverige under olika tidsperioder (%) (Från Franklin opublicerade data och Franklin, 1984; Melin *et al.*, 1996)

Substance	Break-point ¹ (µg/ml)	Resistance (%)		
		Year (No. of isolates investigated)		
		1981-82 (n=200) ³	1989-91 (n=248)	1994 (n=464)
Ampicillin	>16	8	6	8
Streptomycin	>32	56	44	41
Tetracycline	>8	40	28	38
Trimethoprim-Sulphonamides	>8 ²	9	15	10
Enrofloxacin	>0.5	ND ⁴	<1	<1

¹Breakpoint = value above which the isolate has been classified as resistant; ² Denotes the trimethoprim concentration; ³ Denotes number of investigated isolates; ⁴ ND= not determined

¹Brytpunkt = värde över vilken isolatet betecknats som resistent; ² Avser koncentration trometoprim ³ n = antal undersökta isolat; ⁴ ND = ej undersökt

Staphylococcus aureus

Table 2.XI. Proportion of *Staphylococcus aureus* (isolated from clinical mastitis in dairy cows in Sweden during different years) with resistance to different antibacterials (Data from Franklin and Horn af Rantzien, 1983; Robertsson and Franklin, 1987; Nilsson, 1996)

Tabell 2.XI. Andel av *S.aureus* isolerade från klinisk mastit hos nöt i Sverige under olika år, vilka uppvisar resistens mot vissa antibakteriella medel (Data från Franklin and Horn af Rantzien, 1983; Robertsson and Franklin, 1987; Nilsson, 1996)

Substance	Break-point ¹ (µg/ml)	Resistance (%) Year (No. of isolates investigated)		
		1982 (n=287)	1985 (n=529)	1995 (n=181)
Penicillin	- ²	10	10	6
Oxacillin	>2	0	3	0
Erythromycin	>4	1	3	1
Spiramycin	>16	ND ³	ND	1
Tetracycline	>8	1	3	1

¹Breakpoint = value above which the isolate has been classified as resistant; ² Resistant isolates determined on the basis of β -lactamase production; ³ ND = not determined

¹Brytpunkt = värde över vilket isolaten bedömts som resistenta; ² Resitens bestämd som produktion av β -laktamas; ³ ND = ej undersökt

Among the udder pathogens causing bovine mastitis, *S. aureus* is the most important causative agent. It gives rise not only to severe acute inflammation but also to chronic subclinical conditions. In Sweden, about 25% of clinical mastitic cases are caused by this species. Besides *S. aureus*, a number of other staphylococcal species are associated with bovine mastitis. About 5 to 10% of the *S. aureus* isolates from clinical cases of mastitis are resistant to penicillin due to penicillinase production (Table 2.XI). Penicillin resistance is more common in coagulase-negative staphylococci (about 25%, L. Nilsson, personal communication²)

Serpulina hyodysenteriae

Serpulina hyodysenteriae is the causative agent of swine dysentery. Minimum inhibitory concentrations (MIC) for 67 Swedish strains were determined for seven antibiotics. The strains were isolated from herds with and without dysentery problems in the period 1988 to 1990 (Gunnarsson *et al.*, 1991). The distribution of MIC values are shown in Table 2.XII. The MIC values of tylosin and virginiamycin indicate that these drugs would no longer be effective for treatment of swine dysentery.

² Lolita Nilsson, Laboratory Veterinary Officer, Department of Mastitis, National Veterinary Institute (SVA), Sweden

Table 2.XII. Antibacterial sensitivity of 67 isolates of *Serpulina hyodysenteriae* from Swedish pigs (Data from Gunnarsson *et al.*, 1991)

Tabell 2.XII. Känslighet för antibakteriella medel hos 67 isolat av *Serpulina hyodysenteriae* från svenska grisar (Data från Gunnarsson *et al.*, 1991)

Antibacterial substance	MIC of different antibacterial substances ($\mu\text{g/ml}$)						
	No. of isolates						
Carbadox	<0.012	0.012	0.025	0.05	0.1	0.2	>0.2
	36	2	15	9	2	1	2
Olaquinox	<0.12	0.12	0.25	0.5	1	2	>2
	14	2	19	19	2	1	0
Tylosin	<1	1	2	4	8	16	>16
	5	1	7	15	11	14	14
Virginiamycin	<0.5	0.5	1	2	4	8	>8
	4	1	19	25	11	3	4
Tiamulin	<0.03	0.03	0.06	0.12	0.25	0.5	>0.5
	4	4	32	20	3	0	4
Tetracycline	<2	2	4	8	16	32	>32
	27	4	19	8	5	4	0
Ronidazol	<0.06	0.06	0.12	0.25	0.5	1	>2
	35	1	19	10	2	0	0
Dimetridazol	<0.06	0.12	0.25	0.5	1	2	>0.2
	0	1	0	2	4	11	49

2.7.3 Animal commensals

Surveys for antimicrobial resistance in commensals, i.e. non-pathogenic bacteria, have only recently been initiated in Sweden. Preliminary results from these studies are given below.

In a study on poultry bacteria, enterococci and *E.coli* were isolated on selective media from neck skin and hindgut samples representing 52 poultry flocks slaughtered in 9 different slaughterhouses (Greko, 1996). A total of 207 isolates of *Enterococcus* spp. and 194 isolates of *E.coli* were investigated for MIC values for a range of antibacterials. Results from this study are shown in table 2.XIII and 2.XIV.

The figures show an overall low level of resistance for both enterococci and *E.coli*. The one exception is the prevalence of resistance against tetracyclines in enterococci. Tetracyclines are not frequently used in Swedish poultry production, and corresponding figures for other bacteria isolated from chickens (*Campylobacter jejuni*, *E.coli*) do not indicate a high exposure to this drug.

Table 2.XIII Resistance in *Enterococcus* spp. isolated from chicken (neck-skin and faecal samples). No. of isolates = 194

Tabell 2.XIII Resistens hos Enterococcus spp. isolerade från kyckling (hals-skin och feces prov), Antal isolat = 194

Substance	Breakpoint (µg/ml) ¹	Proportion of isolates resistant (%)
Vancomycin	>4	0
Erythromycin	>2	17
Spiramycin	>16	15
Spiramycin	>8	15
Ampicillin	>8	0
Neomycin	>128	0
Streptomycin	>128	2
Chloramphenicol	>8	0
Tetracycline	>4	64
Trimetoprim-Sulphonamide	>0.5	0

¹Breakpoint = value above which the isolate has been classified as resistant

¹Brytpunkt = värde över vilket isolaten bedömts som resistenta

Table 2.XIV. Resistance in *E.coli* isolated from chicken (neck-skin and faecal samples). No. of isolates = 194

Tabell 2.XIV. Resistens hos E.coli isolerade från kyckling (hals-skin och feces prov). Antal isolat = 194

Substance	Breakpoint (µg/ml) ¹	Proportion of isolates resistant (%)
Ampicillin	>8	5
Neomycin	>64	0
Streptomycin	>64	7
Gentamicin	>8	0
Tetracycline	>4	17
Trimethoprim-Sulphonamides	>0.5	4
Chloramphenicol	>16	1
Enrofloxacin	>0.25	4
Nalidixic acid	>32	6
Olaquinox	>32	<1 ²

¹Breakpoint = value above which the isolate has been classified as resistant; ²One isolate, also resistant to enrofloxacin (MIC=1µg/ml)

¹Brytpunkt = värde över vilket isolaten bedömts som resistenta; ² Ett isolat, även resistent mot enrofloxacin

The antibacterial resistance of enterococci and *E.coli* isolated from piglets has recently been investigated in a cohort study (Greko, 1997; Melin *et al.*, 1997). Briefly, in a study aiming to investigate the possible occurrence of resistance to zinc oxide among commensal *E.coli* of piglets in Sweden, a

total of 300 faecal samples were examined. The samples were collected 2-3 weeks after weaning from piglets in a total of 30 herds; 10 herds were using olaquinox as medicated feed (160 ppm), 10 herds were using zinc oxide (2500 ppm) and 10 herds used no medication. No isolates of coliforms with resistance to zinc oxide were found (Melin *et al.*, 1997). All samples were also cultured on selective media containing 50µg/ml of vancomycin. No vancomycin-resistant enterococci were found.

From non-antibiotic containing media, enterococci and coliforms were selected at random from each sample for further investigation. Table 2.XV shows the results of determinations of minimum inhibitory concentration (MIC) of different antibiotics for *Enterococcus* spp. and table 2.XVI the corresponding results for *E.coli*.

Taken together, the figures are comparatively favourable. The number of isolates investigated from each group is too small to validate any conclusions with regard to differences between groups. Some conflicting observations on differences between groups can be made. For enterococci, the figures on resistance to neomycin and streptomycin in the non-medicated group seem higher than those from the other groups. On the other hand, such differences cannot be observed for *E.coli*. Similar observations can be made for *E.coli* and tetracycline resistance. Resistance to macrolides in enterococci seem to be more frequently of constitutive type in non-medicated groups. A lower prevalence of resistance to chloramphenicol in *E.coli* from the non-medicated group is not reflected in the figures for enterococci.

Table 2.XV. Resistance to different antibacterials in *Enterococcus* spp. isolated from weaned pigs in herds using different regimes

Tabell 2.XV. Resistens mot olika antibacteriella medel hos *Enterococcus* spp. isolerade från avvanda grisar i besättningar med olika regim

Substance	Break-point (µg/ml)	Resistance in isolates from sampling group (%): ¹			
		Olaquin- dox (n=69) ²	Zinc- oxide (n=57)	No medi- cation (n=92)	Total (n=218)
Erythromycin	>2	29	33	36	33
Spiramycin	>16	6	12	22	14
Spiramycin	>8	14	26	38	27
Ampicillin	>8	0	4	0	0
Neomycin	>128	3	2	10	6
Streptomycin	>128	9	5	18	12
Chloramphenicol	>8	0	2	1	1
Tetracycline	>4	42	54	33	41
Trimethoprim-Sulphonamide	>0.5	0	0	0	0

¹Breakpoint = value above which the isolate has been classified as resistant; ² n= number of isolates

¹Brytpunkt = värde över vilket isolaten bedömts som resistent; ² n= antal undersökta isolat

Table 2.XVI Resistance to different antibacterials in *E. coli* isolated from weaned pigs in herds using different regimes

Tabell 2.XVI Resistens mot olika antibakteriella medel hos E. coli isolerade från avvanda grisar i besättningar med olika regim

Substance	Break-point (µg/ml)	Resistance in isolates from sampling group (%): ¹			
		Olaquin- dox (n=60) ²	Zinc- oxide (n=73)	No medi- cation (n=85)	Total (n=218)
Ampicillin	>8	2	4	2	3
Neomycin	>64	10	12	8	10
Streptomycin	>64	13	26	13	17
Gentamicin	>8	0	4	0	1
Tetracycline	>4	12	4	26	10
Trimethoprim-Sulphonamides	>0.5	2	7	0	3
Chloramphenicol	>16	15	26	7	16
Enrofloxacin	>0.25	0	1	1	1
Enrofloxacin	>0.12	15	11	1	8
Nalidixic acid	>32	2	1	1	1
Olaquinox	>32	35	19	32	28

¹Breakpoint = value above which the isolate has been classified as resistant; ² n= number of isolates

¹Brytpunkt = värde över vilket isolaten bedömts som resistent; ² n= antal undersökta isolat

2.7.4 Some comments on the resistance figures

As shown above, available data indicate that antibacterial resistance is comparatively low in most bacteria from Swedish food producing animals. For most substances this reflects the consumption figures. However, other factors than antimicrobial usage may also contribute to keeping resistance to certain substances at a high level (see chapter 4). Continuous monitoring for antimicrobial resistance is necessary for detecting trends in resistance development. It is important to remember, though, that resistance data only shows the phenotypic expression of resistance traits. Thus, further research on the genes behind the resistance is also necessary.

2.8 Summary comments

Antibacterial substances are valuable therapeutic tools in both veterinary and human medicine. Their use both as therapeutics and as feed additives have contributed to the development of the contemporary animal industry.

In Sweden, all antimicrobial substances used in animals are classified as pharmaceutical specialities and are only available on veterinary prescription. The ban on antimicrobial feed additives in 1986 was associated with animal health problems in certain production systems. These health problems have to a large extent been overcome, and today's total consumption of antibacterials in veterinary medicine is considerably lower (50%) than what was used before the ban. The lower consumption is reflected in a comparatively favourable resistance situation in most animal bacteria. The Swedish experience shows that changes in production systems are necessary in order to adjust to animal production without antimicrobial feed additives, but also that such adjustments are possible and might pay off in a better situation regarding antibacterial resistance among zoonotic and animal bacteria.

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3 Mode of action and effects of antibacterial feed additives

3.1 Mode of action

The mode of action of antibacterial feed additives (AFA) has been the subject of numerous scientific reports. However, most of these deal with effects, rather than modes of action. The exact mechanisms regulating the growth promoting effect of AFA is not precisely known (for a review see Thomke and Elwinger, 1997c).

3.1.1 Effects

Rosen (1995) has compiled responses in poultry and pigs associated with dietary supplements of AFA (table 3.I). This table illustrates the diversity of the responses to AFA.

AFA will alter the intestinal bacterial flora and reduce the number of sensitive microorganisms. Most AFA in use within the EU have effects on gram-positive bacteria. An early and fundamental finding was that young germ-free chickens, that grew approximately 20% faster than conventionally-reared chickens, did not respond to dietary inclusion of AFA (Forbes and Park, 1959; Eyssen and De Somer, 1967). Inoculation of germ-free chickens with *Enterococcus faecalis* - a common inhabitant of the intestinal tract - lowered growth performance significantly. Dietary inclusion of AFA, however, restored growth performance of inoculated birds (Lev and Forbes, 1959; Eyssen and De Somer, 1967). Some other micro-organisms that have been associated with growth suppression are: *Enterococcus faecium* (Coates, 1980; Fuller, 1994) and *Clostridium perfringens* (Lev and Forbes, 1959; Stutz *et al.*, 1983a; Stutz *et al.*, 1983b). The mechanism for the proposed growth suppressing effect has not been clarified.

In animals given AFA, reduced weight of the intestine, thinning of the intestinal wall and shortening of the gut has been recorded (Jukes *et al.*, 1956; Stutz *et al.*, 1983b). These results suggest a lowered number of mucosal cells which, in turn, possibly also reflect the mucosal cell turn-over rate. Research has shown a similar lowered mucosal cell turn-over rate as well as a thinner intestinal wall in germ-free chickens, as compared to conventionally reared chickens (Coates, 1980). This indicates that the dietary inclusion of AFA will alter these intestinal characteristics towards the properties seen in germ free animals.

Table 3.I. Some physiological, nutritional and metabolic effects ascribed to AFA (modified from Rosen, 1995)

Tabell 3.I. Några fysiologiska, nutritionella och metaboliska effekter som tillskrivits AFT (modifierat från Rosen, 1995)

Physiological effects ^a		Nutritional effects		Metabolic effects	
Gut food transit time	▲	Energy retention	↗	Ammonia production	▲
Gut wall diameter	▲	Gut energy loss	▲	Toxic amine production	▲
Gut wall length	▲	Nitrogen retention	↗	Alpha-toxin production	▲
Gut wall weight	▲	Limiting amino acid supply	↗	Fatty acid oxidation	▲
Gut absorptive capacity	↗	Vitamin absorption	↗	Faecal fat excretion	▲
Faecal moisture	▲	Vitamin synthesis	▲	Liver protein synthesis	↗
Mucosal cell turnover	▲	Trace element absorption	↗	Gut alkaline phosphatase	↗
Stress	▲	Fatty acid absorption	↗	Gut urease	▲
Feed intake	↗,▲,→	Glucose absorption	↗		
		Calcium absorption	↗		
		Plasma nutrients	↗		

^a▲ denotes a reduction; ↗ denotes an increase; →denotes no change

It seems likely that the change of the intestinal flora and the subsequent changes in the intestine, including a slower intestinal passage rate, associated with AFA usage are linked to the many reported effects on nutrient breakdown, nutrient losses and nutrient absorption. Examples are the lowered breakdown of easily fermentable nutrients and the restriction in breakdown of essential amino acids, leading to a general nutrient saving effect.

3.1.2 Suggested main mode of action

As AFA are antibacterial substances, it is likely that their growth promoting effect is associated with their inhibitory effect on intestinal microbes. As discussed above, at dosages permitted for growth promotion, AFA will alter the intestinal microbial flora. Increased understanding of the complex relationships between the immune system and other functions of the body offer possibilities to better understand the mode of action of AFA.

Infections and growth rate

It is well established that growth rate and feed efficiency are reduced as a sequel to infection. This is why for example specific pathogen free (SPF) animals grow faster than animals in a conventional environment. Similarly animals reared in carefully cleaned and disinfected units grow faster than animals reared under a lower level of sanitary conditions. It has been shown that absence of disease increases the capacity of pigs to grow (Young *et al.*, 1959; Caldwell *et al.*, 1961). In Danish SPF systems, feed conversion is 3.9% lower and growth rate 6.4% higher than for conventionally reared pigs (Jorgensen, 1987). Similar observations have been reported in Swedish studies by Wallgren (1993a) (Figure 3.I). Chickens housed in clean, disinfected quarters also grow faster and more efficiently than chickens housed in less sanitary conditions, even in the absence of any clinical signs of infectious diseases (Roura *et al.*, 1992).

Immune responses and growth rate

An infectious challenge is a common form of stress encountered by growing animals. Infectious challenges may, or may not, result in clinical diseases, depending on the pathogenicity of the challenging microorganism and the immunocompetence of the animal. A stress response is indicated by decreased growth rates and quantitative changes in nutritional requirements (cit. from Klasing *et al.*, 1987). In humans, reduced food intake is considered to play a major role in infection-induced weight loss (Grunfeld *et al.*, 1996). This has also been shown in animals. Studying chickens, Klasing and co-workers (1987), found a growth depressive effect associated with an immune response. This effect was correlated to the immunogenic strength of the agent tested, the duration and the intensity of the immune response. The response to combinations of immunogens was additive. The dominating factor in the reduction of the animals' weight gain was shown to be a decrease in feed intake, while the remainder was the effect of less efficient routes in the intermediary metabolism (Klasing *et al.*, 1987). Roura and co-workers (Roura *et al.*, 1992) showed the influence of the animal environment on weight gain and feed efficiency in chickens (see table 3. IIb.). Studies in pigs

with a low or high level of chronic immune system activation revealed lower feed intake, reduced body weight gain, lower daily protein accretion and more feed required per kg weight gain in pigs with a high level of chronic immune system activation (see table 3.IIa.)(Stahly, 1996).

Table 3.IIa. Responses of pigs with a low or high level of antigen exposure to dietary antibacterial agents (carbadox). Adopted from Stahly (1996)

Tabell 3.IIa. Effekt av den antibakteriella fodertillsatsen carbadox på tillväxt och foderutnyttjande hos grisar med hög eller låg grad av antigenexponering. Anpassad från Stahly (1996)

Growth and feed utilisation	Antigen exposure	Carbadox		Change (%)
		0-0 ^a	55-0 ^b	
Daily gain, g	Low	845	858	+1.5
	High	686	735	+7.1
Kg feed/ kg gain	Low	2.75	2.67	-2.9
	High	3.12	2.90	-7.1

^a Non-medicated control group.

^b Pigs fed 55 ppm carbadox from 5 to 34 kg body weight and then placed on the control diet without carbadox from 34 to 115 kg body weight.

^a Omedicinerad kontrollgrupp

^b Grisar som fått 55 ppm carbadox från 5 till 34 kg kroppsvikt och därefter foder utan carbadox från 34 till 115 kg kroppsvikt

Table 3.IIb. Responses of chickens with a low or high level of antigen exposure to dietary antibacterial agents (streptomycin and penicillin) Adopted from Rour (1992)

Tabell 3.IIb. Effekt av de antibakteriella substanserna penicillin och streptomycin på tillväxt och foderutnyttjande hos slaktkyckling med hög eller låg grad av antigenexponering. Anpassad från Rour (1992)

Treatment ¹	Weight gain (g/day) ²	Feed efficiency (g gain/g feed)
Clean	12.65 ^a	0.66 ^a
Unsanitary	12.10 ^b	0.54 ^b
Clean+Antibacterial	12.72 ^a	0.67 ^a
Unsanitary+Antibacterial	12.57 ^a	0.63 ^a

¹Sixty-four chickens were raised 14 days in each of the 2 different environments (clean or unsanitary) and fed diets either without antibiotic or with streptomycin (100 ppm) and penicillin (100 ppm); ²Means in a column with different superscript letters differ significantly ($p < 0.05$).

¹Sextiofyra kycklingar vistades 14 dagar i 2 olika miljöer (låg eller hög antigenexponering) och gavs foder antingen utan antibiotika eller innehållande streptomycin (100 ppm) och penicillin (100 ppm); ²Medelvärden i en kolumn med olika bokstavsmarkeringar skiljer sig signifikant ($p < 0.05$).

The relationship between cytokines, immunologic stress and growth is complex. Responses in the brain and immune system are expressed as effector

signals in the form of regulatory hormones from the hypothalamus and pituitary and as cytokines from the immune system. Several hormones including corticotrophin releasing hormone, prostaglandins, glucagon, insulin and corticosteroids are induced by cytokines (for ref. see Grunfeld *et al.*, 1996). In this respect the release of corticotrophin releasing hormone and corticosteroids is of special interest since corticosteroids have a catabolic effect, thereby reducing muscle tissue. An activated immune system releases cytokines, that mediate the host response to infection and/or inflammation. Cytokines may also have direct effects on the brain, resulting in, for example, a decreased appetite.

In summary, cytokines released as a consequence of a stimulated immune system mediate profound host responses including decreased appetite and a general catabolic effect. Many extrinsic stimuli such as bacterial toxins, yeast cell walls, silica particles, bacterial (gram-positive and gram-negative) or viral antigens have the capacity to elicit these responses (Mahé and Oppenheim, 1992; Degré, 1996).

Immune responses and AFA

Two recently published studies report on the effects of AFA on the immune system in relation to performance in clinically healthy animals. In one, avoparcin (having a gram-positive spectrum) was used and in the other carbadox (having a gram negative spectrum).

In the study on avoparcin, Krinke and Jamroz (1996) compared some immunological parameters in avoparcin-fed chickens with a control group. In birds from the medicated group, most chickens had a suppressed reactive lymphoid tissue of the bursa of Fabricius. This result indicates that there may be a lack of stimulation of the immune system in the antibiotic-treated birds.

The effects of carbadox in a model using pigs with low or high level of chronic immune system activation was studied by Stahly (1996 see table 3.IIa). The magnitude of the performance enhancement induced by carbadox was greater in the pigs with high level of chronic immune system activation than in pigs with low level of immune system. Similar results were obtained for pigs fed tylosin at 110 ppm (Stahly *et al.*, 1995). In chickens fed therapeutic levels of antibiotics (Table 3.IIb Roura *et al.*, 1992), the effects of antibiotics on immune stressed animals was further confirmed.

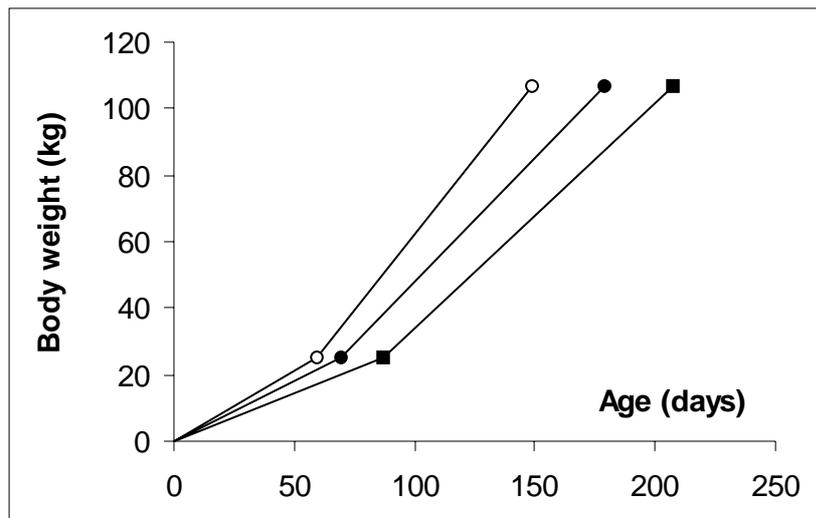


Figure 3.I. Growth rate of pigs in farrow to finish herds with different pathogen loads. ○ denotes specific pathogen free pigs representing the lowest pathogen load, ● denotes conventional pigs reared from farrow to finish but applying an age segregated system representing an intermediate pathogen load; ■ denotes conventional pigs reared from farrow to finish employing a continuous rearing system representing the highest pathogen load (Wallgren, 1993b).

Figur 3.I: Tillväxt hos grisar uppfödda i integrerade besättningar med olika smittämnestryck. ○ visar SPF-grisar (Specific Pathogen Free) representerande det lägsta smittrycket; ● visar konventionella grisar inom en besättning som tillämpar ålderssektionering, representerande ett intermediärt smittryck, ■ visar konventionella grisar som tillämpar ett kontinuerligt uppfödningssystem, representerande det högsta smittrycket (Wallgren, 1993b).

AFA improve growth rate and feed efficiency.

The effects of AFA are inversely related to animal health status. The poorer the health status the better the effect.

Challenge of the immune system will reduce growth rate and feed efficiency. This response is mediated by cytokines. Cytokines have direct effects on the brain, inducing a decrease in appetite, and stimulate the release of catabolic hormones, reducing the mass of muscle tissue.

AFA, through their antimicrobial effects, will alleviate immune system challenges from the intestinal tract on the immune system. This appears to be the major effect of AFA.

3.2 Prophylactic and therapeutic effects of AFA

3.2.1 Introduction

Most substances used as AFA are also used, or have been used, for therapeutic purposes. For some AFA the maximum dosage permitted for growth promotion is the same or nearly the same as what is recommended for prophylaxis and treatment of disease (table 3.III.). It has been pointed out that one expected consequence of abstaining from use of AFA is increased animal health problems due to infectious diseases (McOrist, 1997; Viaene, 1997). Indeed, the Swedish experience from banning AFA in 1986 was the emergence of clinical problems and disturbances of the health status of piglets and broilers which initially created a demand for antibiotic medicated feed at therapeutic dosages. The response to withdrawal of AFA in 1986 on piglet health and performance in 220 Swedish piglet producing herds was statistically evaluated by Robertson and Lundeheim (1994 see chapter 2). Removal of AFA was followed by a doubling of incidence of diarrhoea and number of medical treatments of post-weaning diarrhoea as well as by an increased mortality by about 1.5%. The age at 25 kg increased by 5-6 days. Since then, efforts have been made to improve the rearing and production systems e.g. sectioning between age groups and planned production. Production data of the pig herds from 1986 (Robertsson and Lundeheim, 1994) have now been re-analysed comparing 1986 to 1995 (Wierup, 1996). The comparison revealed a drop in post-weaning mortality from 1.5% in 1986 to 0.9% in 1995 and a reduction of the age at 25 kg by 1-2 days in 1995, as compared to 1986.

This poses questions as to what degree AFA, at their permitted dosages for growth promotion, also have therapeutic and/or prophylactic effects against certain economically important intestinal diseases in pigs and poultry.

Table 3.III. Recommended dosages of AFA for treatment, prophylaxis and growth promotion in swine and poultry. (Från Allen *et al.*, 1992; Prescott and Baggot, 1993; FASS VET., 1997 and 70/524/EEC). Dosages have been converted to mg/kg and rounded off

Tabell 3.III. Rekommenderade doser av AFT för behandling, profylax och som tillväxtbefrämjare hos svin och fjäderfä. (Från Allen *et al.*, ; Prescott and Baggot, 1993; FASS VET., 1997 och 70/524/EEC). Doseringar har räknats om till mg/kg och avrundats

Substance	Indication	Animal species	Dosage for treatment and prophylaxis mg/kg feed	Permitted dosage as AFA mg/kg feed
Avilamycin	<i>growth promotion</i>	poultry		2.5-10
		swine		10-40
Bacitracin	bacterial enteritis	poultry	55-220	
		swine	280	
	<i>growth promotion</i>	poultry		5-50 (100)
		swine		5-20
Carbadox	SD ¹ , PPE ² , bacterial enteritis	swine	50-55	
	<i>growth promotion</i>	swine		20-50
Flavomycin	<i>growth promotion</i>	poultry		1-20
		swine		1-25
Olaquinox	SD, bacterial enteritis	swine	100-160	
	<i>growth promotion</i>	swine		50-100
Spiramycin	mycoplasmosis	poultry	400	
	<i>growth promotion</i>	poultry		5-20
		swine		5-50
Tylosin	mycoplasmosis	poultry	900-1100	
	erysipelas, mycoplasmosis, SD, PPE	swine	40-110	
	<i>growth promotion</i>	swine		5-40
Virginiamycin	necrotic enteritis, respiratory infections, SD, other bacterial enteritis	poultry	20-40	
	<i>growth promotion</i>	swine	28-150	
		poultry		5-20
		swine		5-50

¹SD= swine dysentery

²PPE= porcine proliferative enteropathy

Below, some studies on therapeutic and/or prophylactic effects of AFA on certain diseases have been reviewed. Studies on this subject where several antimicrobial treatments are administered simultaneously to the same animals have not been included, as they are impossible to evaluate.

3.2.2 Necrotic enteritis in poultry

Necrotic enteritis (NE) is an intestinal disease affecting poultry. It is associated with *Clostridium perfringens*.

Wicker and co-workers (1977) demonstrated a reduced mortality ($p < 0.01$) due to NE in broiler chicks fed bacitracin at 11 ppm. In chickens with experimentally induced disease, a prophylactic effect of bacitracin administered in water at 100 mg/gallon and a therapeutic effect at 200 mg/gallon was demonstrated (Prescott *et al.*, 1978).

In a larger study, Jansson and co-workers (1992) induced NE by feeding chickens a diet high in barley. Virginiamycin given in feed at 20 ppm effectively prevented the disease. Another study by Elwinger and Teglöf (1991) showed similar results; virginiamycin at 20 ppm in feed notably reduced mortality due to necrotic enteritis in chickens ($p < 0.001$) compared to non-medicated groups.

Stutz and co-workers (1983a) showed that bacitracin, carbadox and virginiamycin, all at the level of 55 ppm in feed, reduced the amount of *C. perfringens* in the intestines of chickens compared to non-medicated controls ($p < 0.05$). The numbers of clostridia were inversely correlated with performance data. A similar reduction in numbers of clostridia and correlation with performance data was observed by Elwinger and co-workers in several studies using avilamycin at 10 ppm or avoparcin at 15 ppm (Elwinger *et al.*, 1993; Elwinger *et al.*, 1995; Elwinger *et al.*, 1996).

From the above, it is clear that some AFA are effective against clostridia, both *in vitro* and *in vivo*, at the levels used for growth promotion, resulting in effective disease prevention. Prevention of subclinical NE is associated with performance enhancement.

3.2.3 Swine dysentery

Swine dysentery (SD) is a contagious diarrhoeic disease in swine, caused by the spirochete *Serpulina hyodysenteriae*. Other forms of spirochetal diarrhoea in pigs are associated with other *Serpulina* spp.

Various studies have shown effects on SD by quinoxalines at growth promoting levels. Hunneman (1980) reported a notable decrease in outbreaks of SD in a region when carbadox was introduced as a feed additive for swine. These observations are supported by the results obtained in two clinical trials

(Anonymous, 1980). Molnar and Magyar (1987) reported an attempt to eradicate SD from swine herds by the aid of carbadox. Complete eradication of clinically apparent SD failed but the course and the severity of the disease was altered. The author also noted that the performance of the trial herds had improved markedly. Successful eradication of SD using carbadox in feed has been reported by other authors (Olson, 1986; Wood, 1987).

A swine dysentery model for evaluation of drug prophylaxis has been developed by Raynaud and co-workers (1980a; 1980b). Using this model, carbadox, and to some extent olaquinox, was found to prevent the outbreak of SD. This was substantiated in a large scale trial in commercial pig herds in France, where 50 ppm carbadox was found to be sufficient for both prophylaxis and treatment of swine dysentery (Raynaud and Bretheau, 1973). Prevention of experimentally induced SD with carbadox or olaquinox at levels used for growth promotion has been reported by several other authors (Davis and Libke, 1976; Williams and Babcock, 1978; Williams and Shively, 1978; Rainier *et al.*, 1980a; Rainier *et al.*, 1980b; Taylor and Davey, 1980; Biehl *et al.*, 1984; Jenkins and Froe, 1985; Jacks *et al.*, 1986). In one of these studies, olaquinox-resistant strains were also used as challenge (Williams and Shively, 1978). In those trials, the disease was not prevented, but morbidity was lower than in the non-medicated control group.

There seems to be a good correlation between MIC values of *S. hyodysenteriae* for tylosin and therapeutic effect of this substance. Williams and Shively (1978) found that tylosin at 100-110 ppm completely prevented SD induced by a tylosin-susceptible isolate of *S. hyodysenteriae*, while it was only partly effective against the disease induced by isolates with higher MIC values for tylosin. Jacks and co-workers (Jacks *et al.*, 1986) also prevented swine dysentery in pigs experimentally infected with a tylosin-susceptible strain, by feeding tylosin at 110 ppm. When a tylosin-resistant strain was used the disease was not prevented, although mortality was lower in the tylosin-medicated group than in the non medicated group. A new tylosin compound, 3-acetyl-4"-isovaleryl tylosin was also tested and found to be effective at 50 ppm.

Miller and co-workers (1972) studied the effect of different levels of virginiamycin and tylosin on pigs experimentally infected with *S. hyodysenteriae*. They found a good prophylactic effect of virginiamycin at about 25 ppm but only a moderate effect of tylosin even at about 100 ppm. The antimicrobial resistance pattern of the infecting strain was not presented, but presumably it was resistant to tylosin. Williams and Shively (Williams and Shively, 1978) fed virginiamycin at 50 to 100 ppm to experimentally infected pigs. Virginiamycin could not control the disease, although clinical signs were a little less common in medicated pigs than in non-medicated animals. This was supported by the observations of Rønne and co-workers (1992) who investigated the effect of 20 ppm of virginiamycin.

3.2.4 Porcine proliferative enteritis (PPE)

Porcine proliferative enteritis (proliferative adenomatosis) is an intestinal disease of pigs characterised by thickening of the intestinal mucosa. *Lawsonia intracellularis* (formerly *Ileal symbiont intracellularis*) is the causative agent. For a long time, the cause of the disease was elusive and various causative agents have earlier been pointed out. Therefore, most of the research bearing to prophylaxis or therapy is recent.

McOrist and co-workers (McOrist *et al.*, 1997) evaluated the efficacy of orally administered tylosin for the prevention and treatment of experimentally induced PPE. They found that tylosin at therapeutic levels could prevent PPE in challenge exposed pigs and could also be used for the treatment of previously induced PPE. Even at growth promoting levels, tylosin was effective in preventing the development of PPE. Moore and Zimmermann (1996) reported successful prevention of PPE by tylosin at about 100 ppm. Fleck and Jones (1994) compared 0, 40 and 100 ppm of tylosin for the treatment and prevention of PPE. The higher level of tylosin was effective as treatment of PPE, while 40 ppm did not quite prevent the emergence of clinical signs.

Kyriakis and co-workers (1996a; 1996b) found that in-feed bacitracin had a prophylactic effect against PPE in swine. The dosages used in this study were mostly substantially higher than what is used for growth promotion, and doses were changed over time. Even in the group receiving the lowest levels of bacitracin (50-200 ppm), a notable prophylactic effect could be seen.

3.2.5 Other bacterial enteritis in swine

Among bacterial enteric diseases in swine, weaning diarrhoea is one of the most important, both clinically and economically. *Escherichia coli* has been implicated in the pathogenesis. Bertschinger (Bertschinger, 1976) found that olaquinox at low growth promoting dosages was effective for both prevention and treatment of experimentally induced *E. coli*-diarrhoea in piglets. Kyriakis (1989) studied the effect of avilamycin and olaquinox on stress-induced post-weaning diarrhoea. At 40 ppm avilamycin or 50 ppm olaquinox, mortality was reduced ($p < 0.05$). The diarrhoea score was considerably lower compared to the control, but the difference was not significant. At 80 ppm avilamycin, both diarrhoea and mortality were reduced ($p < 0.05$).

Holmgren (1994) found that prophylactic treatment with 122 ppm or 173 ppm olaquinox in commercial swine herds reduced ($p < 0.01$) the incidence of post-weaning diarrhoea in all but one of the herds investigated. From the herd where olaquinox had no prophylactic effect, olaquinox-resistant *E. coli* were isolated.

Troutt and co-workers (1974) found that carbadox at maximum AFA dosage reduced clinical signs and intestinal lesions in pigs experimentally infected with *Salmonella Cholerasuis*.

Most AFA are, or have been, used for therapeutic purposes and for some AFA the maximum dosage permitted for growth promotion is the same or nearly the same as recommended for prophylaxis and treatment of disease.

Most AFA have prophylactic and/or therapeutic effects on enteric diseases, at permitted dosages.

3.3 Growth and feed efficiency responses to AFA in pigs and poultry

AFA were introduced in the early 1950s and have played a major role in the development of intensive and industrialised livestock production.

There is a great variation in responses of animals to AFA (CEC, 1993, see table 3.IV). When judging these promoting or enhancing effects one has to consider the age and type of animals, the duration of AFA administration and the performance level of the control group. The major part of the research in this area with different animal species has been performed by the manufacturing and feed industries, whereas a relatively limited part has been performed by independent research bodies (Brenninkmeijer, 1996).

3.3.1 Growth and feed efficiency responses to AFA in pigs

The response of younger animals, piglets as well as broiler chickens and calves, to AFA is superior compared to responses in older animals. In piglets the growth responses to AFA varies between 9 and 30% and the feed efficiency responses between 6 and 12%, whereas for growing-finishing pigs the level of response is inferior (Thomke and Elwinger, 1997b).

There has been a tendency to increase the level of AFA with time (Rosen, 1995). Some studies indicate a decreasing effect of AFA on daily weight gain and feed efficiency over time (Gruber, 1986). However, Schneider (1992) was unable to find differences in growth rate and feed efficiency for tylosin in growing-finishing pigs comparing the period of 1969/79 versus 1980/90.

A general opinion is that growth and feed efficiency responses of pigs and broiler chickens to AFA is lower under improved environmental conditions than in poorer environments. Rosen (1995) estimated the ratio in response between a very good and a poor environment to 1:2.

Table 3.IV. Percentage responses in growth performance and feed efficiency as a result of AFA usage in livestock production, compared with unsupplemented control diets, as reported by different authors (sources cit. by De Craene and Viaene, 1992; CEC, 1993)

Tabell 3.IV. Sammanställning av procentuell effekt av AFT på tillväxt och foderutnyttjande hos olika husdjur, jämfört med obehandlad kontroll, enligt vad som rapporterats av olika författare (källor cit. av De Craene and Viaene, 1992; CEC, 1993)

<i>Animal species</i> Source	Growth, daily weight gain	Feed efficiency
<i>Piglets</i>		
CEC (1993)	16	9
<i>Growing pigs (20-50 kg)</i>		
CEC (1993)	9	5.5
<i>Finishing pigs (50 kg-slaughter)</i>		
CEC (1993)	0	0
<i>Growing-finishing pigs</i>		
Bickel (1983)	5-10	5-7
Hudd (1983)	4-5	4-5
Mordenti et al. (1979)	6.5	4.1
Weiss (1989)	1.9-8.6	0.7-5.1
Robinson (1969)	10-20	5-10
CEC (1993)	3.5	3
<i>Broiler chickens</i>		
Birzer and Gropp (1991)	4	4
Hudd (1983)	3-4	3-4
Mordenti et al. (1979)	5.0	3.5
Robinson (1969)	5-10	5
CEC (1993)	4	4
<i>Laying hens</i>		
CEC (1993)	2	1
<i>Veal calves</i>		
Birzer and Gropp (1991)	10	5
CEC (1993)	7	4

Some discrepancy exists between different reports on the effect of AFA on sow performance. Speer (1974) concluded that the farrowing rate was improved by the use of AFA. However, others state AFA had no effects on oestrus, mating behaviour or breeding efficiency (Myers and Speer, 1973). Soma and Speer (1975) reported improved litter weights at birth and at

weaning This could not be corroborated by Frölich and co-workers (1974) under Swedish conditions.

Based on the available literature data, Thomke and Elwinger (1997b) estimated the average response in growth rate to 17% and in feed efficiency to 9%, when piglet diets were supplemented with AFA. Corresponding responses for growing-finishing pigs were inferior, and averaged 3.6% for growth rate and 3.1% for feed efficiency.

Thomke and Elwinger (1997b) also tried to predict the responses on performance in the Swedish pig industry to a re-introduction of AFA. They assumed a lower response level on performance as a result of changes in the animal welfare rules, the current rearing and production models and performance levels in Sweden. For the piglet sector they calculated a response to AFA of 4-5% for growth performance and feed efficiency. For growing-finishing pigs a lower level of responses to AFA, of 1.5-2% was estimated.

3.3.2 Growth and feed efficiency responses to AFA in poultry

In broiler production the administration of AFA can yield an effect in comparison with the unsupplemented control of between -1 and 6% (Brenninkmeiyer, 1996).

Swedish and Danish experiments, performed during the period 1967-76 with broiler chickens fed bacitracin have been reviewed by Elwinger (1976). This antibacterial improved growth rate on average by 2.0% and feed efficiency by 1.3%. There were no differences in mortality. Similar to the result for pigs, responses in broiler chickens to AFA are superior in the first growth phase, as compared with the second.

In a comprehensive review, Rosen (1996), arrived at responses in growth rate and feed efficiency of 2 and 3%, respectively. It was also observed that AFA were more effective with respect to live weight gains when used in diseased than in apparently healthy birds. When including anticoccidials, the growth-promoting effect of the AFA themselves was clearly limited, due to the fact that some anticoccidials have antibacterial effects as well.

Based on available literature data, Thomke and Elwinger (1997b) estimated the overall responses of broiler chickens to AFA as regards growth rate and feed efficiency and found it to be on average 3.9 and 2.9%, respectively.

A re-introduction of AFA into today's Swedish broiler chicken industry would induce responses in performance and feed efficiency at a lower level than those reported above. An explanation for this is that the Swedish broiler industry has introduced production models without AFA, with a very high standard of hygiene and by the use of feeding programmes including a mixture of enzyme preparations. A tentative response level for performance and feed efficiency of 1-1.5% may be assumed.

In pigs the responses to AFA in terms of growth performance and feed efficiency are higher in piglets than in growing pigs and for finishing pigs the effects seem to be minute.

A re-introduction of AFA into Swedish pig and broiler meat production would most likely lead to lower levels of responses for growth performance and feed efficiency than what is reported in the literature. This is because these production systems have adjusted to production without AFA usage.

3.4 Alternatives to AFA

Livestock performance and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the host animal, including the alimentary tract and the animal environment. Appropriate nutrient supply and choice of ingredients and their proper preparation with respect to the animal's digestive capacity will minimise nutrient losses, overloading, disturbances and intestinal overcrowding by harmful microbial flora. Hence, improvements in feed composition and feeding strategies is an alternative to usage of AFA.

Despite a vast body of information, details on the mode of action of both enzymes and probiotics are still lacking. Obviously, more research is needed to develop more efficient enzymes and to find out efficient combinations of enzyme preparations suitable for individual and compounded feedstuffs

3.4.1 Enzymes

Supplementing feed with enzymatic preparations may improve the digestive capacity, particularly in young animals. Thereby, nutrient utilisation in the anterior part of the gastrointestinal tract may be improved, possibly limiting the incidence of intestinal perturbation.

Some experiments demonstrate that the beneficial effects achieved by AFA can at least partly be obtained by the inclusion of dietary enzymes affecting the physical properties of the ingested non-starch polysaccharides (Elwinger and Teglöf, 1991; Kronseder, 1993; Miles *et al.*, 1996; Vranjes and Wenk, 1996).

Thomke and Elwinger (1997a) assessed the effects of various enzyme preparations on the nutrient digestibility and performance in pigs, based on available literature data, and arrived at a value for the average feed efficiency response of approximately 4% in piglets and about 2% in growing-finishing

pigs. For broiler chickens the average improvement of feed efficiency was about 4% (Thomke and Elwinger, 1997a) which is similar to the figure arrived at in the extensive compilation undertaken by Kronseder (1993).

In terms of improved digestibility and feed efficiency a fair estimate of the improvement by enzyme supplementation for young pigs and poultry according to the literature reviewed above would be approximately 3-4%. Calculating with an average digestibility of organic matter in cereal-based diets for these animal species of 80%, one arrives at a decrease in animal nutrient voidings by 15-20%.

3.4.2 Probiotics

Fuller (1992) recently redefined "probiotic" as a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance.

The probiotic concept is generally based on a viable micro-organism culture with capacity to adhere to structures in the intestine. In selecting microbial strains with probiotic potentials, their genetic stability and intestinal colonising capacity as well as their stabilising properties are of main concern.

The efficacy of administering probiotics seems to be dependent on a number of conditions, e.g. the physiological or health status of the animal, environmental factors such as feed regimen and microbial load. Beneficial effects are more often observed in neonatal animals and in weaners than in older animals.

In evaluating the great number of reports on the efficacy of probiotic organisms and substances in poultry, Barrow (1992) points out a number of factors hampering a critical appraisal. The vast majority of results on the efficacy of probiotics in poultry is reported as abstracts with essential information lacking, e.g. on the identity of strains used and on experimental design. The implantation in the gastrointestinal tract of the strains used is rarely assessed. The microbiological results in nutrition-oriented papers are often poorly interpreted. A further comment is that in a number of reports in which performance and health could be improved by probiotics, the general conditions seem to have been poor and non-representative with respect to production level and morbidity. Moreover, Barrow (1992) in his review considers that some of the interpretations of the results are obviously over-optimistic and arise mainly from a naive and uncritical acceptance of the data or speculations by previous workers.

According to the review by Mead (1995), there are a number of commercial preparations available that have been successfully used in the prophylaxis of *Clostridium* and *Salmonella* infections in poultry. Recently, Abu-Ruwaida and co-workers (1995) confirmed the potential of this method

against salmonellosis. The prophylactic treatment of salmonellosis is based on the principle of early establishment of an adult intestinal microflora in the young bird by supplying a suspension of anaerobic cultures of intestinal material, thereby competitively excluding pathogenic organisms. However, criteria on which to select micro-organisms for protective purposes are lacking at present and will remain so until more is known about factors influencing salmonella colonisation at the cellular level and the protective mechanism(s) involved (Mead, 1995).

Swedish experimental results when using the probiotic Broilact[®] have been promising, with a significantly lowered mortality and lowered caecal counts of *C. perfringens* (Elwinger *et al.*, 1992).

Supplementing feed with enzymatic preparations may improve the digestive capacity, particularly in young animals.

Enzyme additives affect the physical properties of ingested polysaccharides.

Some probiotics have been demonstrated to improve piglet performance and health. The efficacy of probiotics is difficult to evaluate in many instances, since experimental details are poorly presented.

More research is needed both on enzymes and probiotics and their mode of action.

3.5 Addendum: A benefit cost analysis of reintroduction of AFA in Sweden.

3.5.1 Executive Summary

If antibacterial feed additives (AFA) were to be used in the Swedish production of pork, egg and broilers (poultry meat) the expected direct savings in the production costs would be between 0.5-1%. However, these estimates do not regard externalities such as the impact on demand for these animal products, nor the costs of increased antibiotic resistance both in veterinary and human medicine.

The economic impact of AFA on the Community level should be scrutinised, having regard to all relevant factors and externalities including (a) the results of the GATT and CAP reform, (b) the costs of increased antibiotic resistance, (c) the dynamic effects for the industry within the Community, and (d) the smaller distortions of the world market due to subsidised exports from the EU. Previous studies have not accounted for the increased opening of the EU internal market to the world market due to the

GATT/WTO agreement and the increased fickleness of the consumers with regard to the perceived safety of the food of animal origin. The gains from lower export restitutions and increased import levies could be used to compensate farmers if AFA were not to be used as feed additives.

In the final analysis one should, moreover, also consider the danger of losing the consumer confidence in the safety of meat and eggs for which the continued use of AFA could be a risk factor.

3.5.2 Introductory comments

This benefit cost analysis will examine the monetary impact for Swedish farmers, if AFA were to be used in the pork and poultry sector.

The estimates presented were derived from the budget sheets from the Swedish University of Agricultural Science's extension service (SLU-Kontakt, 1996) and the budget sheets were adopted to capture the variation of estimates concerning the effects of AFA under Swedish conditions. The results were presented as estimated savings in production costs in SEK per kg produced meat or eggs, and also as the estimated monetary gain for the whole segment of the industry.

Since Sweden is a member of the EU, the market prices could be assumed to be constant with regard to the putative use of AFA. In consequence, decreased production costs would benefit the Swedish agricultural industry as such. The distribution of benefits between the primary producers, and the industry was not possible to estimate, and was not within the purview of this report.

Another uncertainty was the consumer response if AFA were to be used in pig and poultry production. The assumption both in the CEC report (1993) and the De Crane and Viane report (1992), that the consumer will only respond to price changes, seems to be contradicted by the BSE experience within the EU and also by the scrapie experience in Norway. In both cases considerable drops in the consumption of meat occurred. There is a risk of damaging the Swedish consumer confidence if AFA were to be used in Swedish animal production. This should not be ignored.

This report presents the models based on the budget sheets for the pig industry, for the egg production and finally for the poultry meat production. Moreover, the De Crane and Viane (1992) report is elaborated on to show an alternative analysis of the impact of AFA on the Community level with the egg market as an example. This is because the GATT agreement resulted in a downward slope of prices within the EU and this exogenous downward pressure on prices was ignored by the same report.

3.5.3 Gains if AFA were to be used in Sweden

Jonasson and Andersson (1996) indicated that the costs for AFA were approximately 0.02 SEK per kg feed for the pig sector, and the same estimate was also used in the poultry sector. The same authors noted that by using AFA, one should be able to use cheaper feed ingredients, thus lowering the net costs of AFA. However, they provided no estimate of this cost reduction.

Simulation of outcomes

All budget sheets were simulated 1000 times using @ Risk (Palisade Corp.) to capture the uncertainties with regard to the effect on growth and feed conversions if AFA were to be used in Swedish pig and poultry production.

Results

The results of the study appear in table 3.V, while the more detailed results for each kind of production appear in tables 3.VI - 3.IX. The detailed budget sheets used for the estimation of the putative benefits appear in Annex F, tables 1-4.

The savings on the supply side is around 1% of the production costs. However, this benefit must in the final analysis be weighed against the possibility of disturbances on the demand side due to adverse consumer reactions and externalities such as the costs of the increased antibiotic resistance both for animals and humans. Based on the experience from the last 10 years of not using AFA, the Swedish pig and poultry meat market could be foreseen to experience disturbances on the demand side if AFA were to be used.

Table 3.V. Possible gains at the supply side if AFA were to be used in the Swedish pork and poultry industry

Tabell 3.V. Möjliga vinster på leverantörssidan om AFA åter började att användas i svensk fläsk- och slaktkycklingproduktion

	Reduced production costs (million SEK)	Cost of AFA (million SEK)	Benefit /cost ratio	Industry net gain (million SEK)
Piglet	47	4	11	43
Slaughter pig	47	16	3	31
Egg	11	5	2.3	6
Broiler	9	3	3	6

If consumers' confidence in the safety of e.g. pig and poultry meat diminishes, then the consequences tend to result in decreased demand. Recent experiences suggest that a drop in demand of 10-25% should be

foreseen. Given a price elasticity of -0.5, this could indicate a decrease in the market price of 20 to 50%. Recent similar experiences include the drop in beef prices and increased market management expenses due to BSE and its impact on the demand for beef within the EU during the 90s, and also the diminished demand for mutton due to the fear of scrapie transmission to humans in Norway during 1996.

3.5.4 The pig industry

Piglet production assumptions

The following assumptions concerning the effects of AFA in piglets were made: the number of sows in Sweden amounts to 237 355 in 1995 according to the official yearbook of agricultural statistics (SCB, 1997). However, not all piglets from these sows should be foreseen to receive AFA. Exemptions include e.g. elite breeding herds and organic farming. It was assumed that the offsprings of around 230 000 sows were candidates to receive AFA.

Earlier estimates of an increase in piglet mortality of 1.5% after the prohibition of AFA in 1986 (Robertsson and Lundeheim, 1994) had to be adjusted to the present Swedish situation (see chapter 2). It was assumed that AFA would lead to a decrease in piglet mortality by 0.6%, based on the calculations by Wierup (1996).

To incorporate all relevant information on feed efficacy and piglet growth weighted approaches were used in which 0% was set as the minimum.

Feed efficacy was assumed to improve on average by 4.6%. The estimated effect on feed efficiency (FEFAC according to CEC, 1993) for Danish piglets was 2%, while the average effect on feed efficiency within the EU was 7.3% according CEC. Thomke and Elwinger (1997b) indicated an effect of feed efficiency of 4.5% if AFA were to be used under current Swedish conditions, and the upper estimate given in the same report was 9%. The average of these estimates was used.

Piglet growth was assumed to improve on average by 6.8%. The estimated growth (FEFAC according to CEC, 1993) for Danish piglets was 2% and the average effect within the EU was 8.5%. Thomke and Elwinger (1997b) indicated an effect on growth of around 4.5% if AFA were to be used under the current Swedish conditions, and the upper estimate given in the same report was 16%. The average of these estimates was used.

Veterinary and therapeutic costs was assumed to be reduced by 2 SEK per piglet, if AFA was introduced (Jonasson and Andersson, 1996).

Table 3.VI: The results of 1000 simulations if AFA were to be used in Swedish piglet production, mean value, with the 5th-95th percentile range in parentheses

Tabell 3.VI: Resultatet av 1000 simuleringar av effekten om AFT åter började användas i svensk svinproduktion; medelvärde, med 5e-95e percentilen inom parentes

	Saved production costs	Cost of AFA	Net benefit
SEK per piglet produced	10.19 (6.76-13.62)	0.93 (0.86-1.01)	9.23 (5.81-12.67)
SEK per sow	205.01 (134-274)	18.75 (18-20)	186.26 (116-255)
Savings in production costs (%)	1.18 (0.78-1.58)	0.108 (0.10-0.11)	1.07 (0.67-1.47)
Industry benefits (million SEK)	47.2 (31-63)	4.3 (4.1-4.5)	42.8 (27-59)

The expected benefit cost ratio was 11 (mean value of simulations) while the minimum benefit/cost ratio was 5.6, the maximum was 15.9 and the 5th to 95th percentile range was 7.2-14.7. In other words, under these assumptions the use of AFA in piglet production should produce a benefit for the piglet producing part of the pork industry of around 43 million SEK. The distribution of the benefits within the industry was not possible to predict.

Slaughter pig assumptions

It was assumed that around 3.5 million slaughter pigs would receive AFA. According to the official year book of agricultural statistics (SCB, 1997) the number of pigs slaughtered in Sweden was 3.7 million in 1995. However, not all herds would use AFA in their pig production. Moreover, it was assumed that if AFA were to be used in piglet production (production of pigs of 25 kg live weight), this would not interfere with the market for piglets and the piglet price which the pig farmers faced. No effect was foreseen on mortality by using AFA in the slaughter pig production stage.

To incorporate all relevant information on feed efficiency and growth, weighted approaches were used in which 0% was set as the minimum.

Feed efficacy was assumed to improve by, on average, 1.9%. The estimated effect for Danish slaughter pigs was 1.5% and the average effect within the EU was 2.1% according to CEC (1993). Thomke and Elwinger (1997b) indicated an effect of feed efficiency of 1.75%, and an upper estimate of 3.1%. The average of these estimates was used.

Table 3.VII. The results of 1000 simulations of the effect of AFA in the slaughter pig production, the values are represented as mean values (5th-95th percentile)

Tabell 3.VII: Resultatet av 1000 simuleringar av effekten om AFT åter började användas i svensk slaktsvinproduktion; medelvärde (5e-95e percentilen)

	Saved production costs	Costs of antibiotics	Net benefit
SEK per slaughter pig	13.5 (7.4-19.7)	4.5 (4.3-4.7)	9.0 (2.3-15.3)
SEK per kg pig meat	0.173 (0.094-0.253)	0.058 (0.056-0.060)	0.115 (0.03-0.195)
Savings in production costs (%)	0.99 (0.54-1.4)	0.33 (0.32-0.35)	0.65 (0.16-1.1)
Industry benefits (million SEK)	47.2 (26-69)	15.8 (15-16)	31.4 (8-53)

Growth was assumed to improve by on average 1.6%. The estimate on growth for Danish slaughter pigs was 1.5% and the average effect within EU was 2.4% (CEC, 1993). Thomke and Elwinger (1997b) indicated an effect on growth of 1.75%, with an upper estimate of the effect of 3.6%. The average of these estimates was used.

The expected benefit/cost ratio was 3 (mean value of simulations) while the minimum benefit/cost ratio was 0.58 with a maximum of 5.6 and a 5th to 95th percentile range of 1.5-4.4. In other words if examining only the production stage, the use of AFA would have a net benefit in most cases. However, in a few scenarios (1%) one would expect a negative benefit in the production stage. The impact on the demand side and possible externalities such as the costs for increased resistance to antibiotics were not assessed.

A brief comment on the results

Gains of such magnitude, around 74 million SEK, for piglet and slaughter pig production could be questioned based on the variations in piglet prices in Sweden and Denmark. If the only difference were the usage of AFA, then the Swedish piglet could be foreseen to be 10 SEK more expensive than its Danish counterpart. However, the market prices have varied significantly and according to the agricultural paper Land, April 11, 1997, the Swedish piglets were 37 SEK more expensive than their Danish counterparts, while last November according to Land, November 29, 1996, the Swedish piglets were 53 SEK less expensive than their Danish counterparts.

3.5.5 Egg production

Assumptions

No effect on the mortality of laying hens due to the use of AFA was foreseen, and the improvements in feed efficiency and laying performance were assumed to be in addition to the use of digestive enzymes and coccidiostats in the feed.

To incorporate all relevant information on feed efficiency and laying performance weighted approaches were used in which 0% was set as the minimum.

Feed efficacy was assumed to improve by on average 1.1%. The estimate by the food industry for Danish layers was 2.2% and the average effect within EU was 1.2% (CEC, 1993). Thomke and Elwinger (1997b) indicated an effect on feed efficiency of 1% using Zn-bacitracin. The average of these estimates was used.

Laying performance was assumed to improve by on average 1.4%. The estimate by the food industry for Danish laying hens was 3% and the average effect within EU was 1.3% (CEC, 1993). Thomke and Elwinger (1997b) indicated an effect on laying performance of 1%, using Zn-bacitracin. The average of these estimates was used.

Table 3.VIII: The results of 1000 simulations of the effect of AFA in the consumer egg production, the values are represented as mean values (5th-95th percentile)

Tabell 3.VIII: Resultatet av 1000 simuleringar av effekten om AFT åter började användas i svensk äggproduktion; medelvärde (5e-95e percentilen)

	Saving of production costs	Costs of antibiotics	Net benefit
SEK/kg egg	0.104 (0.052-0.158)	0.0455 (0.0450-0.0460)	0.0587 (0.006-0.113)
SEK/100 hens	165 (83-255)	72 (70-75)	93 (10-183)
Savings of production costs (%)	1.20 (0.87-1.85)	0.52 (0.51-0.54)	0.68 (0.07-1.32)
Industry benefits (million SEK)	10.9 (5.5-17)	4.75 (4.7-4.8)	6.1 (0.7-12)

Results

The expected benefit/cost ratio was 2.3 (mean value of simulations) while the minimum benefit/cost ratio was 0.37. The maximum was 4.4 and the 5th to 95th percentile range was 1.1-3.5. In other words, if examining only the production stage the use of AFA would have a net benefit in most cases. However, in some scenarios, 4 out of 100 egg producing farms, one would expect a negative benefit in the production stage. The impact on the demand side and possible externalities such as the costs for increased resistance to antibiotics were not assessed in this analysis.

3.5.6 Production of poultry meat (broilers)

Assumptions

No effect of AFA on the mortality of broilers was foreseen. The effect of AFA on feed efficacy was assumed to be in addition to the use of digestive enzymes and coccidiostats.

To incorporate all relevant information on feed efficiency and growth promotion, weighted approaches were used in which 0% were set as the minimum.

Feed efficacy was assumed to improve by on average 1.4%. The estimate by the food industry for Danish layers was 1.85% and the average effect within the EU was 2.0% CEC (1993). Thomke and Elwinger (1997b) indicated an improved feed efficiency of between 1-1.5%, while the upper estimate according to same authors was 2.9%.

Growth performance was assumed to improve by on average 2.1%. The estimate by the food industry for Danish broilers was 2.5% and the average effect within EU was 2.5% (CEC, 1993). Thomke and Elwinger (1997b) indicated an effect on growth performance of 2%, using Zn-bacitracin in 1976, while the usage of AFA in general would indicate an effect of 1-1.5% while the upper estimate for the effect on growth was 3.9% according to the same authors.

Results

The expected benefit/cost ratio was 3.2 (mean value of simulations) while the minimum benefit/cost ratio was negative and the maximum was 9.4, and the 5th to 95th percentile range was 0.4-7.0. In other words, if examining only the production stage the use of AFA would have a net benefit in most cases. However, in some scenarios, 18 out of 100 farms, one would expect a negative result. The impact on the demand side and possible externalities

such as the costs of increased resistance to antibiotics were not assessed in this analysis.

Table 3.IX. The results of 1000 simulations of the effect of AFA in the poultry meat production, the values are represented as mean values (5th-95th percentile)

Tabell 3.IX: Resultatet av 1000 simuleringar av effekten om AFT åter började användas i svensk slakkycklingproduktion; medelvärde (5e-95e percentilen)

	Savings of production costs	Costs of antibiotics	Net benefit
SEK/ kg meat	0.107 (0.0-0.22)	0.034 (0.031-0.038)	0.73 (-0.02-0.19)
SEK/ batch of 80000 chicken	98000 (0-202000)	31000 (28200-33700)	66700 (-28000-173000)
Savings of production costs (%)	1.5 (0.0-3.1)	0.47 (0.44-0.50)	1.0 (-0.4-2.7)
Industry benefits (million SEK)	8.5 (0-18)	2.7 (2.5-3.0)	5.8 (-2.5-15)

3.5.7 A brief comment on an earlier report

In the following, a brief comment on the report by De Crane and Viaene (1992) dealing with the economic effects of performance enhancers in food animal production will be given.

Two issues are elaborated here i. the impact on the market of the withdrawal of performance enhancers and ii. the impact of the GATT-WTO regime and reform of the common agricultural policy (CAP).

An alternative point of view is presented, in which the adaptation will fully be on the supply side, since prices are presumed to be constant and unchanged regardless of whether AFA are permitted or not within the EU. This assumption is based on the GATT agreement for agriculture and the CAP reform, where the previous variable import levies and export restitutions are fixed and thereafter shall be reduced annually. Moreover, the market shall be opened for a small, but increasing share of imported animal products including meat and eggs. Hence, there will be a downward pressure on the prices during the next years. The De Crane and Viaene report (1992) mentions these effects in an appendix, but does not account for them in the substantive analysis.

The removal of AFA will result in an upward shift in the supply curve, initially it is here assumed as a parallel shift of the curve. This means that for the same price a smaller quantity will be supplied, or that a higher price is required for the same quantity to be supplied. In the long run, dynamic effects will dominate, and possibly change the slope of the supply curve.

Notice that the producer surplus change is estimated as the increase in production cost times the decrease of the amount supplied, in the case of AFA being prohibited.

The impact on the market of the withdrawal of AFA

The report has examined the impact for each country and we will elaborate this for the egg market within the EU. The price elasticity of demand is assumed to be -0.5 and the price elasticity of supply is assumed to be 0.5.

For eggs the impact would be an increase in production costs of 1.2%, which would result in an increase of market price of 0.6%, which would result in a decrease in supply of 5300 tonnes (0.1% of 4.466 million tonnes) while demand would also decrease 5200 tonnes (0.1% of 4.439 million tonnes). This would increase the need for export by 100 tonnes to 27700 tonnes, and a drop in economic surplus of 28.7 million ECU, i.e., the market seems to be broadly in balance.

Comments: In other words, the impact of the use of AFA in addition to the use of coccidiostats and digestive enzymes would be hard to detect at the market level. Moreover, due to the CAP and GATT reform, an increase in prices is unlikely. The effect of increased production costs would thus be a decrease of supply, i.e., a shift of the supply curve, meaning less surplus export and less subsidies paid (Gardner, 1988). If the market price was kept unchanged, how much would the supply drop due to the cost increase?

If one could use the supply elasticity 0.5, i.e. % change in quantity/ % change in price, which is derived from the aggregate cost function as an estimate for the response to an increase in production costs, the supply would drop by roughly $1.2\% \times 0.5$ which is 0.6%, or approximately 53000 tonnes, i.e., the surplus situation would change to an import situation, with 25300 tonnes of eggs in net import. If one assumed an import levy/export restitution of 10% or 90 ECU/tonne, this would lead to a savings at the community level of 53000 tonnes times 90 ECU/tonne or 4.8 million ECU. In this case the consumer surplus would be unchanged, while the producer surplus would decrease by 4.4 million tonnes times 10 ECU/tonne or 44 million ECU. A net loss of 39 million ECU could be foreseen, if the prices were assumed unchanged or 0.9% for the producers.

In other words using De Crane and Vianes (1992) assumptions, not using AFA in egg production would have insignificant impact on the economics of egg production within the EU. This does not account for the possible costs of

a loss of consumer confidence, for which the continued use of AFA is a risk factor.

Economic studies of the use of AFA has often arrived at positive conclusions for the use of AFA. Nevertheless the assumptions underlying the studies, in particular about the ignorant consumer, are probably too restrictive and at variance with the experience in the beef market regarding BSE. Hence one could question whether the results are of any significant relevance for future decisions concerning AFA.

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4 Microbiological aspects on antibacterial feed additives

4.1 Introduction

Antibacterial substances (antibiotics and synthetic substances) exert their activity by inhibiting or disturbing vital bacterial processes. The effect is concentration dependent. At levels above the minimum inhibitory concentration (MIC), bacterial growth will cease or the bacteria will be killed. The only way for sensitive bacteria to avoid elimination is to develop resistance. This can be achieved by mutation or by acquisition of genes from other bacteria. Transfer of genes occurs by several mechanisms, namely transduction, transformation, and conjugation mediated by plasmids and/or transposons. The latter mechanism is the most studied and possibly the most important. Horizontal gene transfer between bacteria is a common event and an important factor in microbial evolution.

Antimicrobial resistance, leading to loss of effectiveness of antibacterial drugs, has been targeted by the WHO as one of the major emerging human health problems (WHO, 1994). The consequences of antimicrobial resistance are seen as an increase in morbidity and mortality due to bacterial diseases. Antimicrobial resistance also has a considerable economic impact and is estimated to cost at least US\$ 4 billion annually in the United States alone.

The possible influence of antibacterial feed additives (AFA) on resistance in animal and human pathogens has been debated for almost four decades. The development of new techniques, particularly in the field of molecular biology, has provided new insights into these problems.

The antibacterial effect of AFA is also manifested in alterations of the intestinal microflora. The intact microflora is an important barrier against colonisation by enteric pathogens. Disruptions may lead to increased prevalence of zoonotic bacteria in food.

At the end of this chapter, a short glossary of terms used has been provided.

4.2 Antibacterial activity of AFA

The concentrations of AFA used in feed for performance enhancement exceed the susceptibility ranges of naturally susceptible intestinal bacteria (table 4.I). Concentrations reached in various parts of the intestine using the approved dosages will depend on a number of factors. Most AFA are poorly,

if at all, absorbed from the gut. They are therefore likely to be present in concentrations well above the MIC of normally susceptible bacterial species at least in part of the intestine.

The fact that AFA exert an inhibitory effect on the intestinal microflora is evident from various types of experiments. Early studies showed that the growth promoting effect could be associated with changes in the microflora, especially the enterococci and the clostridia, of the animals (see chapter 3.1). This is further substantiated by the preventive effect of AFA against certain intestinal diseases (see chapter 3.2). For therapy, higher doses are generally needed. By definition, therapy will be instituted once the disease is overt. When the infectious agent to be combated is already established in tissues, higher doses will be needed in order to have a satisfactory effect. If the infectious process is located in an internal organ other than the intestine, the administered substance must reach this organ at concentrations well above MIC. As the amount of substance given will then have to be absorbed in adequate amounts and be distributed in a larger volume (the whole body as opposed to only the intestine), higher doses are needed.

Table 4.I. Normal susceptibility ranges of clostridia and enterococci for some AFA compared to permitted levels in feed for performance enhancement (modified from Dutta and Devriese, 1984; Devriese *et al.*, 1993)

Tabell 4.I. Känslighet hos klostridier och enterokocker för några AFT jämfört med tillåtna halter i foder för avkastningsökning (modifierat från Dutta and Devriese, 1984; Devriese et al., 1993)

Antibacterial substance	Range of minimum inhibitory concentration (ppm) for:		Dosages used for performance enhancement (ppm)
	Clostridia	Enterococci	
Avilamycin	<0.25-0.5	NA ¹	2.5-40
Avoparcin	0.5-2	1-2	5-40
Bacitracin	<1-4 ²	<0.5-16 ²	5-100
Flavomycin	<1-8	0.25-4	1-25
Monensin	0.5-4	1-2	10-40
Spiramycin	0.25-8	0.5-4	5-80
Tylosin	<1	1-4	4-40
Virginiamycin	0.25-1	0.25-8	5-80

¹ NA = no information available; ² Given in IU/ml

¹ NA = information saknas; ² Uttryckt i IE/ml

The mechanisms of action of the different types of substances used as AFA have been studied in detail, with the exception of the quinoxalines. Currently approved AFA exert their effect on bacteria by one of four mechanisms (table 4.II):

- inhibition of protein synthesis
- inhibition of cell wall synthesis
- inhibition of DNA synthesis
- alterations of the cytoplasmic membrane

Concerning information on resistance mechanisms and their genetic background, practically all published studies on resistance mechanisms have focused on substances, or classes, also used in human therapy and mostly on resistance in human pathogens. The characterised resistance genes and mechanisms are probably only "the tip of the iceberg".

It is often stated that the AFA used in animal production are not used as therapeutics in humans. This is true only for avilamycin, flavomycin and the quinoxalines. Spiramycin is used in human therapy. So is bacitracin, although mainly for local application. Small chemical differences between substances within the same class of antibacterials may have profound effects on their pharmacokinetics but their mode of action on the bacterium will usually be the same. Therefore, the bacterial defence in the form of resistance will often confer cross-resistance to all or most substances of the same class. Tylosin, avoparcin, ardacin and virginiamycin all belong to classes of antibacterials that include valuable therapeutics.

Old, presently not used substances may become valuable therapeutics in the future. Such is the case with everninomycins, a substance of the orthosomycin class. This substance is presently undergoing trials as a new drug candidate. Another member of the orthosomycin class is avilamycin.

Most AFA are similar to, or identical with, antibacterials used for therapy.

AFA are, in concentrations given for performance enhancement, inhibitory to inherently susceptible intestinal bacteria.

4.3 AFA and development of resistance

It has been argued that the concentrations of antibacterials used for performance enhancement are too low for any development of resistance to occur. As noted in 4.2, they are high enough to inhibit the growth of susceptible microorganisms. The usage of AFA would therefore be expected to favour the occurrence of resistant strains in exposed populations.

Table 4.II. Mode of action of substances used as AFA and known mechanisms of resistance (modified from Witte, 1996)

Tabell 4.II. Verkningsmekanismer hos substanser som används som AFT och kända resistensmekanismer (modifierad från Witte, 1996)

Class	Substance	Examples of other substances in the class	Mode of action	Known resistance mechanisms	Known genetic background of resistance	Cross-resistance (to substances of other classes)
Glycopeptides	avoparcin ardacin	vancomycin, teicoplanin, daptomycin	inhibition of cell wall synthesis by preventing transglycosylation	modification of binding site (peptidoglycan precursor)	<i>van</i> genes	
Ionophores	monensin salinomycin	other ionophores	disaggregation of cytoplasmic membrane			
Macrolides	tylosin spiramycin	erythromycin, azithromycin, clarithromycin	inhibition of protein synthesis by stalling the ribosome	modification of binding site (methylation of 23S rRNA) drug inactivation, efflux	<i>erm</i> genes, point mutation	lincosamides, streptogramins
Orthosomycins	avilamycin	everninomycins	inhibition of protein synthesis by preventing elongation			
Phospho-glycolipids	flavomycin		inhibition of cell wall synthesis by preventing transglycosylation			no information available
Polypeptides	bacitracin		inhibition of cell wall synthesis by preventing transpeptidation	active efflux, alteration of binding site (IPP), reduced membrane permeability	<i>bcr</i> gene, <i>bacA</i> gene	no information available
Quinoxalines	olaquinox carbadox	cyadox	inhibition of DNA synthesis			no information available
Streptogramins	virginiamycin	pristinamycin, quinupristin-dalfopristin	inhibition of protein synthesis by stalling the ribosome	modification of binding site (methylation of 23S rRNA), drug inactivation, active efflux	<i>erm, sat, vat, vga, sbh</i> genes	macrolides, lincosamides

Substances with well known therapeutic use in human and/or veterinary medicine are in bold characters.

Substanser med välkända terapeutiska applikationer inom human- och/eller veterinärmedicinen är skrivna med fetstil

4.3.1 Prospective studies

Experimental studies

Several studies by the research group of Linton (Hinton *et al.*, 1986b; Kaukas *et al.*, 1987; Kaukas *et al.*, 1988) illustrate the influence of AFA on development of resistance in enterococci.

In two of these studies (Hinton *et al.*, 1986b; Kaukas *et al.*, 1987), the control groups were given a diet containing growth promoters (bacitracin or virginiamycin) and/or a coccidiostat with antibacterial activity. The confounding effect of growth promoters and anticoccidials in the control groups of these latter studies cannot be ignored. Consequently, only comparisons between the groups in the study from 1988 are deemed to be valid and will, in the following, be discussed in detail.

In this study (Kaukas *et al.*, 1988), small groups of chickens were given feed containing either avoparcin, nitrovin, virginiamycin or bacitracin or a diet without AFA during the first three weeks of life. The resistance to virginiamycin, bacitracin or nitrovin in *E. faecium* increased in the groups receiving the respective substance. Moreover, the incidence of resistance to therapeutic antibiotics, expressed as Antibiotic Resistance Index (ARI), was higher ($p=0.003$) in all groups receiving antibacterials. This increase could be associated with an increase in the proportion of *E. faecium* in the enterococcal population of the treated birds.

An experimental study on germ-free mice fed carbadox, olaquinox, flavomycin and chlortetracycline was reported by Corpet (1984). The mice were inoculated with intestinal microflora from 4 piglets, and the drugs were given at dosages corresponding to those used for growth promotion in livestock. Little effect on coliform resistance against olaquinox and carbadox was observed, while resistance against tetracycline increased in the flora of mice fed this drug. In the group given flavomycin, resistance to tetracycline was lower than in the control group. Resistance against flavomycin was not tested.

Dealy and Moeller (1977a) investigated the effect of in-feed flavomycin on the antibacterial susceptibility patterns of faecal *E. coli* from calves. They found an increase in resistance to flavomycin, but a decrease in resistance against streptomycin and tetracycline in the medicated group.

Observations on an apparent suppression of coliforms carrying resistance plasmids has been suggested in reports for both flavomycin, carbadox and bacitracin (Pohl *et al.*, 1975; Walton, 1978; Sepulchre, 1979; Gedek, 1981; Walton, 1984; Walton and Wheeler, 1987; Brophy, 1988). Some of these reports, however, merely studied the synergistic effects of the AFA and other

antibacterials and in others, the information provided is not enough to fully evaluate the issue.

Only for flavomycin have the possible mechanisms for a direct effect on numbers of plasmid carrying strains been addressed. George and co-workers (1984) investigated several possible mechanisms for the effects of flavomycin in concentrations from 1-4 ppm on *E. coli* strains harbouring different types of plasmids. The growth of some, but not all, of the plasmid carrying strains was inhibited in the presence of flavomycin. The plasmid transfer rates were mostly reduced, but also in some cases increased, depending on the type of plasmid. The authors suggest, among other explanations, that the differences observed could be due to flavomycin interacting with transfer-related cell wall structures, such as pili, coded for by certain plasmids. Publications concerning possible effects on gram-positive plasmid-carrying bacteria have not been found. The transfer proteins implicated in the mechanism have not been demonstrated in gram-positives and therefore, such effects would not be expected.

Field studies

Linton and co-workers (1985b) studied antibiotic resistance in the enterococci of commercial broiler flocks fed virginiamycin, bacitracin and avoparcin, and of pigs from commercial farms using in-feed tylosin and virginiamycin. In short, feeding of virginiamycin and/or tylosin resulted in increased resistance to tylosin and variable effects on virginiamycin resistance in both pigs and poultry. Feeding of bacitracin led to an increased resistance to bacitracin. Resistance to avoparcin or other glycopeptides was not tested. Unfortunately, only the pig study included a non-medicated farm as control group, outcome was somewhat variable, and the enterococci were not identified to the species level.

The influence of virginiamycin on the prevalence of resistant enterococci within flocks of turkeys has recently been reported by Thal and co-workers (1996). Different turkeys from the same flock were found to share the same type of streptogramin-resistant *E. faecium*, and the prevalence of resistance to both streptogramins and ampicillin increased over time. All flocks received in-feed virginiamycin, plus other AFA and coccidiostats.

A study to monitor the development of olaquinox resistance in coliforms following the introduction of olaquinox as a feed additive was conducted in commercial farms in Suffolk by Linton and co-workers (1988). The results concerning olaquinox resistance in coliforms in farms with and without use of the antibacterial are shown in table 4.III. As is often the case in field studies, there were problems with sampling variability and difficulties to control the management on the farms. In spite of this, the overall results were consistent and showed an increasing level of resistance to olaquinox in

coliforms from farms using olaquinox. Incidence and level of resistance increased on neighbouring farms not using olaquinox as well, but to a lesser extent. The latter finding is not surprising as the herds were not isolated from the environment.

Table 4.III. The average percentage of coliforms resistant to olaquinox in each year of the survey (from Linton *et al.*, 1988)

Tabell 4.III. Genomsnittlig procentandel av koliformer resistent mot olaquinox under respektive studieår (från Linton *et al.*, 1988)

Year	Resistance (%)	
	Control farms	Test farms
1981 ¹	0.00	-
1982	0.03	0.04
1983	0.12	5.63
1984	0.68	6.14

¹ Sampled before olaquinox was used in the UK

¹ Provtagna innan olaquinox användes i Storbritannien

4.3.2 Retrospective studies

Devriese and co-workers (1993) studied the antibacterial resistance patterns for some AFA in *C. perfringens* isolated from various animal sources and found no notable increase in resistance between 1979 and 1992.

Ohmae and co-workers (1981; 1983) reported the incidence of carbadox resistance in *E. coli* from cattle, pigs and chickens, collected during 1976-1980. Carbadox resistance was only found in isolates from pigs. All carbadox-resistant isolates originated from 6 farms, where carbadox was used for preventing swine dysentery or promoting piglet growth. All isolates from one farm harboured a conjugative plasmid, carrying carbadox, spectinomycin, streptomycin and ampicillin resistance.

An increase in carbadox resistance in salmonellae over time was noted in a study by Mills and Kelly (1986). The study included clinical isolates from necropsied swine in Kansas during 1980-1983. A steady increase in carbadox resistance, from 37% 1980 to 61 % 1983 was noticed. The authors note that in-feed carbadox in Kansas is labelled for prevention of swine dysentery and for treatment of salmonella infections.

A strong association between the use of avoparcin and prevalence of vancomycin resistant enterococci (VRE) in animals has been reported. Bager and co-workers (1997) investigated poultry and pig farms in a retrospective cohort study. The relative risk for occurrence of vancomycin resistant *Enterococcus faecium* was 3.3 (0.9-12.3) for pig herds exposed to avoparcin. The corresponding figure for poultry flocks was 2.9 (1.4-5.9). These findings are supported by observations from countries where avoparcin is not used, no

VRE have yet been isolated from animals (Coque *et al.*, 1996; Greko and Lindblad, 1996).

4.3.3 Point-prevalence studies

Shortly after the introduction of avoparcin, no glycopeptide resistance was found among 15 strains of *E. faecium* isolated on vancomycin-free media (Dutta and Devriese, 1982), nor was resistance found in other enterococcal species. In table 4.IV, recent results concerning glycopeptide resistance from the Danish surveillance system are presented. As in the above mentioned study, the isolates investigated were obtained without the use of antibiotic containing media in the course of a monitoring programme.

Studies using media favouring resistant isolates indicate that enterococci with high level resistance to glycopeptides (VRE) are widespread among animals including pets and horses (Bates *et al.*, 1994; Klare *et al.*, 1995b; Devriese *et al.*, 1996). The lack of earlier data on VRE in animals precludes conclusions on whether the resistance trait was present in animal populations at the time of introduction of avoparcin in animal husbandry.

As mentioned in 4.3.2, in studies from USA where avoparcin has never been used and from Sweden where avoparcin has not been used for 10 years no VRE were found in samples from animals using selective techniques (Coque *et al.*, 1996; Greko, 1996). Thus, in the absence of avoparcin, the prevalence of VRE in animals is, at most, very low.

Table 4.IV. Frequency of resistance to glycopeptides (vancomycin) in *E. faecium* from different sources¹

Tabell 4.IV. Frekvens av resistens mot glykopeptider (vancomycin) hos *Enterococcus faecium* från olika provtyper¹

Animal source	No. of isolates	Resistance in %
Cattle	13	0
Beef	40	0
Swine	58	20
Pork	23	0
Poultry	54	59
Poultry meat	71	18

¹ Data from DANMAP (1997). In samples from food, only high-level resistance was reported whereas in animals samples, all resistant isolates are included

¹ Data från DANMAP (1997). För prover från livsmedel har endast höggradig resistens rapporterats, från djurprover inkluderades däremot alla resistenta isolat

It has often been assumed that resistance observed in animal bacteria is entirely a result of the use of therapeutics. A comparison between the situation in countries where macrolides are used both for therapy and as

AFA, and where they are only used for therapy is therefore of interest. In table 4.V, results from investigations in Denmark, Finland and Sweden are presented. Spiramycin, being a 16-membered macrolide was chosen in the example as indicator of constitutively expressed *erm*-genes, encoding for MLS_B type resistance (see annex E). A lower prevalence of resistance is reported from Finland and Sweden, using only therapeutical macrolides compared to Denmark where they are also used as AFA.

The tables above illustrate phenotypic expression of resistance. However, information on phenotype at best only permits inferential conclusions about the genes conferring these resistance traits. All the resistant isolates from Sweden and Finland were cross-resistant to erythromycin (a 14-membered macrolide). The enterococci are inherently resistant to moderate concentrations of lincosamides (e.g. clindamycin) but high level resistance can be acquired (Murray, 1990). For all the Swedish isolates that were resistant to 16-membered macrolides, MIC values higher than for the remaining group of isolates were noted. This leads to the assumption that the resistance shown is mediated by constitutively expressed *erm*-genes. MICs for clindamycin were not given for the Finnish isolates.

Table 4.V. Resistance to MLS_B antibacterials (indicated by spiramycin resistance) in enterococci from various species and countries (From Greko, 1996; DANMAP, 1997; Greko, 1997; MAF, 1997)

Tabell 4.V. Resistens mot MLS_B antibiotika (indikerat av spiramycinresistens) hos olika djurslag (Från Greko, 1996; DANMAP, 1997; Greko, 1997; MAF, 1997)

Bacterial species	Animal source	No. of isolates	Year	Resistance in %	Country
<i>E. faecium</i>	poultry	54	1995-96	54	Denmark
<i>E. faecium</i>	poultry	234	1996	10	Finland
<i>E. faecalis</i>	poultry	299	1996	9	Finland
<i>Enterococcus</i> spp.	poultry	207	1996	15	Sweden
<i>E. faecalis</i>	swine	123	1995-96	89	Denmark
<i>E. faecium</i>	swine	58	1995-96	88	Denmark
<i>E. faecium</i>	swine	89	1996	12	Finland
<i>E. faecalis</i>	swine	85	1996	15	Finland
<i>Enterococcus</i> spp.	swine	92	1996	14	Sweden

Further information on frequency of resistance to AFA can be found in annexes A-F.

4.3.4 Some comments on study design

Studies performed to date are extremely difficult to compare. Selection criteria, methods used and definition of resistance vary. Further, a serious limitation of most studies on antibacterial resistance is the restricted range of microbes surveyed (Salyers, 1995). This is particularly true if the question asked is how the use of a substance affects the evolution and spread of resistance genes in a certain environment. Inclusion of numerically predominant organisms, such as certain anaerobic genera, would give a more complete picture. For practical reasons, most studies focus on easily cultured bacteria. Until further knowledge about the role of the predominant part of the flora is obtained, we have to accept that much information concerning gene transfer in natural settings is missing (Salyers, 1995).

Some species are inherently (naturally) resistant to certain antibacterial substances. For example, *C. perfringens* and *E. faecium* have been reported to be inherently resistant to flavomycin and enterococci, apart from *E. faecium*, are inherently resistant to streptogramin A. Such species will be favoured if a selective pressure is applied. Other species, that are inherently susceptible to the substance in question, may become resistant either by mutation or by the acquisition of already existing resistance genes. When the aim is to assess the influence of antibacterial usage on the prevalence of antibacterial resistance, bacterial populations with a potential for acquiring resistance should be targeted. Enterococci are inherently susceptible to most AFA and are known to easily acquire resistance genes. In view of this, and bearing the above mentioned limitations in mind, the choice of enterococci as one of the indicators of the development of resistance seems acceptable for most AFA.

The study time in both experimental and field exposure studies is generally short. Dissemination of antibacterial resistance will be governed by two main factors; presence of the antibacterial in concentrations high enough to inhibit susceptible bacteria and presence of resistance traits (intrinsic or acquired) in bacteria. If either is continually absent, a resistance problem will not emerge (Levy, 1996). Acquisition of the relevant gene and its subsequent adaptation to a new host bacterium may take time (years). As closed experimental herds are often used, resistance may in fact never appear, even if it would with time in a field situation where herds are expected to have numerous direct and indirect contacts with potential gene reservoirs.

Given the presence of both a selective pressure and resistance, the resistance trait will be selected for and propagated. The spread of resistant bacteria themselves, as well as cell to cell spread of genes conveying resistance, further adds to the equation. Longer study periods (years) would be expected to give more applicable results, but may be impractical for other reasons.

In view of the long standing debate on usage of AFA and resistance, there are surprisingly few studies including data on prevalence of resistance against the substances in question. Even less data is available on development over time. Well-planned screening studies, conducted in several countries and combined with data on consumption of antibacterial feed additives in these countries (amount given to different animal species), as well as therapeutic usage might provide at least some of the information on exposure necessary for an accurate analysis of potential hazards and associated risks. Programmes, such as the Danish monitoring (DANMAP, 1997), as well as the one lately initiated by the European Commission, are therefore to be commended.

Various types of studies show that exposure of bacterial populations to AFA in approved dosages favours resistant clones or subpopulations.

4.4 Acquisition of resistance in bacteria

Acquired resistance can arise in a bacterium through mutation(s) in existing genes or through the uptake of a pre-existent gene. In the first case, the resistance trait will be confined to the mutant clone. Spread of resistance will depend on the ability of that clone to multiply (vertical transmission) and infect new hosts. In the second case, the resistance trait can also spread to other bacterial clones, to other bacterial species and genera (horizontal transmission).

Following uptake, acquired resistance genes may be modified, or optimised, by means of mutation. Once the optimal configuration has been accomplished, little change would be expected within the gene, although transfer to a new host species or genus may require some adaptation for maximum expression. "Silent" resistance genes, i.e. non-expressed genes, may be "activated" by mutations.

4.4.1 Transfer of resistance genes

Pre-existent resistance genes are mostly associated with transfer elements such as plasmids or transposons, residing in plasmids or on the chromosome. Detailed studies concerning transfer elements associated with relevant genes have only been published for antibacterials in clinical use. As acquired resistance has been reported for other AFA as well, there is no reason to believe that resistance against these substances would be any different in this respect. In table 4.VI, described genes conveying resistance to macrolides and/or streptogramins and examples of their localisation have been compiled.

The plasmids and transposons mentioned are often of a conjugative type, meaning that in addition to the resistance genes, they carry the information necessary to initiate and complete their own transfer to new hosts.

Transfer of resistance determinants with relevance for AFA has been shown *in vitro* and, for some, *in vivo*.

Resistance to the A component of streptogramins is mediated by various genes carried on plasmids. The *vatB* gene has been shown to transfer between coagulase-negative staphylococci and *S. aureus* (Allignet *et al.*, 1996).

Resistance to streptogramin B and 16-membered macrolides (spiramycin, tylosin) is mostly mediated by constitutively expressed *erm*-genes of different classes (Leclercq and Courvalin, 1991b). The product of the gene, a ribosomal methylase, alters the ribosome slightly with the result that macrolides, lincosamides and streptogramin B cannot bind (MLS_B resistance). The *erm*-genes in staphylococci are often inducible only by 14-membered macrolides (erythromycin), meaning that the methylase will only be produced in presence of erythromycin. The gene can convert to constitutive expression by a single or two point mutation rendering the bacterium phenotypically resistant to all macrolides, to lincosamides and to streptogramin B (see annex E).

Poyart-Salmeron and co-workers (1990) showed by way of *in vitro* mating experiments that a self-transferable plasmid designated pIP811 carrying an *ermB* gene (also carrying resistance determinants for chloramphenicol, tetracycline and streptomycin) could transfer from *Listeria monocytogenes* to, among others, *E. faecalis* and vice versa. The donor efficiency³ depended on the combination of donor-recipient tested and varied from 10⁻³-10⁻⁹ with enterococci and streptococci being the most and staphylococci the least efficient. The plasmid, originally identified in *Listeria monocytogenes*, was suggested to have originated from streptococci or enterococci as it is similar to pAMβ1, the prototype for broad-host range plasmids in those genera.

Acquisition of macrolide resistance through transfer of the chromosomally carried transposon Tn1545, harbouring MLS_B (*ermB*), kanamycin (*aphA3'*) and tetracycline (*tetM*) resistance determinants from *E. faecalis* to *L. monocytogenes* has been demonstrated both *in vitro* by mating experiments and *in vivo* in gnotobiotic mice (Doucet-Populaire *et al.*, 1991). The *in vitro* and *in vivo* transfer frequencies were around 10⁻⁷ and 10⁻⁸, respectively. In both cases, subinhibitory concentrations of tetracycline increased the transfer frequency around 10 times.

³The donor efficiency expresses the number of successful transmissions from donors to recipients per total number of recipients. The donor:recipient ratio in experiments is often 1:1

Table 4.VI. Examples of resistance to macrolides and streptogramins by different mechanisms and genes¹

Tabell 4.VI. Exempel på resistens mot makrolider och streptograminer, via olika mekanismer och gener¹

Phenotype ²	Mechanism	Gene	Described localisation	Examples of bacterial hosts
MLS _B	target modification	<i>ermA</i>	Tn554	<i>S. aureus</i> , coagulase negative staphylococci
		<i>ermB</i> , <i>ermAM</i>	Tn551, pAMβ1, Tn917, Tn1545, plus various other plasmids and transposons	<i>S. aureus</i> , <i>S. intermedius</i> , <i>S. hyicus</i> , <i>Streptococcus</i> spp, <i>S. pneumoniae</i> , <i>E. faecalis</i> , <i>Lactobacillus</i> spp.
		<i>ermC</i>	pE194, pLM13, pE5, pNE131	<i>S. aureus</i> , coagulase negative staphylococci, <i>S. hyicus</i> , <i>Bacillus subtilis</i>
		<i>emrCX</i>	Tn5432	<i>Corynebacterium xerosis</i>
		<i>ermD</i>	chromosome	<i>B. licheniformis</i>
		<i>ermBC</i>	pIP1527	<i>E. coli</i>
		<i>ermF</i>	pBF4	<i>Bacteroides</i> spp.
		<i>ermE</i>	chromosome	<i>Streptomyces erythreus</i>
		<i>ermP</i> , <i>ermQ</i>		<i>Cl. perfringens</i>
		<i>ermZ</i>	Tn5398	<i>Cl. difficile</i>
		<i>ermJ</i> <i>ermM</i>		<i>Bacillus anthracis</i> <i>S. epidermidis</i>
S _B	drug inactivation	<i>sbh</i>	pIP524	<i>S. aureus</i>
S _A		<i>vgb</i>	pIP680	<i>S. aureus</i>
		<i>satA</i>	pAT424	<i>E. faecium</i>
		<i>vat</i>	pIP680, IS257	<i>S. aureus</i>
		<i>vatB</i>	pIP1156	<i>S. aureus</i>
S _A	active efflux	<i>vga</i>	plasmids	<i>S. aureus</i> , <i>S. epidermidis</i>

¹Data compiled from Leclercq and Courvalin, 1991a; 1991b; Brisson Noel *et al*, 1988; Arthur *et al*, 1987; Eady *et al*, 1993; Weisblum, 1995; Mullany *et al*, 1995; Allignet *et al*, 1996; Tauch *et al*, 1995

² Resistance phenotype; MLS_B = macrolide-lincosamide-streptogramin B; S_B = streptogramin B; S_A = streptogramin A

¹Data sammanställda från Leclercq and Courvalin, 1991a; 1991b; Brisson Noel *et al*, 1988; Arthur *et al*, 1987; Eady *et al*, 1993; Weisblum, 1995; Mullany *et al*, 1995; Allignet *et al*, 1996; Tauch *et al*, 1995

² Resistensfenotyp; MLS_B = makrolid-linkosamid-streptogramin B; S_B = streptogramin B, S_A = streptogramin A

In the above cited experiment, germ-free mice were used. McConnell and co-workers (1991) used mice with a complex microflora functionally equivalent to that of conventional mice. They investigated transfer of the plasmid pAM β 1, carrying *ermB*. Transfer of the plasmid from *Lactobacillus reuteri* to *E. faecalis* was detected within days after birth in the offspring of the mice inoculated with the donor strain. The parent animals were given lincomycin at subtherapeutic concentrations. No transfer was detected in offspring of mice not given lincomycin.

The fact that conjugal transfer was easily detectable within a relatively short time period in both *in vivo* experiments cited above confirms that the intestinal environment is highly conducive to conjugal transfer, even when the donor and recipient are of different genera. Further, they show that *in vitro* results on transfer and transfer rates can be predictive of *in vivo* results.

Transferable glycopeptide resistance is usually mediated by the gene clusters *vanA* or *vanB*. These are generally located on plasmids and/or transposons (Arthur and Courvalin, 1993). High level resistance to glycopeptides mediated by the *vanA*-gene cluster has been detected in *E. faecium*, other enterococcal species (Arthur and Courvalin, 1993), *Oerskovia turbata* and *Archanobacterium haemolyticum* (Power *et al.*, 1995). The gene cluster is mostly associated with the conjugative transposon Tn1546 and/or self-transferable plasmids (Arthur *et al.*, 1996). Transfer of the *vanA*-gene cluster has been shown *in vitro* from *E. faecium* to *Listeria monocytogenes*, *Staphylococcus aureus*, and various streptococci (Leclercq *et al.*, 1989). Transfer frequencies were 10^{-4} for *E. faecium* to *E. faecium* and 10^{-6} - 10^{-9} for transfer to other species. When resistance to MLS antibiotics was also present, the two traits were transferred *en bloc* (Leclercq *et al.*, 1989). Conjugal co-transfer of resistance to high levels of glycopeptides, erythromycin and chloramphenicol, from *E. faecalis* to *S. aureus* on the skin of hairless obese mice was demonstrated in an experiment by Noble and co-workers (1992). The mice used cannot be regarded as "normal" mice. Nonetheless, they are a better model for the *in vivo* situation than a petri dish.

Resistance to glycopeptides mediated by the *vanB* gene cluster has, in relation to AFA, attracted less attention. The *vanB*-gene cluster is transferable either directly from the chromosome by a transposon (Tn1547) or through plasmids (Quintiliani and Courvalin, 1994; Woodford *et al.*, 1995; Quintiliani and Courvalin, 1996) at a low frequency. The *vanB*-gene cluster has been found in *E. faecalis*, *E. faecium* and, recently, in *S. bovis* (Arthur and Courvalin, 1993; Poyart *et al.*, 1997). The gene usually confers inducible resistance to glycopeptides by a mechanism similar to that of the *vanA* gene-cluster. The *vanB*-gene cluster is induced by vancomycin but not by teicoplanin, meaning that when strains carrying the gene cluster are exposed to teicoplanin, the gene will not be activated and the strain phenotype will remain susceptible (Arthur and Courvalin, 1993). According to available

information, the *vanB* gene cluster does not seem to be inducible by avoparcin. No information is available on the capacity of ardacin to induce *vanB*.

In vitro as well as during therapy, mutants expressing the *vanB* gene constitutively have been reported (Hayden *et al.*, 1993; Green *et al.*, 1995). Recently, transfer experiments with a strain expressing *vanB* constitutively were reported (Hayden *et al.*, 1997). The resulting transconjugants were either of constitutive or of inducible type. The use of a non-inducing antibacterial such as avoparcin, and possibly ardacin, could favour strains harbouring *vanB*-gene clusters with the mutation required for the gene to be constitutively expressed should the gene be present in animal populations or their environments. Further information is needed on this topic.

4.4.2 Bacterial interspecies transfer of resistance genes

As evident from 4.4.1, similar or identical resistance genes can be found in various bacterial species, indicating that transfer of resistance genes between different bacterial species is not uncommon in nature. Conjugal transfer systems are able to cross genus, phylogenetic, and even kingdom lines (Amabile-Cuevas and Chicurel, 1992). There are broad host range genes that can be expressed in a variety of species as well as broad host range gene transfer elements.

As an example, high level resistance to MLS_B antibacterials in a clinical isolate of *E. coli* was characterised by Brisson-Noël and co-workers (1988). The resistance trait was found to be due to the presence of an *erm*-gene highly homologous to *ermB*, previously described in *E. faecalis* and *S. sanguis*. The occurrence of *ermB*-like genes in enterobacteria was confirmed in other *E. coli* strains and in *Klebsiella*. The findings indicate that the gene pool of gram-positive cocci has extended to gram-negative bacteria, that such transfer can occur in nature and that the extension is recent. Further inferential evidence of such trans-gram promiscuity exists for other resistance genes (for a review see Courvalin, 1994).

4.4.3 Co-transfer of genes and multiresistance

A plasmid or transposon may gradually acquire one gene after another. Integrons and transposons appear to be largely responsible for this phenomenon. When they are located on the same transferable element, the transmission of different genes is linked and transfer leads to co-resistance. This means that a bacterium concurrently becomes resistant to two or more different antibacterials, mediated by different resistance mechanisms,

governed by different genes. Co-transfer, linked transfer or transfer *en bloc* are different expressions for this phenomenon.

The transmission of resistance genes with relevance for AFA can be linked to the transfer of other genes. The *E. coli* plasmid pNV13 conveys resistance to carbadox, streptomycin, spectinomycin and ampicillin (Ohmae *et al.*, 1981; Ohmae *et al.*, 1983). Conjugal transfer of resistance to streptogramin A, between *S. aureus* strains, linked to lincosamide, trimethoprim and penicillin resistance has been reported (Allignet and El Solh, 1995; Allignet *et al.*, 1996). Enterococcal plasmids from several host species have been shown to harbour and transfer MLS_B, kanamycin, streptomycin and, sometimes, tetracycline resistance (Rollins *et al.*, 1985; LeBlanc *et al.*, 1986; Christie *et al.*, 1987). The transposon Tn1545 has been shown to carry and transfer resistance genes to kanamycin, macrolides and tetracycline (Doucet-Populaire *et al.*, 1991). Genes conveying resistance to glycopeptides and macrolides have been found to co-reside on plasmid pIP819 (Leclercq *et al.*, 1989). Other examples of co-transfer, have been given in 4.4.1.

A similar linkage between different genes has been shown between some resistance and virulence genes, such as haemolysin- or toxin-encoding genes. Co-transfer of virulence and resistance genes has been well studied in enteric pathogens (among others Franklin and Möllby, 1983). Less information is available concerning gram-positive bacteria. Christie and co-workers (1987) demonstrated *in vitro* co-transfer of genes encoding for macrolide and tetracycline resistance and haemolysin production from *E. faecalis* to *Bacillus subtilis*. *In vivo* transfer of pheromone-responsive plasmids carrying genes conferring cytolytic properties and macrolide resistance (*ermB*) between strains of *E. faecalis* has been studied in a model using Syrian hamsters (Huycke *et al.*, 1992). Transfer occurred in high frequencies (10^{-1} - 10^{-2} per donor). Even more worrying is the transfer *in vitro* of macrolide resistance (*ermBZ*) and toxin A, apparently carried on a transposon (Tn5398) from *C. difficile* to non-toxigenic *C. difficile* strains and to *B. subtilis*, reported by Mullaney (1995).

Consequently, a selection for antibacterial resistance may, in some cases, also select for virulence. The danger of transfer elements carrying multiple genes is evident; a single transfer event conveys not only resistance to the selector but also other properties to the recipient bacterium. The recipient may acquire multiresistance and/or increase its virulence. Any advantage given by one of the transferred genes helps to maintain all of the transferred genes in the new host.

4.4.4 Resistance genes and animal hosts

Highly homologous resistance genes and their associated transfer elements have been found in natural isolates not only of different bacterial species but also in bacteria from a variety of host species. The macrolide resistance gene

ermB has been demonstrated in bacteria of different species originating from pigs, chickens, cattle, dogs and humans (Rollins *et al.*, 1985; Arthur *et al.*, 1987; Stuart *et al.*, 1992; Eady *et al.*, 1993; Roberts and Brown, 1994; Wasteson *et al.*, 1994).

As an example, the results of Eady and co-workers (1993) who screened for resistance determinants in macrolide resistant isolates of staphylococci (excluding *S.aureus*) from humans, pigs and dogs are shown in table 4.VII.

Table 4.VII. Distribution of macrolide resistance genes in staphylococci from various hosts given as percent of total number of genes identified in isolates from respective animal host¹ (modified from Eady *et al.*, 1993)

Tabell 4.VII. Fördelningen av makrolidresistensgener hos stafylokocker från olika värddjur uttryckt som procent av totalantalet identifierade gener¹ (modifierat från Eady *et al.*, 1993)

Gene ¹	Swine	Dogs	Humans	
			group 1 ²	group 2 ²
<i>ermA</i>	0	6	12	6
<i>ermB</i>	20	65	0	0
<i>ermC</i>	63	6	41	54
<i>msrA</i>	6	6	38	39
<i>erm</i> untypable	11	18	9	2

¹No. of genes identified and No. of investigated isolates: swine 35 and 33, dogs 17 and 16, humans group 1 58 and 55, humans group 2 121 and 117, respectively.

²Group 1 includes isolates from patients undergoing ambulatory peritoneal dialysis (n=27) and from blood cultures (n=28); group 2 includes patients with acne (n=117).

¹ Antal identifierade gener respektive antal undersökta isolat: svin 35 och 33, hundar 17 och 16, människor grupp 1 58 och 55, människor grupp 2: 121 och 117.

² Grupp 1 inkluderar isolat från patienter i ambulatorisk peritonealdialys (n=27) samt blododlingar (n=28); grupp 2 inkluderar patienter med akne (n=117).

The suggested spread of vancomycin resistance between enterococci of various origin is another example of the spread of resistance among gram-positive bacteria. VRE harbouring the *vanA* gene cluster have been isolated from humans, both in hospitals and community, from pig, rabbit, dog, cat, horse chicken, turkey, pheasant, duck, food of animal origin and sewage (Bates *et al.*, 1994; Torres *et al.*, 1994; Klare *et al.*, 1995b; Chadwick *et al.*, 1996; Devriese *et al.*, 1996; DANMAP, 1997). A polyclonal nature of the VRE strains has been demonstrated (Klare *et al.*, 1995b). The *vanA* gene cluster consists of 7 gene components and two transposition gene sequences. It is extremely unlikely that such a complicated gene should have developed separately in so many different host populations. Its occurrence therefore strongly suggests a spread between bacteria of different host species.

4.4.5 The encumbrance of resistance for bacteria

In an environment that contains antibacterials, possession of a corresponding resistance gene is clearly beneficial for the bacterium. The resistance traits have, however, been considered to impose a burden on their bacterial hosts. Synthesis of plasmids require energy and metabolites. The products encoded for by resistance genes may interfere with the bacterium's physiology alterations of the cell wall may lead to loss of adhesive properties and alternative metabolic pathways may be less efficient. Constitutive expression of resistance genes encoding for such traits would be a handicap in the absence of antibacterials. For such reasons, resistant clones have been thought to suffer a competitive disadvantage in the absence of a selective pressure. Even so, this might not always be the case. Much evidence of the cost for the bacterium of harbouring the resistance trait has been obtained in laboratory experiments with bacteria that have recently acquired the resistance gene.

Other experiments show that, given time, bacteria can eliminate the cost of resistance by adapting their system in order to counteract the harmful side-effects of the resistance genes. This will result in increased fitness of resistant strains with consequent persistence of the resistance trait in the microbial population even after the selective pressure is removed. Such adaptation has been shown for resistance acquired through mutations as well as through plasmids (for reviews see Lenski, 1996; Gillespie and McHugh, 1997). As an example, McConnell and co-workers (1991) demonstrated the need of a selective pressure for the establishment of a resistance plasmid in the microflora of mice. However, once established, the strain originally carrying the plasmid adapted and competed well with susceptible strains in the absence of a selective pressure.

4.4.6 Some remarks on horizontal transmission of genes

Transmission of genes between bacteria occurs readily *in vitro*. Experimental transfer of a conjugative element may require little manipulation besides the mixing of two cultures. The rates, or frequencies, of such transmissions will depend on the transfer element involved and the donor-recipient combination as well as on the conditions of the transfer experiments. In this respect it should be noted that failure to demonstrate transfer does not mean it cannot take place, simply that it did not occur under the conditions of the experiment. On the other hand, demonstration of transfer indicates that the event can take place.

Bacteria are equipped with very efficient gene transfer systems. Similar, or identical, resistance genes and associated transfer elements are present in field isolates from different host species as well as in different bacterial

species. A multitude of transfer elements are found in bacteria isolated from natural settings. Resistance traits arise within species that were formerly considered uniformly susceptible.

The experiments demonstrating *in vivo* transfer in laboratory animals have been conducted to test a hypothesis based on firm observations from "real life" of emerging resistance. An example of this is the studies on macrolide and tetracycline resistance in *Listeria monocytogenes* cited in 4.4.1. The settings are, albeit artificial, certainly closer to the real situation than the test tube.

The opinion that the transfer rate in nature would be significantly lower than what is obtained *in vitro* is based on the assumption that laboratory settings provide optimal conditions, and that such conditions are rare in real life. This is not necessarily true. The laboratory conditions normally used to test DNA transfer employ rapid growth, aerobic conditions and nutrient excess. Such conditions might enhance transfer rates, but it might also repress gene transfer in some bacteria (Salyers, 1995). Further, the event does not have to be frequent in order to have an effect. Once the resistance gene is present in a recipient, given a selective pressure the resistant clone will multiply.

Taken together, it is evident that transfer of genes does occur in nature (in/on living hosts or in their environments). The question that remains is how often it occurs.

Resistance mechanisms and resistance genes have only been investigated for AFA belonging to classes containing therapeutics.

Described resistance genes are generally associated with transfer elements. Transfer of resistance has been shown experimentally both *in vitro* and *in vivo*.

Transfer of resistance has been shown between bacteria of different genera.

The same resistance genes are found in bacteria from different host species.

Co-transfer of multiple genes is common.

Resistant bacteria are not necessarily less competitive in the absence of a selective pressure.

4.5 Transfer of resistance between various host species

There are two theoretically possible ways for transmission of antibiotic resistance from bacteria of one host species to those of another; firstly, direct

transmission of resistant bacteria, and secondly, transmission of resistance genes between bacteria colonising different host species. The debate has mostly been concerned with transmission of resistant bacteria from animals (taken as a group) to man. It has been questioned whether such transmission can occur. Logically, if transfer did not occur between bacteria from a specific animal species to man, then transfer between different animal species would not be expected to occur either. After all, humans are the only species in constant contact with all the other species.

4.5.1 Transmission of bacteria

Studies concerning transmission of resistant bacteria from animal to man have mainly focused on gram-negative enteric pathogens such as *Salmonella* spp. and *Campylobacter* spp. For antibiotic resistant salmonellae, the pathways from animals to man and subsequent infections have been clearly demonstrated (Bezanson *et al.*, 1983; Holmberg *et al.*, 1984; Spika *et al.*, 1987; Mishu *et al.*, 1991). Several reports suggest the transfer of other antibiotic-resistant enteropathogens such as quinolone-resistant campylobacter (Endtz *et al.*, 1991) and chloramphenicol-resistant *Yersinia enterocolitica* (Perez Trallero *et al.*, 1988).

Transfer of *E. coli* between various animals and humans has been demonstrated in various studies. The spread of a labelled multiresistant *E. coli* from cow to pig, mouse, fowl, fly, water and human, and from pig to fly, mouse, fowl, water and human was reported by Marshall and co-workers (1990). Levy (Levy *et al.*, 1976) reported the transfer of a labelled multiresistant *E. coli* from chicken to a farm worker. Linton and co-workers (1977) demonstrated the transfer of sulphonamide-resistant *E. coli* from a chicken carcass to the faecal flora of five human volunteers handling the chicken.

Although the above cited studies concern resistance to antibacterials not used as AFA in the EU, they demonstrate that bacteria can be transmitted from animals to man directly, via food, or by other routes and in the case the bacterium carries resistance this trait will not disappear along the line. Transfer of gram-positive bacteria has been less studied. Hummel and Witte (1981) found macrolide-resistant *S. aureus* of human biovariant in farm workers and in occasional pigs on farms using in-feed tylosin and tetracycline. This seems to indicate a human to animal transmission. As staphylococci are known to be relatively host specific, it is likely that the finding was merely the result of a transient colonisation. Interestingly, no macrolide-resistant strains were found among the family members of the farm workers, indicating the need for a selective pressure for transferred bacteria to persist in the new host. In the case of the farm-workers, they were likely to be exposed to tylosin through dust.

Direct transmission from chicken to man has been implicated in a recent report on a case of wound infection with a vancomycin resistant *E. faecium* in a worker at a chicken packaging plant (Das *et al.*, 1997). Available information strongly suggests that the infection was occupationally derived (contracted from chicken carcasses).

Human *E. faecium* strains have been widely used as probiotics and do, at least transiently, colonise animal intestines. Further, human VRE have successfully been used to colonise mice experimentally (Whitman *et al.*, 1996). This indicates that at least certain enterococcal strains can colonise, or transiently inhabit, a variety of hosts. Another report on occupational exposure provides further evidence (van den Bogaard *et al.*, 1997). The prevalence of VRE in turkeys, turkey farmers, turkey slaughterers and urban residents was found to be 50%, 39%, 20% and 14%, respectively. Further investigations showed that VRE isolated from one of the farmers and his turkeys could not be differentiated by phenotypic or genotypic (pulsed-field gel electrophoresis) methods. Investigations of the *vanA*-gene cluster by polymerase chain reaction (PCR) and hybridisation showed the two strains to be identical in the tested areas, having an insertion in a previously nondescribed position, between the *vanX* and *vanY* gene, and a deletion in the right end of the cluster.

4.5.2 Transfer of genes

The majority of the bacteria in the animal intestinal microflora are relatively host specific and would not be expected to colonise other animal species or man. Similar restrictions do not apply to the bacterial host range of resistance genes. Therefore, transfer of genes between animal and human bacteria is more likely to have an impact on interspecies exchange of resistance. Transfer of resistance genes between animal and human microflora has been shown in several studies. Again, many of those deal with resistance genes related to therapeutical antibacterials.

However, in the light of the close relatedness between resistance genes in human and animal bacteria and the current knowledge of gene transfer mechanisms and genetic drift, it would be naïve to presume that there is no exchange of resistance genes between animal and human microflora. On the contrary, this is the most, if not the only, plausible explanation for the spread of antibacterial resistance against both therapeuticals and AFA in various populations. The majority of the studies addressing this topic concern resistance in gram-negative bacteria against therapeutic antibacterials. Nonetheless, they illustrate the fact that transmission of genes occurs between animal and human bacteria.

Resistance genes identified in both animal and human bacteria

Levy and co-workers (Levy *et al.*, 1976) reported spread of an *E. coli* multi-resistance plasmid between chickens and from chicken to man.

The spread of streptothricin resistance from animal to human bacteria has been documented in former Eastern Germany. A streptothricin antibiotic, nourseothricin, was commonly used in pig feed from the beginning of the 80s. A novel transposon-encoded resistance mechanism was identified in *E. coli* from pigs in 1983. Subsequently, this transposon, Tn1825, has been found in the normal microflora of pig farmers and their families and of healthy unrelated adults in the community, and in urinary tract infections in humans. Finally, the resistance determinant has been detected in salmonella and shigella from human cases of diarrhoea (Hummel 1986, Tschäpe 1994). Streptothricin antibiotics are not used in humans. In areas where the antibiotic was not used in animals, the resistance determinant was not found.

Another example is the spread of aminoglycoside-acetyl-transferase IV (apramycin resistance) and hygromycin B phosphotransferase (hygromycin B resistance). The corresponding genes, *aacC4* and *hphB*, form part of one resistance gene operon associated with an insertion sequence IS140, probably part of a transposon, that is found on plasmids. The organisation of *aacC4* and *hphB* is such that they are always co-transferred. Apramycin and hygromycin B are used exclusively in animals. The gene *aacC4*, conferring cross-resistance to gentamicin, was first identified in *E. coli* and *Salmonella typhimurium* from animals in France and the United Kingdom (Chaslus-Dancla *et al.*, 1986; Wray *et al.*, 1986). It has since been demonstrated in various enterobacteria, including salmonella, from human as well as environmental sources in different countries (Threlfall *et al.*, 1986; Chaslus-Dancla *et al.*, 1989; Hunter *et al.*, 1993; Hunter *et al.*, 1994). A high degree of genetic homology between plasmids harbouring *aacC4* and *hphB* of human and animal origin has been demonstrated (Salauze *et al.*, 1990; Chaslus-Dancla *et al.*, 1991). In a prospective study on a pig farm, Hunter (1994) demonstrated a widespread dissemination of plasmids harbouring *aacC4* in *E. coli* from pigs, calves, the stockman and a variety of environmental sources including rainwater puddles and water from a stream nearby. *Klebsiella pneumoniae* with a slightly smaller conjugative plasmid and similar resistance pattern was isolated from the stockman's wife.

As mentioned in 4.3.4, investigations concerning antimicrobial resistance tend to focus on a limited range of microbes. Few investigations concerning transfer of genes between the predominant, anaerobic, part of the microflora of man and animals are available. Nikolich and co-workers (1994) examined the possible gene transfer between microflora of ruminants and man, by using the *tetQ* gene, conveying tetracycline resistance, as indicator. By DNA sequencing of the gene from *Bacteroides* spp. and *Prevotella intermedia* of human origin and *Prevotella ruminicola* of bovine origin, they showed

identical, or nearly identical, gene sequences in bacteria of the two hosts from different geographic origin. The findings indicate that extensive transmission of the *tetQ* gene has occurred in nature between bacteria normally colonising different hosts and that the transfer is recent.

No similar investigations with direct relevance for AFA used in Europe have been found, nor studies concerning dominant microflora of other animal species. *In vitro* co-transfer of macrolide and tetracycline resistance between *Bacteroides* spp. and *Prevotella ruminicola* has been shown (Shoemaker *et al.*, 1992), indicating a strong possibility of similar *in vivo* transfer events related to macrolides and streptogramins. Further, successful transfer of *ermF* genes from *Treponema denticola* to *Enterococcus faecalis* has been demonstrated (Roberts *et al.*, 1996a)

The question of "identity" of genes has been a matter of debate in relation to the discussion on the use of avoparcin in animal husbandry. It is important to point out that the bacterial genome is subject to changes over time. Mobile gene-sequences may insert into or close to non-essential parts of a gene, and deletions or point-mutations may take place. Consequently, the sequence of a specific gene may differ from one point in time to another.

The *vanA* gene cluster contains 9 genes (7 *van* and two transposition genes). Between those genes are intergenic, non-coding regions. The coding regions would be expected to be highly conserved once their sequences are optimal for function. As the intergenic regions are not essential for the function of the gene cluster, they are more likely to vary. Several recent studies have addressed the matter by amplification by polymerase chain reaction (PCR) and sequencing of the genes and/or their intergenic regions (Jensen, 1996; Haaheim *et al.*, 1997; Kirk *et al.*, 1997).

In a study from Norway (Haaheim *et al.*, 1997), PCR for the *vanA* and *vanB* genes combined with restriction fragment analysis of a long PCR covering the entire gene cluster and sequencing of the intergenic *vanS-vanH* region were used to analyse the *vanA* gene cluster from VRE of Norwegian poultry and humans of various nationality (Swedish, Norwegian and American). In 9/12 human isolates and 7/10 poultry the results were identical, indicating horizontal transfer of the gene cluster.

Kirk and co-workers (1997), investigated 37 VRE isolates from one UK hospital and 36 VRE isolates from poultry meat bought in national supermarkets. By PCR, three intergenic regions and three genes of the *vanA*-cluster were investigated (*vanS-vanH*, *vanX-vanY*, *vanY-vanZ*, *vanX*, *vanY* and *vanZ*). In the chicken isolates, all three investigated genes were amplified as well as the three intergenic regions. Evidence of a not previously described insertion sequence in various locations of the intergenic region between *vanX* and *vanY* was found in some of the chicken isolates. The gene sequences of the strains from humans in this study were clearly atypical.

The focus of a Danish study, reported by Jensen (1996), was slightly different. In order to investigate the degree of variation within the *vanA* gene cluster, isolates from different animals and humans from a wider geographic range were investigated. Similar to Kirk and co-workers (1997), Jensen found evidence of an insertion sequence in the *vanX-vanY* region in 7 of 12 British human isolates. Based on sequencing of coding and non-coding regions, the remaining isolates could be divided into 3 groups, each containing isolates both from man and animals from different countries. The designation of one of the groups was based on the presence of a point mutation in *vanX* and an insertion sequence (IS1216V) in a specific position in the transposon (Tn1546). The group contained isolates from humans (Denmark and USA) and pigs (Denmark and UK). Mutations within the coding regions appear to be rare. Insertion sequences are highly mobile, and frequently vary in their location. The occurrence of an insertion sequence in the same location in strains of different origin could either be interpreted as evidence of an epidemiological relationship, or as the site being a "hot spot" for insertion of the specific sequence. However, the likelihood of both a point mutation and an insertion in a specific location occurring independently in different strains is extremely low. Therefore, the genes present in those isolates must be very closely related and their presence the result of horizontal transfer.

Experimental transfer studies

Lacey (1980) examined the possibility of transfer of macrolide resistance between animal and human isolates of *Staphylococcus aureus*. Transfer by phage conjugation from human to animal isolates was shown at low frequencies (10^{-8} - 10^{-9}) for some of the isolates (see comments on transfer rates under 4.4.6 and 4.5.4). No transfer from animal to human isolates could be demonstrated. The genetic nature of the resistance traits for which transfer was attempted is not known. The same author also investigated transfer of resistance between animal and human streptococci, including *Streptococcus* group D (now *Enterococcus*) (Lacey, 1984). In about 10% of the animal strains investigated, transfer to human recipients was demonstrated. Transfer of resistance occurred more readily from the enterococci. No transfer to group A or group C streptococci was demonstrated. The most common transfer frequencies were between 10^{-6} and 10^{-8} . However, the experiments were conducted in broth, filter matings being used only for isolates that had failed to transfer initially. Under optimal conditions, the transfer rates would be expected to be considerably higher (by up to 3 logarithms). Further, the experiments also failed to demonstrate conjugative transfer from human enterococci to human group A or group C streptococci. Such transfer has, contrary to the statement of Lacey, been demonstrated by other authors

(Malke, 1979; Horodniceanu *et al.*, 1981; Ravdonikas, 1983; Horaud *et al.*, 1985). This indicates that the conditions of the experiment may not have allowed optimal transfer rates.

4.5.3 Routes of transmission - food as a vehicle

Direct contact with animals carrying resistance genes provides excellent opportunity for uptake of these genes. Examples of farmers carrying the same resistance genes in their microflora as do their animals have been given in 4.5.1 and 4.5.2. Logically, this transfer works both ways, resistance genes may also spread from humans to animals. However, as animals are in many ways part of the human food chain, transfer from animals to humans would be expected to occur more easily.

Enteric pathogens are readily transmitted through foods as are antibiotic resistant pathogens and commensals. It has been suggested that, in the normal human population, most resistant enterobacteria in faeces come from contaminated food (Corpet, 1988). In an experiment (Corpet, 1988), 7 healthy volunteers were given a control diet for 3 weeks followed by a sterile diet for 2.5 weeks. During both periods, total and antibiotic resistant *Enterobacteriaceae* in stools were counted. A drastic drop in faecal concentrations of antibiotic resistant enterobacteria was observed during the sterile diet period ($p < 0.05$).

Antibiotic resistant gram-negative bacteria are present in animal products as well as on vegetables and fruits (Levy, 1983). Antibiotic-resistant gram-positive bacteria have also been isolated from various foods (Roosen *et al.*, 1989; Franco Abuin *et al.*, 1994; Perreten and Teuber, 1995; DANMAP, 1997; Perreten *et al.*, 1997; Wegener *et al.*, 1997). Moreover, various transferable resistance genes, including *vanA*-genes in enterococci and *erm*-genes in *Listeria monocytogenes*, have been found in gram-positive bacteria isolated from food (Bates *et al.*, 1994; Aarestrup, 1995; Klare *et al.*, 1995a; Perreten and Teuber, 1995; Roberts *et al.*, 1996b; Perreten *et al.*, 1997).

Most foods are heat treated before consumption and hence, no viable resistant bacteria would be expected to be present in the final product. However, food-borne infections with infectious doses as high as 10^6 - 10^9 (as for salmonellosis) are relatively common. This proves that recontamination is common and viable bacteria can be present in relatively large numbers in food when consumed. Certain bacteria, such as *E. faecium* may also have an increased heat tolerance (Panagea and Chadwick, 1996).

The origin of these resistant bacteria is difficult to determine but there can be no doubt that they at least partly originate from contamination of meat and vegetables with animal bacteria. Exchange of resistance genes between bacterial species has been demonstrated in water, soil, on kitchen towels, on cutting boards, and on the surface of food (Kruse and Sørum, 1994).

Epidemiological studies comparing the prevalence of antibiotic resistant bacteria in vegetarians and meat-eaters are of limited value for quantifying the spread of bacteria from animals to man via the food chain. Vegetables are likely to become contaminated by animal microbes through manure. The higher prevalence of resistance in vegetarians found in some studies is therefore not surprising as vegetables are less often heat treated, as compared to meat.

4.5.4 Likelihood of resistance being transferred from animals to man

It has been proposed that both types of transfer discussed above are extremely rare events (Knothe, 1977; Lacey, 1980; Lacey, 1984; Lacey, 1988; Shah *et al.*, 1993). The basis for this opinion is the rarity of isolation of the same bacteria from animals and man (Lacey, 1988; Shah *et al.*, 1993), the rarity of gene transfer *in vitro* between human and animal isolates of some bacterial species (Lacey, 1980; Lacey, 1984) and lack of association between drug consumption in veterinary medicine and resistance patterns in human pathogens (Knothe, 1977). Acknowledging the logic of these arguments, they are mainly based on observations on staphylococci and to a lesser extent streptococci and enterococci. Staphylococci and streptococci are indeed rather host specific, and transfer frequencies between staphylococci are, compared to other genera, rather low irrespective of host origin.

Nevertheless, it is doubtful whether the finding of a low number of animal isolates capable of transferring resistance to human isolates, and vice versa, in the laboratory at a frequency in the order of 10^{-6} - 10^{-8} (Lacey, 1980; Lacey, 1984) under suboptimal conditions should be regarded as proof of a rare event. Considering the vast amount of bacterial subpopulations present in the intestinal flora, even a comparatively low transfer frequency may result in a large number of successful transfer events. The rarity of macrolide resistance in *Campylobacter* spp. isolated from humans has also been pointed out (Lacey, 1988). The species distribution of the bacterial isolates investigated was not given, but as the vast majority of human isolates are *C. jejuni*, this species can be assumed to be the predominant in the material. It was stated that in animal husbandry, macrolides are mainly used as AFA in pigs. As the main animal host of *C. jejuni* is poultry, prevalence of macrolide resistance in this bacterial species provides little information about the effects of macrolide use in pigs. In *C. coli*, which is mainly found in pigs, a high prevalence of macrolide resistance has been reported (Wang *et al.*, 1984; Burrige *et al.*, 1986; Jacobs-Reitma *et al.*, 1994; DANMAP, 1997). This has also been shown in *C. coli* isolated from humans (Reina *et al.*, 1992).

As stated by Lacey (1988), it is known that animal bacteria can colonise man, at least transiently, and that resistance genes can be transferred between animal and human bacteria, but the frequency of these events in nature is unknown.

Antibiotic-resistant bacteria and transferable resistance genes in food are ingested by humans.

Transmission of resistant bacteria between host species occurs, although depending on bacterial species the resulting colonisation may be transient.

Transmission of resistance genes can and does occur in a variety of surroundings.

The question that remains is how often successful transfers cause clinical problems.

4.6 Epidemiology of antibacterial resistance

The causal relationship between use of antibacterials and resistance has been shown both for human and animal bacteria (among others Richmond, 1972; Levy *et al.*, 1976; Seppälä *et al.*, 1997). In evolutionary terms, exposure to antibacterials exert a selective pressure on bacterial populations, giving bacteria with advantageous traits a competitive advantage (survival of the fit).

All factors influencing the usage of antibacterials and/or the spread of infectious agents will also affect the emergence of resistance. In human medicine, such factors are; changes in human demographics and behaviour, changes in technology and industry, economic development and land use, international travel and commerce, microbial adaptation and change and breakdown of public health measures (Cohen, 1996). Corresponding factors are known to influence epidemiology of contagious diseases in animals. Apart from the two latter, these factors all influence the degree of contact between individuals, either by affecting the population density or by providing new contact routes. This emphasises the similarity between spread of resistance and spread of infectious diseases.

4.6.1 Reservoirs - the gene pool

As noted in 4.3.4, dissemination of resistance requires both presence of the antibacterial in concentrations high enough to inhibit growth of normally susceptible bacteria and presence of resistance genes (Levy, 1996). This spread involves a variety of commensals and environmental bacteria (see 4.3, 4.4 and 6.4) which act as reservoirs.

In order to create a problem, or even to be noticed, the resistance genes have to pass from the reservoirs into clinically relevant bacterial hosts. The size and accessibility of the reservoir available will determine the likelihood of such events. The prevalence of resistance genes (i.e. the size of the

reservoir) will primarily depend on the selective pressure applied in different microbial habitats. In most surveys on antibacterial resistance, only potential pathogens are included and thus, the bulk of the resistance gene pool will go unnoticed.

4.6.2 Risk factors for spread of resistance

The kinetics of resistance spread in bacterial populations will be dependent on the total time and degree of exposure to risk factors, and on the sizes and numbers of populations exposed (Levy, 1996).

The selective pressure

The main risk factor for increased resistance is, as mentioned, exposure of bacteria to the specific antimicrobial under study. The selective pressure can be defined as the product of exposure dose and exposure time. The importance of time is illustrated by the investigations by Garber (1989) on the association between antibacterial use and the nosocomial spread of infections with resistant gram-negative bacteria. By using a mathematical model, he found that the risk of an individual acquiring an infection with a resistant strain was directly proportional to the length of time the patient was treated with the drug in question. Further, for some antibacterials, the risk for such infections also increased for non-treated individuals in the same ward.

The number of bacteria exposed is important, but even more so the number of separate bacterial subpopulations. Orally administered antibacterials will exert a larger selective pressure than for instance if locally applied on the skin, as the intestine contains a larger number of various bacterial species (Salyers, 1995).

The proportion of individuals exposed in a subpopulation, as well as the number of subpopulations exposed, is of importance for the spread of resistance between the microflora of individuals.

When antibacterials are used as AFA, they are administered for a long period of time, and usually to all individuals in a group or even herd. As discussed in 4.2, the doses given are certainly inhibitory enough to disrupt the initial balance between resistant and susceptible strains in the normal flora. With time, resistance will and does evolve (see 4.3).

Population factors

The spread of resistance genes between the microflora of different individuals depends on the number of direct or indirect contacts between the bacteria of the individuals. As for infectious diseases, the incidence of resistance genes will depend on size, and structure of the host population.

The degree of contact between individuals and subpopulations, population density, is also crucial (Schwabe *et al.*, 1977). As an example, studies on human skin flora show that sequential therapy for acne promotes the carriage of resistant staphylococci on the skin of close contacts (Miller *et al.*, 1996).

In animal husbandry, high stocking densities, frequent indirect contacts with other herds, frequent trading of animals, and a low level of hygiene will favour the spread and maintenance of resistance traits. Other factors such as feeding practices, management, temperature, humidity and light intensity may influence the composition of the bacterial population and thus, to some extent, influence the incidence of resistance.

Individual factors

Transfer of resistance and/ or colonisation with resistant strains appears to occur more readily in the intestine of young animals as compared to adults (Langlois, 1988). McConnell and co-workers (1991) found, in previously cited experiments (see 4.4.1), that transfer of a resistance plasmid could only be detected in the offspring of the mice originally inoculated. The authors suggested that this might be correlated to the differences in the composition of the intestinal flora between suckling and adult mice. This was thought to affect the donor-recipient ratio. Other explanations might be that colonisation by resistant bacteria is favoured in young animals. This may be ascribed to factors favouring adhesion, including less competition from established microflora (Corpet, 1986).

Individual features of the combination of bacterial species and specific antibacterial involved also play a role. For example, penicillin resistance developed rapidly in human *Staphylococcus aureus* following the introduction of penicillin, whilst in group A streptococci, such resistance has not yet been demonstrated in spite of 4 decades of use of the drug. On the other hand, group A streptococci seem to readily acquire resistance to macrolides (Hamilton-Miller, 1990; Seppälä *et al.*, 1997). Certain genera, such as enterococci, seem to have a high capacity for both acquisition and transfer of resistance (Murray, 1990).

4.6.3 Persistence of resistance

Field observations indicate that the relation between antibacterial use and resistance is not as clear-cut as it may seem (Leak *et al.*, 1986; Langlois, 1988). Whether resistance would regress in the absence of antibacterials depends, among other things, on the availability of susceptible strains to replace the resistant ones. The importance of the environmental reservoir is illustrated by some experiments by Levy (1976) in which chickens excreting multiply resistant *E. coli* were studied. Despite repeated cleaning of the

cages, over several months, the resistance level did not decline. However, when the cages were relocated to different sites, the flora slowly returned to more susceptible levels. This was interpreted as the first environment lacking susceptible strains to replace the resistant ones.

The concept that susceptible strains will replace the resistant ones once the selective event has passed relies on the idea that resistant strains will be at a disadvantage once the antibacterial is withdrawn. This might not always be the case, as a long-term selective pressure can lead to bacterial adaptation (see 4.4.5).

Another factor to be considered is co-selection (see 4.4.3). Due to this phenomenon, exposure to factors other than the substance in question might help sustain antibacterial resistance within a bacterial population.

4.6.4 Some comments on epidemiology

While each single use of an antibiotic has only a small effect on the probability of a resistant strain developing, the consequences add up across all other users in the community. Logically, the selective pressure within a dense population, such as patients in a hospital or animals in a herd, is the major factor contributing to amplification of resistance genes within that particular population. However, very few populations of either animals and humans are isolated from others and transmission of resistance genes can easily occur between groups. This is illustrated in the findings of Linton (1988 see 4.3.1). A resistance gene originating in an animal population may spread to a population of humans and persist there, under the selective pressure from antimicrobials used for human therapy (for example apramycin resistance, see 4.5.2), and vice versa.

Disproportions in the usage of antibacterials between different populations of humans or animals may cause "spill-over" of resistance genes to other populations. Hypothetically, if a gene from animal bacteria, conferring resistance to virginiamycin, were to enter a hospital where regular streptogramin therapy had recently been introduced, the gene would be likely to spread within the hospital and soon become a problem. In this case, the animal population would have served as a reservoir for the resistance gene

subsequently maintained within the hospital population. This illustrates the fact that although the antimicrobial usage within a certain population contributes to most of the antimicrobial resistance within that population, other populations serve as reservoirs for resistance genes.

Commensals and environmental bacteria act as reservoirs for resistance genes.

In the presence of antimicrobials, resistant bacteria will have a competitive advantage.

The effects of the selective pressure on bacterial populations will depend on the exposure dose and exposure time, and on the sizes and numbers of populations exposed.

If susceptible strains are available in the environment, these may replace the resistant ones when the selective pressure is removed.

Bacterial adaptation may result in increased fitness of resistant strains.

Factors influencing the usage of antibacterials and/or the spread of infectious agents will also affect the emergence of resistance.

Microbiota of animals serve as resistance gene reservoirs for human bacteria and vice versa.

4.7 Effects of an increase in resistance

Presence of resistance genes in the human or animal microflora is not in itself a problem. The problem arises when bacteria causing a disease withstand antibiotic therapy.

For substances that are used both as AFA and for therapy or prevention (tylosin, spiramycin, virginiamycin, olaquinox, carbadox) the consequences for animals of resistance in animal pathogens are obvious. As an example, for important swine pathogens such as *Serpulina hyodysenteriae* and *Lawsonia intracellularis* tylosin or tiamulin are the drugs of choice (Fellström, 1996; McOrist *et al.*, 1996; McOrist *et al.*, 1997). Tylosin resistance in *S. hyodysenteriae* is already widespread, leaving tiamulin as the only available alternative (Gunnarsson *et al.*, 1991; Buller and Hampson, 1994; Molnar, 1996). In table 3.III (chapter 3) some indications for treatment and prophylaxis with substances also used as AFA are listed.

In human medicine, macrolides, streptogramins and glycopeptides all have important indications such as respiratory infections, mycoplasmas, chlamydiae (macrolides only) and other infections with staphylococci, streptococci enterococci and clostridia, (see annexes D and E). For some of

these indications, other drugs might be the first choice but substances in the groups listed constitute valuable second- or even last-resort choices. For example, in infections such as streptococcal infections (common in children), penicillins are generally considered first choice. When this is not an option, for instance due to penicillin allergy, macrolides are often used (Huovinen *et al.*, 1996). Macrolide resistance in streptococci is currently regarded as an emerging problem (Coranaglia *et al.*, 1996).

An increase in resistance against these drugs may lead to therapeutic failures and will substantially diminish the available therapeutic arsenal. As long as there are alternative drugs available, the situation can still be managed, although possibly at a higher cost.

The relative importance of the different therapeutical substances is subject to change over time. Vancomycin became a last resort antibiotic as a consequence of the spread of multiresistant staphylococci in hospitals. Emergence of resistance is one explanation, but also that new pathogens emerge due to a combination of technological and societal changes (Cohen, 1992).

Some potential alternatives have not yet been introduced into human medicine. Old, underexploited antibiotic classes such as orthosomycins, phosphoglycolipids and elfamycins as well as antimicrobial peptides all have modes of action that make them interesting as templates for therapeutic substances (van den Bogaard and Stobberingh, 1996; Chopra *et al.*, 1997). Cross-resistance between old and new substances of the same class is to be expected. The most recent examples of this are quinpristin-dalfopristin and everninomycins. Quinpristin-dalfopristin is a streptogramin now launched in human medicine for the treatment of infections with vancomycin resistant bacteria, and everninomycins, an orthosomycin compound, is a promising candidate for the same area (Nicas *et al.*, 1997). Unfortunately, as these agents are widely used as AFA, their lifespan as therapeutics may be shortened by rapidly emerging resistance problems.

Although safe and effective new antibacterial substances may still be found, the efficacy of the older drugs is imperative in keeping fatal infections at bay.

Resistance becomes a problem when bacteria causing a disease withstand antibiotic therapy.

Some substances used as AFA are also used for therapy in animals and humans. Increased resistance to AFA may therefore cause clinical problems

Other substances used as AFA may serve as templates for future drugs. An increase in resistance may shorten the life-span of the drugs.

4.8 Other effects on the microflora

The normal intestinal flora of healthy animals and humans does not cause the host any harm. On the contrary, it protects against colonisation with enteric pathogens. Intensive livestock production systems can, however, induce imbalance in this normal flora, thereby making way for enteric disease (McOrist, 1997). All exposure of the normal flora to antibacterials, for growth promotion or for therapy, will disturb its balance (Corpet, 1996). Depending on the antibacterial spectrum, different microbes will be suppressed. This may lead to a loss of the protective role of the normal flora, impairing resistance to colonisation. As many of the enteric pathogens are of zoonotic character and are known to cause foodborne infections, this might present a hazard for human health.

If the antibacterial feed additive is effective against certain pathogenic bacteria, it would be expected to prevent intestinal colonisation by these organisms and perhaps even remove already established infections. In this case, however, feeding the antibacterial substance must be regarded as prophylaxis or therapy and cannot be categorised as growth promotion.

4.8.1 Salmonella

Concern over potential salmonella contamination of human food has led to the instigation of various measures for its control. Reports in the medical literature that antibiotics may prolong the course of salmonella colonisation has led to the scrutiny of the effects of AFA on salmonella in animals.

The protective effect of the normal microflora against salmonella infection was demonstrated by Nurmi and Rantala in the early 70s (Nurmi and Rantala, 1973). As most AFA, apart from quinoxalines and perhaps flavomycin, are not active against salmonella, their presence in the intestine would be expected to favour rather than disfavour salmonella colonisation.

The main concern regarding human health is contamination of the animal products. There are numerous ways by which salmonellae can contaminate animal products. Stress during transportation may lead to translocation of enteric bacteria with subsequent contamination of internal organs at slaughter. Faecal material on skin or from the intestine can spread to the meat of several carcasses along the processing line (Lillard, 1990). This problem is particularly important when chickens are slaughtered. Rigorous measures following Hazard Analysis Critical Control Points (HACCP) may reduce the problem.

The important factor when assessing the overall risk of contamination of food is the total amount of salmonella organisms brought into the processing plant by animals (Jordon, 1990). Thus, the epidemiological unit of concern is the flock or herd.

Theoretical effects of AFA on salmonella colonisation could be a lowering of the infectious dose necessary to achieve colonisation, an increase in the amount of organisms shed by colonised animals or a prolonged duration of shedding by colonised animals.

A lowered infectious dose will increase the probability of the flock becoming infected by exposure to low doses of salmonella. Moreover, a lowered infectious dose will facilitate the subsequent spread within the flock.

Likewise, an increased amount of organisms shed will contribute to the spread of the infection within the flock. More importantly, animals shedding a large amount of salmonella organisms can have a substantial impact on the cross-contamination of carcasses at slaughter and on the spread to subsequent flocks through contamination of the environment.

A prolonged duration of shedding also contributes to the spread within the flock, as well as the proportion of animals shedding salmonella at slaughter.

A substantial amount of studies have been performed on the effects of AFA on salmonella colonisation. Most of these studies are, however, inconclusive and while some are excellent, many have considerable shortcomings regarding study design and conclusions drawn. As the study designs used are very different, it is almost impossible to compare the results of one study to those of another. Further comments on the different studies can be found in annexes A-F. In table 4.VIII an attempt has been made to summarise the results of some studies in this area. One must remember, though, that do not allow for comparisons.

Table 4.VIII. Results from various studies on the effects of AFA on No. of animals shedding salmonella.

Tabell 4.VIII. Resultat från olika studier avseende effekten av antibakteriella fodertillsatser på antal djur som utsöndrar salmonella

Study	Animal species	Serovar	AFA	Conclusions drawn¹	Comment
Abou-Youssef <i>et al</i> , 1979	swine	<i>S. Typhimurium</i>	virginiamycin	→ No statistical difference between medicated and controls	
Abou Youssef <i>et al</i> , 1983	chickens	<i>S. Typhimurium</i>	virginiamycin	→ No statistical difference between medicated and controls	
Barrow, 1989	chickens	<i>S. Typhimurium</i> , <i>S. Pullorum</i> , <i>S. Choleraesuis</i> , <i>S. Dublin</i> , <i>S. Arizonae</i>	avoparcin	↗ Increased No. of birds shedding salmonella in medicated group	Dose-response relationship established for avoparcin
Dealy and Moeller, 1976	swine	<i>S. Typhimurium</i>	flavomycin	▲ Reduced duration and prevalence of salmonella shedding by medicated animals	Infecting organism susceptible to flavomycin
Dealy and Moeller, 1977	calves	<i>S. Typhimurium</i>	flavomycin	▲ Reduced duration and prevalence of salmonella shedding by medicated animals	Infecting organism susceptible to flavomycin
George <i>et al</i> , 1982	chickens	<i>S. Typhimurium</i>	flavomycin	→ No statistical difference between medicated and controls	
Gustafson <i>et al</i> , 1981	chickens	<i>S. Typhimurium</i>	avoparcin, virginiamycin	→ No statistical difference between medicated and controls →	All birds also received monensin
Gustafson <i>et al</i> , 1983	chickens	<i>S. Typhimurium</i>	avoparcin	→ No statistical difference between medicated and controls	Also tested effect of clean versus used litter

Table VIII. Continued

Tabell VIII. Fortsättning

Study	Animal species	Serovar	AFA	Conclusions drawn ¹	Comment
Hinton <i>et al</i> , 1986	chickens	<i>S. Bredeney</i> , <i>S. Cubana</i> , natural infection via feed	avoparcin, virginiamycin	? Results inconclusive	All birds also received monensin
Hinton, 1988	chickens	<i>S. Kedougo</i>	avilamycin	→ No statistical difference between medicated and controls	
Humbert <i>et al</i> , 1991	chickens	<i>S. Typhimurium</i>	avoparcin, bacitracin, flavomycin, virginiamycin	→ No significant effect of AFA alone, → interaction with CE treatment → →	All birds shed salmonellae, comparisons based on amount of organisms in faeces
Jacks <i>et al</i> , 1988	swine	<i>S. Typhimurium</i>	efrotomycin	→ No statistical difference between medicated and controls	Both quantity, duration and prevalence of shedding evaluated
Latour and Barnum, 1981	ducks	<i>S. Typhimurium</i>	bacitracin	↘ Increased prevalence in medicated group in 2 experiments, no statistical difference in 2 experiments →	
Leuchtenberger, 1981	chickens	<i>S. Typhimurium</i>	avoparcin, tylosin, virginiamycin	↘ Increased prevalence and duration of shedding in medicated groups ↘ ↘	
Linton <i>et al</i> , 1985	chickens	Various, natural infection via contaminated feed	avoparcin	→ No statistical difference between medicated and controls	All groups except one of the controls also received monensin
Manning <i>et al</i> , 1994	chickens	<i>S. Enteritidis</i>	bacitracin	↘ Increased prevalence of salmonella shedding in medicated group	All birds kept on used litter

Table 4.VIII. Continued

Tabell 4.VIII. Fortsättning

Study	Animal species	Serovar	AFA	Conclusions drawn ¹	Comment	
Matthes et al, 1981	chickens	not given	avoparcin, tylosin, virginiamycin	↘ ↘ ↘	Prolonged salmonella shedding in medicated groups	Possibly identical to one of the experiments reported by Leuchtenberger
Nurmi and Rantala, 1974	chickens	<i>S. Infantis</i>	bacitracin	▲	Reduced prevalence and amount of salmonella shedding in medicated group	
Smith and Tucker, 1975	chickens	<i>S. Typhimurium</i>	bacitracin, flavomycin, tylosin, virginiamycin	→ ↘ ↘ →	Small or no increase in prevalence of shedding in groups fed virginiamycin or bacitracin. Larger increase in most groups fed flavomycin and tylosin	
Smith and Tucker, 1978	chickens	<i>S. Typhimurium</i>	avoparcin, tylosin	↘ ↘	Increased prevalence of salmonella shedding in medicated groups	
Smith and Tucker, 1980	chickens	<i>S. Typhimurium</i> , <i>S. Heidelberg</i> , <i>S. Oranienburg</i> , <i>S. Infantis</i> , <i>S. Senftenberg</i>	avoparcin, bacitracin	↘ →	Increased prevalence and duration of shedding in groups fed avoparcin. Little or no increase in groups fed bacitracin	Effect of infectious dose, poultry breed and feed type also investigated
Troutt <i>et al</i> , 1974	swine	<i>S. Choleraesuis</i>	carbadox	▲	Diminished clinical signs in medicated group	

¹ Arrows indicate conclusions as drawn by the authors regarding effect of AFA on colonisation; → denotes no difference, ▲ denotes a decrease, ↘ indicate an increase in shedding time and/or prevalence when medicated groups were compared to control groups

The interest in these studies has mainly been focused on prevalence and duration of salmonella shedding in experimentally infected animals. Most studies have been conducted for 40-50 days and evaluated at the end of the study period. Roughly, 18 experiments show non-significant results, 14 experiments indicate an increase in prevalence/duration of salmonella shedding among medicated animals, and 4 trials indicate a decrease in shedding by medicated animals (see table 4.VIII). Only one study (Barrow, 1989) established a dose-dependent response, to in-feed avoparcin.

Three studies (Nurmi and Rantala, 1974; Barrow, 1989; Humbert *et al.*, 1991) evaluate the amount of organisms shed. Two indicate a possible decrease in numbers shed by bacitracin-fed animals and two an increase in the amount shed by animals receiving avoparcin. However, none of these studies investigate the amount of organisms shed over time.

Regarding changes in the infectious dose, or increased susceptibility of infected animals, the prevalence of salmonella in the different experimental groups shortly after infection may give a hint. One study, by Smith and Tucker (1980) clearly showed, by dose-titration, a decrease (from 10^4 to 10^3) in the infectious dose necessary for colonising avoparcin-fed birds as compared to non-medicated birds.

Some substances do appear to affect the course of salmonella infection within an animal herd. Gustafson and co-workers (1981) used an inoculation dose optimised to achieve colonisation of about 50 % of the animals, which resulted in a faster spread of the infection, with an earlier peak of the epidemic curve, in birds receiving either avoparcin or virginiamycin, compared to the control group. At day 47, the prevalence of salmonella in samples from live birds was larger in the control group than in the medicated groups, but the prevalence of salmonellae in caecal contents after slaughter was larger in the medicated groups. This illustrates that there is sometimes a difference between the epidemic curves of the control group and the medicated group.

Comments on study design

One problem with many of the studies in the table above is that the groups of animals are too small. Population size and density are important factors influencing the spread and persistence of an infection within a herd (Schwabe *et al.*, 1977). Studies performed on small groups of chickens are not necessarily applicable to real poultry flocks with tens of thousands of birds.

The need for a study design as realistic as possible must be balanced against the need for a controlled environment. Practices vary in animal husbandry and what is a realistic study design in one part of the world may

be unrealistic in another area. For example, Gustafson (1983) conducted a series of studies on the effect on salmonella shedding if chickens were raised on clean or used litter and found a higher prevalence of shedding among birds raised on clean litter.

As evident from table 4.VIII, most investigators use *S.Typhimurium* for the experimental inoculation, and most strains are laboratory mutants resistant to nalidixic acid. It is unclear whether the results obtained with such strains are applicable to all salmonella serovars. Further, the properties of an old laboratory strain are not necessarily comparable to wild strains. *S. Enteritidis* is currently the main cause of poultry-derived salmonellosis in humans. As this serovar has a distinctly different behaviour compared to *S. Typhimurium* in the host animals in natural infections (Barrow and Lovell, 1991), separate studies should be conducted. Similarly, for swine, other serovars may be of interest.

Titration of infectious dose and establishment of dose-response relationships is an essential part of risk assessment (see chapter 8). This type of investigation is crucial when determining causal relationships and also for explaining the seemingly conflicting results obtained in different studies. If the doses used are close to the threshold value of response/no response, different studies are bound to show equivocal results. In all dose-response experiments, Barrow (1989) found significant increases in salmonella shedding by chickens in all groups receiving concentrations of avoparcin from 15 ppm. For lower concentrations, variable results were obtained. This explains the contradictory results obtained in studies where 10 ppm has been used.

Doses used for inoculation are often large enough to cause colonisation of almost every animal in the control groups. While this allows for a maximum number of animals to monitor the shedding time, differences in susceptibility or epidemic curves are impossible to detect.

From a statistical point of view, these studies were designed to demonstrate differences between experimental groups, not to prove the absence of differences. In the former type of experiments, the null hypothesis is that there is no difference. In such cases, most statistical analyses use figures for significance and power that accept a 5% risk of falsely rejecting the null hypothesis (type I error) and a 20% risk of falsely maintaining the null hypothesis (type II error). Consequently, results calculated under these assumptions cannot simply be reversed to argue that the null hypothesis is true. When trying to prove safety, the allowances for type I and type II errors should be reversed. Alternatively, other statistical methods can be used, such as the detection level (a parameter indicating the minimum effect that could have been detected) suggested by Hansson (1995).

4.8.2 Other pathogens

In addition to various *Salmonella* spp., colonisation of the animals with other zoonotic and animal pathogens could also be affected. Little is known of such effects in relation to pathogens such as *Campylobacter* spp., *Yersinia* spp., verotoxin-producing *Escherichia coli*, *Clostridium perfringens*, *Serpulina hyodysenteriae* and *Lawsonia intracellularis*. Increased presence in the animal intestine of these bacteria could have serious consequences for either human or animal health. No studies specifically addressing this topic have been found.

4.8.3 Other alterations of the microflora

Resistant bacteria may carry other traits that are disadvantageous for the host, such as other resistance genes, or virulence genes. An increase of the population of naturally resistant bacteria following exposure to antibacterial substances can be expected. Moreover, the prevalence of species with a high capacity for acquisition of resistance genes can be expected to increase. Although this may indirectly lead to an overall increase in resistance, this issue has not attracted much attention.

In an earlier cited study by Kaukas and co-workers (1988, see 4.3.1), the feeding of AFA increased the proportion of *E. faecium*. As this species was generally more resistant than the other enterococcal species, the ARI increased in groups fed AFA. Resistance to the antibiotics studied is mainly conferred by acquired resistance genes in *E. faecium*.

AFA cause disruption of the intestinal microflora which may increase the susceptibility to colonisation by enteric pathogens.

Avoparcin enhances intestinal colonisation with salmonella.

Some other AFA also affect salmonella colonisation.

Information on enteric pathogens other than salmonella is scarce.

Published studies do not directly address the influence of AFA on contamination of food of animal origin with zoonotic pathogens.

Well-designed studies, and use of appropriate statistical methods, are needed in this area.

4.9 Summary comments

Use of AFA results in dissemination of resistance to AFA and related substances. Resistance against AFA can and does transfer between different bacteria and between different human and animal hosts. The use of AFA will thereby contribute to the increase of the environmental pool of resistance genes. The relative importance of this contribution for the increased resistance in animal and human pathogens cannot be determined. In a situation where the substance in question is not used for therapy, the bulk of the resistance genes will be directly attributable to AFA usage. At least two of the classes formerly primarily used as AFA are today options for new therapeutical drugs.

The possible effects of AFA on prevalence of food-borne pathogens in animal products have not been fully addressed. For salmonella, the effects would be expected to be more important in areas where the prevalence is low, or where control programs are in force. Regarding other pathogens, such as *Campylobacter* spp. and *Yersinia* spp., no information has been found.

Glossary

active efflux - active transportation of a substance out of the bacterial cell, mostly by a membrane "pump"

chromosome - circular DNA structure, the major part of the bacterial genome

co-resistance - simultaneous resistance to several different antibacterials by different mechanisms, encoded by different genes

co-transfer - simultaneous transfer of different resistance genes, located on the same mobile element

conjugation, or conjugal transfer - direct transfer of plasmids or transposons between different bacteria via cell-cell contact

conjugative plasmids and transposons - mobile DNA elements that carry genes encoding the mechanisms for their own conjugal transfer

constitutive expression - the gene product is synthesised regardless of the presence of an inducer

cross-resistance - resistance to different antimicrobials by the same resistance mechanism

deletion - disappearance of DNA within or between genes

gene - DNA sequence that encodes a single protein; promoter plus open reading frame

gene cassette - gene lacking promoter, that encodes a specific mechanism, and can be inserted into integrons

gene cluster - several genes located together, forming a functional gene with products acting together to exert an effect

inducible resistance - the product of the resistance gene is only synthesised in the presence of the inducing substance, usually the antimicrobial that the resistance mechanism counteracts

insertion - addition of DNA within or between genes

integron - small mobile element carrying gene cassettes and genes encoding its own transfer and insertion into the bacterial genome, as well as genes encoding rearrangement, expression and uptake of gene cassettes

intergenic sequence - DNA sequence separating functional genes

multiresistance - resistance to a large number of different antimicrobials by various resistance mechanisms encoded by different genes

mutation - random change in DNA sequence, can change the amino acid of an encoded protein

open reading frame (ORF) - DNA sequence that encodes a single protein

operon - gene plus the operator controlling its expression

phage - virus that infects bacteria and inserts its own DNA into the bacterial genome

plasmid - extrachromosomal small, covalently closed, usually circular DNA element that can replicate autonomously and be transferred between bacteria

ribosome - cytoplasmic structure where protein synthesis takes place through translation of mRNA

transduction - phage mediated gene transfer

transformation - gene transfer via bacterial uptake of free, extracellular DNA

transposon - small, mobile DNA element that carry one or several genes, plus genes encoding for its on transposition between various locations in the bacterial genome

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