ANTIBIOTICS IN MANURE AND SOIL – A GRAVE THREAT TO HUMAN AND ANIMAL HEALTH

PREAMBLE

Growth promoting properties of antibiotics in farm animals were first discovered in the late 1940's in chickens and pigs. Feeding of sub-therapeutic doses of antimicrobials to the farm animals was readily adopted and it has now become an integral part of the farm animal/fish production systems. In spite of tremendous beneficial effects in improving feed efficiency and live-stock productivity, antibiotics are now found loosing ground as additives in animal feed, because of their effect on development of resistance in some deadly bacteria in the animal gut and in the terrestrial environment. Today, there is a growing concern over the use of antibiotics to promote growth of animals.

Use of antibiotics as growth promoter is loosely defined as administration of antibiotics to healthy animals at concentrations below 200 ppm in feed for more than 14 days. This feed dose, in terms of animal body weight will be around 200 mg per 100 kg, which works out to a concentration of 2 ppm in animals. This much dose is well distinguished from therapeutic and prophylactic antibiotic use, which is generally delivered at a higher minimum dose of about 20 ppm in animals and are generally administered in water.

Recommended inclusion levels in poultry and pig diets were 4 ppm for the narrow spectrum and 10 ppm for the broad-spectrum antibiotics in 1950's. Since then these levels have increased 10 to 20 folds. In case of fish and fish products certain antibiotics are permitted up to 100 ppm.

The lower concentration (non-lethal dose for any bacteria) through animal feeds over long periods results in a condition conducive for the development of resistance in bacteria. The continued feeding of antibiotics in feed also introduces low levels of antibiotics in the soil and water through the animal excreta. This in turn produces antibiotic resistance in soil bacteria including pathogenic bacteria. Overuse of prescribed drugs in human and veterinary medicine, their use as growth promoters in live-stock feeds has all been blamed as the cause of growing antibiotic resistance.

Some of the antibiotics approved for use as feed additives in livestock production and acqua culture are Beta-Lactams (Cefadroxil, Ceftiofur, Penicillin, G benzathine, G potassium, G procaine, G sodium, Amoxicillin, Ampicillin), Macrolides (Erythromycin, Tilmicosin Phosphate, Tylosin) Spectinomycin, Chloramphenicol, Florfenicol (Aqua-flor, Nuflor), Nitrofurans (Furazolidone, Furaltadone, Nitrofurazone, Nitrofurantoine), Tetracycline, Oxytetracycline, Chlortetracycline, Quinolones (Enrofloxacin, Sarafloxacin, Oxolinic acid, Flumequine), Sulphonamides (Sulphonamides, Sulfachlorpyridazine, Sulfadimethoxine, Sulfamethazine, Sulfanilamide, Sulfaquinoxaline, Sulfathiazole) and Aminoglycosides (Aminoglycosides, Amikacin, Apramycin Dihydrostreptomycin, Gentamicin, Kanamycin, Neomycin, Streptomycin,)

Some of the bacterial infections which could normally be treated by specific antibiotics have turned out to be untreatable. For example, *methicillin* was introduced in 1960 for the treatment of *Staphylococcus aureus* infection and with in a few years, *methicillin* resistant *Staphylococcus aureus* (MRSA) strains were reported. Then in 1980, *fluoroquinolones* were introduced for treatment of MRSA, but a majority of *Staphylococcus* strains became resistant to *fluoroquinolones* with in a year. A national news paper (The Telegraph, Kolkata, Monday, 11 December, 2006) reported that a bacteria displaying resistance to virtually all antibiotics known to humans has surfaced in the country, sending ripples of alarm among medical researchers. A nationwide surveillance was conducted and it stepped up efforts to stem its emergence. Scientists of Institute of Medical Sciences, Varanasi detected the “super bug" among strains of *Staphylococcus aureus.* These bacteria can cause life-threatening infections such as pneumonia and septicemia. Two strains of *Staphylococcus aureus* were found resistant to *Vancomycin,* the drug of last resort in the arsenal of conventional antibiotics.

Worldwide legislation to control use of antibiotics has eventually fuelled the reduction of antibiotic use, at least their non-therapeutic use. Consumers in many advanced countries are no longer keen to eat meat / livestock products from the animals raised on feeds containing antibiotics and efforts are being made to ban their use in many countries. World Health Organization of United Nations (WHO), American Medical Association, and the American Public Health Association have urged a ban on use of antibiotics as growth promoting additives (GPAs) due to increased antibiotic-resistant infections in humans. Some of the warnings / actions against the use of antibiotics in animal feed are in Table 1.

Table 1. Warnings/Actions against the use of antibiotics as animal feed additives

Sr. No	Year	Warnings / action
1	1945	Alexander Flemming warns against misuse of penicillin as ‘microbes are educated to resist’
2.	1950s	Antibiotic resistance widely recognised — vertical transmission.
3.	1950	Tuberculosis bacteria resistant to Streptomycin.
4.	1953	Certain strains of dysentery bacillus was found resistant to Chloromphenicol, Tetracycline, Streptomycin, and Sulphanilamides
5	1958-59	Tetracycline resistant to poultry.
6	1960s	Horizontal transmission recognized.

Sr. No	Year	Warnings / action
7	1969	Swann Committee recommends severe restrictions on antimicrobial supplementations in animal feeds.
8	1970s	Swann committee recommendations implemented in the UK and EU
9	1975	Swann committee recommendations relaxed: tolysin and spiramycin still permitted as growth promoters; vancomycin comes into use.
10	1977	Swedish Agriculture Board considers potential risk of antibiotic resistance, but concludes it is negligible
11	1984	Swedish farmers ask for government ban on antimicrobials in animal feed because of health and consumer concerns.
12	1985	Swedish ban on grounds of antibiotic resistance in animals and it's 'uncertain' long-term effects
13	1995	Avoparcin and Vancomycin resistant Enterococci in pigs and poultry.
14	1995	Norway banned Avoparcin
15	1996	German government banned Avoparcin
16	1997	EU banned Avoparcin
17	1997	Swedish report concludes that risk of antibiotic resistance in humans is 'far from negligible'
18	1997	WHO scientific meeting concludes that it is 'essential to replace growth promoting antimicrobials'
19	1998	Danish government banned Virginiamycin due to Streptococcus resistance.
20	1998	EU bans five antimicrobials in animal feed as 'precautionary' measure, such as Avoparcin, Bacitracin Zn, Spiramycin, Virginiamycin and Tylosin in animal feedstuffs.
21	1999	EU Scientific Steering Committee recommends phase-out of antimicrobials that may be used in human/animal therapy
22	1999	Pharmaceutical industry opposes EU bans and takes EU to the European Court; judgement expected end 2001
23	2000	WHO recommends ban on antimicrobials as growth promoters if used in human therapy and in absence of risk-based evaluation.

Sr. No	Year	Warnings / action
24	2004	U.S. Food and Drug Administration banned the non-therapeutic use of Eenrofloxacin for growth promotion in food animals on the grounds that its use has contributed to fluoroquinolone-resistance in human pathogens.
25	2006	European Commission authorized use of Flavophospholipol, Monensis Na, Salinomycin Na and Avilamycin in poultry, beef cattle, pigs, rabbits and calves diets, as these are not used in human medicine. The act is Animal Drug user Fee Act (ADUFA), Strategies to address antimicrobial drug resistance (STAAR) and Preservation of Antibiotics for Medical Treatment Act (PAMTA)
26	2008	US congress passed a legislation regarding AGP's

Source :

Purpose of this policy paper is to invoke general awareness about the indiscriminate use of antibiotics in agriculture and its impact on human health and terrestrial environment.

A brief account of information that emerged during the deliberations is given below:

Use of Antibiotics in Animal Feed for Growth Promotion

The overall outcome of use of antimicrobial growth promoters (AGPs) is the availability of more nutrients for growth and production of livestock and poultry. Improvement of growth rate and feed conversion ratio (feed : gain) has been reported as 16% and 9% in piglets, 9% and 5.5% in growing pigs, 3-10% and 3-5% in broiler chickens, 2% and 1% in layers and 7-10% in veal calves. Antimicrobials for therapeutic purposes can be purchased only on prescription of a registered medical practitioner, however, as growth promoters; these are freely accessible and sold over the counter. Effects of uses of AGP's on broad issues are depicted in Table 2.

Table 2. Effects of AGP in relation to some broad issues of animal production.

Sr. No.	Broad issue	Positive effect	Negative effect
1	Health	Control of certain diseases (primarily enteric) to some extent	1. Development of antimicrobial resistance 2. Masks sub-clinical disease and infection 3. Limits incentives for hygienic improvements
2.	Welfare	Alleviates and dampens disease signs	1. Camouflages stress associated with sub-clinical disease 2. Allows higher stocking rates

Sr. No.	Broad issue	Positive effect	Negative effect
3	Husbandry	Increases production & productivity	Stimulates, increases and intensifies animal production
4	Feed	Enhances shelf life.	1. Camouflages bad feed quality. 2. Hampers improvements in feed formulation 3. Development of alternatives
5	Production system	Lowers labour demand due to more intense production methods. Betters crop security.	1. Hampers the development of animal-friendly production systems. 2. Development of antibiotic resistance even for pathogens of farm animals.
6.	Environment	Better utilization of feed; less manure.	Increases the environmental pool of antibiotic resistance genes; antibiotic residues.
7.	Human health	None	1. Threat of infection by antibiotic resistant pathogens. 2. Increased health care cost due to unmanageable infections. 3. Shortens economic life of medical antimicrobials. 4. Occupational hazards through exposure to aerosol and dust contaminated with antimicrobials.

Exact mechanism as to how AGP's promote growth is not entirely clear. It is widely assumed that AGP's act mainly through their effect on intestinal flora. With less than 10 % of intestinal micro-flora identified, there has been little chance of fully understanding AGP's mode of action. It is postulated that AGP's allow the animal to express their natural potential for growth through their direct influence on gut bacteria. AGP's are beneficial to the host as these reduce the total number of intestinal microorganisms. Secondly, these may also create a more favourable balance between beneficial and non-beneficial ones. Thirdly, there will be a proportional sparing of nutrients, which are utilized by the animal for absorption and weight gain.

In general, the antibiotic feed will be beneficial to the animal because of inhibition of sub clinical infections, reduced gut motility, reduced mucin secretion, reduced toxin (eg. ammonia and biogenic amine from proteins) production, bile salts modification, thinning intestinal wall, increase in digestive enzyme output, improved digestibility, increased uptake of nutrients along the alimentary canal, reduced opportunity for harmful bacteria to establish in the gut, activation of the intestinal immune system and reduction in microbial use of nutrients, thus sparing the nutrients for the host. The ultimate impacts of antimicrobials as growth promoters in farm animal/aquaculture production systems are increased growth rate of animals/fish/shell fish, better feed conversion, improved egg production in laying hens, increased litter size in sows, early weaning of piglets, increased milk yield in dairy cows, economized animal production systems, reduced incidence of disease in aquaculture, high stocking rates and some protection against certain diseases.

Antibiotics in Soil Environment and Food Chain

A major portion i.e.30- 80 percent of antibiotic dose fed to the animals as growth promoters may be excreted as waste because of poor absorption. When antibiotic-laden manure is used to fertilize crop lands, antibiotics in the manure may get into the soil and eventually end up in streams, lakes or rivers. Antibiotics enter the environment by two ways, (i) directly when using the drugs i.e. the unabsorbed as waste and (ii) subsequent excretion of absorbed antibiotic residues and their metabolites through urine and feaces of the animal. The dominating pathways of environmental release of antibiotics in the terrestrial compartments are through application of FYM in arable soil and in fish farms. An unknown part of food-pellets containing the medical compound may not be eaten by the animals and hence will reach the sediment directly without any change. Antibiotics after consumption may be excreted partly as unchanged compounds or as metabolites, which finally reach the sediment.

No information is available on the fate of veterinary medicinal products with antibiotics during storage of manure/cow dung slurry. Two types (hydrophilic and hydrophobic) of substances are available in the manure /slurry. The antibiotic residues present in the dung depending on its chemical properties, either undergo degradation or leach to the soil. In grazing animals drugs released via the urine immediately reach the soil and if water soluble, leaching down to ground water or adjacent water systems is a rapid process. Most drugs excreted through the urine are water soluble, whereas drugs excreted via feaces, in general, are less soluble.

Antibiotics, which kill disease causing bacteria, especially broad spectrum antibiotics, could work havoc on natural microbial communities in the soil. Antibiotics might disrupt essential biological activity in the soil. One major consequence of this phenomenon is the emergence of antibiotic-resistant bacteria that could infect humans, livestock, fish & shellfish and wildlife. After consumption of antibiotic laced feed to the animals, unused antibiotics enter the environment. In fish and shellfish farming, antibiotics are given as feed additives and approximately 70 to 80 % are directly released into the aquatic environment. Antibiotic residues with significant antibacterial activity have been reported in the sediments from fish and prawn hatcheries and farms in our country.

Tetracyclines (oxytetracycline and chlortetracycline), tylosin, sulfamethazine, amprolium, monensin, virginiamycin, penicillin, and nicarbazine are the most common antibiotics present in swine, beef, and poultry/turkey manures. The concentration of these antibiotics varies from traces to as high as 216 (mg L^{-1}) of manure slurry. These antibiotics generally remain stable during manure storage and end up in agricultural fields on manure applications. Soil and water contamination from manure fertilization has been frequently reported. It is a matter of great concern that residual concentrations of antibiotics in soils can easily reach levels similar to pesticides. Such a contamination by veterinary antibiotics exposes humans and animals to a constant threat of unknown consequences due to the presence of low concentrations of antibiotics in the environment. This threatens the human and animal health by diminishing the success of antibiotic treatment. Evidences show that antibiotic resistant genes from microorganisms in the environment can transfer directly to humans. Leaching and runoff of antibiotics from manure-fertilized lands is threatening the quality of drinking water. Effects of long-term exposure to low concentrations of antibiotics are not yet clear, but the potential danger resulting from veterinary antibiotic contamination to human and animal health cannot be neglected.

Persistence of Antibiotics in Soil

There are very few studies on reaction of antibiotics in soil. Photolysis, hydrolysis, bio-degradation and binding on to soil particles through adsorption process are some of the reactions of these antibiotics and their products that can take place and influence their persistence in soil. They may form complexes with soluble organic materials and become more mobile and contaminate even groundwater while still in its parent form.

Antibiotics like ciprofloxacin, ofloxacin, and virginiamycin degrade very slowly and may persist in soil in its original form up to 30-80 days while bambermycin, tylosin, and erythromycin completely degrade in a period of one month at temperatures ranging from 20-30^{0}C. Persistence of an antibiotic in the terrestrial environment is the key factor determining its environmental impact. Most of the antibiotic residues in manure generally remain stable during manure storage until its application to agriculture fields.

So far, there have been very few studies on the impact of the antibiotics added to the soil through manure, sludge and waste waters on the environment and perhaps none in this country. We need to collect precise data on antibiotic use in animal farming, aquaculture and agriculture and the potential reservoir for residual antibiotics in the terrestrial environment in the country. Besides, research work is needed to understand kinetics of biodegradation and potencies of degraded products of various antibiotics in different soils, manures and waste water. This would help us to better understand the eco-toxicological impacts of various antibiotic residues in the environment.

Effects of some soil properties on the persistence of anti biotics in soil are briefly described.

Effect of soil pH

Soil pH plays an important role in ionization of most of the antibiotics. Besides molecular structure, molar mass, physico-chemical properties and their dissociation constant (pKa) value of antibiotics determine as to how they will ionize in soil due to pH variation. The anti-microbial properties of the antibiotics in soil are determined by their active functional groups.

Most of the antibiotics of tetracycline group are amphoteric in nature and are stable under acid conditions. These compounds can form chelates with divalent metal ions. Sulfonamides, on the other hand, have two pKa values, they behave as weak acids and form salts both under acid and basic conditions. Amino-glycosides being polar compounds move easily with the percolating water and can contaminate the ground water resources. But they are photodegradable therefore, easily decompose when subjected to sun-light. Penicillin belongs to ß-lactam class of antibiotics. It is stable under a wide range of pH values from strong acid to strong alkali conditions. This speaks of its long persistence in soil as parent molecule and is a potential health hazard. Fluoroquinolones, also resist break down through hydrolysis and therefore are highly stable in soil.

Adsorption reactions

Antibiotics in soil can be retained on the mineral and organic colloids. In this form, they are less liable to degradation forces and less potent towards its targets. Antibiotics that can ionize in soil to furnish positive charges can be retained on colloidal soil surface through the adsorption process. Binding strength of antibiotics with soil is determined by the negative charge in the soil and positive charge developed in antibiotic molecule. The extent of such binding can be quantified by taking the ratio of antibiotic concentration adsorbed in soil to the same in water in equilibrium with soil. This is also called distribution coefficient (*Kd*). Antibiotics with higher *Kd* value are strongly bound with the soil and are less mobile. Compounds with less *Kd* value are less strongly bound and more mobile in the soil. The later group of antibiotics can be easily transported to contaminate the ground as well as surface waters. Strongly bound antibiotics can however, be transported mainly to surface waters with the sediments during run off losses of soil. Some of the antibiotic compounds form complexes with soluble organic matter in the soil. This increases their mobility and they easily find their way to contaminate ground waters. Under ordinary conditions, they are strongly bound to soil solids and thus highly immobile. In highly developed dairy farming countries like Germany, sulfamethoxazole concentration as high as 40 ng L^{-1} has been reported in almost 10% of the ground water samples tested. Sulfonamides have little adsorption tendency and do not form immobile complexes in soil. Therefore, sulfonamides are strong contaminators of ground and surface waters while tetracycline is likely to contaminate mainly the surface water bodies.

The tenacity with which the antibiotics are held on the soil solid surfaces is also determined by pH, clay and soil organic matter contents. Amphoteric antibiotics like tetracycline which is most widely used in animal feed and fish feed, may exist as anions and cations depending on pH of the medium. Cationic antibiotics bind to the soil particles through ionic interaction, while acidic and amphoteric antibiotics may bind to the soil through non-ionic interaction.

Effect of soil texture

Soil type, whether loam, silt loam or sandy loam matters in the persistence of antibiotics in soil. For example, it has been found that ciprofloxacin was mineralized to CO_2 less than 1% in all the three soils in 80 days of incubation. Strong binding of this antibiotic was sited as the reason for its slow degradation. Half-life of ceftiofur was more than 49 days in sand and only 22 days in clay loam. Half-life of oxytetracycline in marine sediments at a depth of 5 to 7 cm was more than 300 days as compared to 87 to 173 days for virginiamycin in sandy soil. This shows that antibiotic persistence in soil is determined by not only the soil type but also soil depth. Antibiotics can persist for longer periods if they are lodged in sub-surface soil layers and deep in waters away from sunlight and aeration.

Effect of type of clay minerals

Depending on the reaction of antibiotics with the clay minerals, antibiotics can be divided into 4 groups:

I	Strongly basic	-	Streptomycin; dihydrostreptomycin; neomycin and kanamycin.
II	Amphoteric	-	Bacitracin; Aureomycin; and Tetramycin
II	Acidic	-	Penicillin
IV	Neutral	-	Chloromycetin and Cyclohexamide

In soils dominated by montmorillonite or illite or kaolinite, clay mineral reacts with the first two groups (strongly basic and amphoteric) of antibiotics to form complexes. But acidic and neutral antibiotics are adsorbed only in soil that dominantly contains montmorillonite type of clay mineral; still the tenacity of adsorption is relatively weak. On an average, the amount of antibiotics adsorbed by clays varies from 9 mg g^{-1} for kaolinite clays and strongly basic antibiotics to more than 300 mg g^{-1} in case of montmorillonite clays and amphoteric antibiotics. Strongly basic antibiotics are so strongly held on the clay surface of montmorillonite, vermiculite or illite minerals, that they are virtually un-releasable as assessed from bioassay studies. In kaolinite, however, where it is not held that strongly, there is some release in case of streptomycin and dihydrostreptomycin. But in case of amphoteric antibiotics, there is easy release from all kinds of clay minerals. Streptomycin that is commonly used as growth promoter in swine is adsorbed strongly on the soil particles, which is high in clay and low in sand fractions. Wide variation in tenacity of their adsorption on soil exchange sites is apparent from a vide range of observed sorption distribution coefficient *Kd* from 0.2 to as high as 6000. Clay adsorption is the main reason for weakly adsorbed antibiotic compounds, such, as metronidazole and olaquindox to be more mobile and can leach with percolating water. However, strongly adsorbed oxytetracycline and tylosin percolate the least in the leachate. In general, affinity of many of the commonly used antibiotics as growth promoters is quite high to soil particles. This indicates that most of the mobility of these antibiotics in terrestrial environment is probably due to run-off losses of antibiotic-laden sediments to surface waters from fields where antibiotic laden manures are applied.

Effect of soil temperature

Most of the degradation process of antibiotics in soil is mediated by soil micro-organisms. Therefore persistence of these compounds in the soil is affected by all those factors that affect the activity of microbes. Soil temperature is an important factor in this respect. As the temperature decreases from the normal range of 25-30^{0}C, persistence of antibiotics increases. At 30^{0}C, 44% of chlortetracycline and 23% of bacitracin remained in the soil after 30days of their application. However, when temperature decreased to 20^{0}C, 88% of chlortetracycline, 33% of bacitracin, 25% of erythrocin remained in soil. At 4^{0}C, almost all chlortetracycline, erythrocin and bambermycin persisted in soil. It is very likely that under north-western Indian conditions, antibiotics finding their way to the fields with manures during *kharif* season, a rapid decomposition may eliminate it from the soil due to prevailing moderate to high temperature. During *rabi* season, when atmospheric temperature is low, significant portions of antibiotics applied through manures may remain intact in their original parent form.

Eco-toxicological Impacts

The soil environment may be impacted by the antibiotics in the following ways:

(i) Alter the composition and diversity of indigenous soil microbial communities which are of fundamental importance for ecosystem,

(ii) Change function in nutrient cycling, especially of nitrifying bacteria.

(iii) Inhibit decomposition of organic matter.

(iv) Change energy flow and

(v) Develop resistance, (even cross and multiple), in organisms in the soil environment.

Antibiotics in soil are well known to inhibit microbial growth. How these affect soil fauna and flora, enzymatic activity and nutrient cycling needs greater emphasis for investigation.

These impacts could be direct, such as, antibiotic toxicity to soil microbes and indirect effect, such as, reduced nutrient availability due to changed microbiological activity and reduced rate of organic matter decomposition in soil. Decomposition of organic matter depends on various microbial processes, which in turn depend on type and population of microorganisms in soil and also by allowing only the antibiotic-resistant microbes to flourish that may affect decomposition differently. Oxytetracycline or chlortetracycline fed animals has been found to result in the manure/feaces which when applied to the soil result in more evolution of CO_2. It has also been reported that feaces or dung from the animals fed with antibiotics contained higher proportion of easily decomposable/degradable carbon compounds. Ionophore antibiotics, such as, monensin favour growth of Gram-negative bacteria in the gut.

Impact on Non-target Microorganisms

Antibiotics like streptomycin-laden manure decrease bacterial count in soil up to 50-75% over several months depending upon the nature of bacterial population. Streptomycin has been specifically found to adversely affect the nitrifying bacteria. Gram negative bacteria like *Nitrosomonas spp* are responsible for nitrification in soil. Therefore, broad spectrum antibiotics like tetracyclines, aminoglycosides, and sulfonamides in manure and soil are expected to inhibit the nitrification process. Narrow spectrum antibiotics such as sefdiazine, oxolinic acid, and tylosin, on the other hand, stimulate the nitrification process. Veterinary antibiotics may also inhibit SO_4 reduction as well. Build up of tylosin in soil can cause shifting of bacterial communities from Gram positive to a Gram negative.

Emergence of Antibiotic Resistance

Widespread use of antibiotics and their subsequent release into the environment may lead to the selection of antibiotic resistant bacteria. There has been a frequent observation on shortening of the time between introduction of a new antibiotic and development of resistance of the targeted microbial species. If spontaneous mutations were the only cause of antibiotic resistance, it would have been restricted to only a few bacterial species amongst the hundreds of billion in one antibiotic-treated host and would not be the epidemic problem as it is to-day. A higher degree of vancomycin resistance (60%) in various enterococci isolates from the broiler droppings has been reported. Similarly, a high level of resistance among Gram-positive and Gram-negative isolates from various meat products have been observed for penicillin, erythromycin, sulfamethoxine, tetracycline, ceftibiotics, and gentamycin. Therefore, animal manures containing such antibiotics can cause elevation of resistance in soil bacteria. Development of resistance is quite rapid as within 3 weeks of antibiotic feeding, more than 70% of feacal bacteria become resistant to penicillin and tetracycline. This indicates that antibiotic feeding provides an environment for selection of resistant strains and may encourage the transfer of genetic information from even unrelated bacterial species. Every time one or the other antibiotic drug becomes ineffective because of the emergence of resistance in the targeted bacteria. Discovery of a new drug is very expensive costing more than one $ billion and may take 10-15 years before getting its regulatory approval. It is thus very important for us to look for safer growth promoters and use antibiotics to a bare minimum. Antibiotics used for managing human infections should be totally banned for use as growth promoters.

Many bacterial species multiply rapidly enough to double their numbers every 20–30 minutes. Their ability to adapt to changes in the environment and survive unfavourable conditions often results in the development of mutations that protect them. In addition, another factor contributing to their adaptability is that individual cells do not rely on their own genetic resources alone. Bacterial capacity to adapt to external changes using these mechanisms is called resistance development and this allows the resistant organisms to proliferate in the prevailing conditions. Resistance takes two forms (i) inherent or intrinsic resistance and (ii) acquired resistance. In intrinsic resistance, the species is not normally susceptible to a particular drug. This may be due to the inability of the antibacterial agent to enter the bacteria to reach its target site. In acquired resistance, species is normally susceptible to a particular drug but certain strains express drug resistance, which may be mediated through a number of mechanisms. When resistance develops, the antibiotic is no longer capable of curing or treating the disease caused by the infective agent. A low level of resistance may be detected by a slight increase in the minimal inhibitory concentration (MIC), which is not necessarily of clinical significance. A higher degree of resistance is characterized by an MIC that exceeds, sometimes by several orders of magnitude, the concentrations of drug safely attainable in the patient's tissues.

Transfer of Resistance

Resistant genes are flowing freely between animal and human bacteria through the food chain, which makes the situation more alarming. Of great concern is the possibility that resistance generated on the farm could lead to a loss of effectiveness of key antibiotics in the management of human diseases. To assess the likelihood of the risk of resistance transfer, two risks have to be considered independently : (i) risk associated with the transmission of resistant bacteria from aquaculture environments to humans, i.e. the increase of resistance in human bacterial pathogens as a direct consequence of the use of antibiotics in aquaculture, and (ii) risk associated with the introduction in the human environment of non pathogenic bacteria containing antimicrobial resistant genes and the subsequent transfer of such genes to human pathogens.

Different mechanisms exist in bacteria that make them resistant to a specific antibiotic of a common chemical group. Antibiotic resistance is conferred by variations in the genetic makeup of bacteria. The genes that confer antibiotic resistance are carried on the bacterial chromosome or on separate plasmids that can be transferred between different bacteria. In addition, genes that confer resistance tend to group together such that resistance to multiple antibiotics is transferred by a single plasmid. These properties of antibiotic resistant genes complicate the control of antibiotic resistance and contribute to the concern over the use of antibiotics in livestock and public health. Antibiotics can promote the establishment of an antibiotic resistant population of bacteria by killing the susceptible bacteria and leaving behind the resistant bacteria. They do not induce the genetic changes but select bacteria that already have the genetic changes responsible for resistance. Indiscriminate or inappropriate use of antibiotics can promote the selection of antibiotic resistant bacteria. It is extremely important that antibiotics are used appropriately and according to the manufacturer's recommendations unless otherwise specified by the animal nutritionist/veterinarian.

Originally it was thought that resistance trait would be confined to the mutant clone and spread of resistance is confined to that clone only (Vertical Transmission). Later on, another type of resistance through mutation in existing genes (Horizontal transmission) was observed and this resistance could also be developed through the uptake of existing genes. In this case, the resistance trait through mobile genetic elements can also spread to other bacterial clones, to other bacterial species and even to other genera. Bacteria when exposed to antimicrobials develop strategies for their survival. Widespread use of antimicrobials in human disease management undoubtedly is of more importance for the emerging antimicrobial resistance problems in humans. Continuous use of antimicrobials in feed is one of the major sources of overuse and misuse of antimicrobials in animal production.

Resistance in Food-borne Pathogens

If there is development of resistance in this way into the food-borne pathogens, then it really becomes a problem, because these infections can become difficult to treat with traditional antibiotics, thus threatening human and animal life. The reports of several incidences of infection by multidrug-resistant *Salmonella typhimurium DT 104* is the result of such a possibility. Around 20% of 120 isolates of *E.coli* from animal food were resistant to multi-antibiotic drugs. Similarly, a wide range of tetracycline-resistant genes of *E.coli* have been observed as isolated from human and animal sources. Gram-negative enterobacteria and Gram-positive bacteria are the major source of antibiotic-resistant integrons in animal litters. More than 40% of bacteria collected from surface waters were resistant to one or more antibiotics in USA. Similarly, 54% of the coli form isolates of Korean river were resistant to at least one antibiotic. In Greece, 20% of the *Salmonella* samples isolated from surface waters were resistant to antibiotics.

Until recently, research on antibiotic use has been mainly directed toward their beneficial and adverse effects on the end user, humans and animals.

Allergic or Toxic Reactions

There have been relatively few studies on the effect of antibiotics on plants raised on manure-amended soils. Consumers may unknowingly be ingesting some of these antibiotics by consuming vegetables grown on manure-applied lands. Some adverse effects of consuming antibiotics in vegetables and fruits are discussed. Some antibiotics when ingested by humans, especially children, cause serious allergies or toxicity. There may be some interaction effects from simultaneous ingestion of two different antibiotics. It has been shown that some of the macrolide antibiotics present in animal feed have interacted with monensin resulting in its toxicity leading to death of affected cattle. Antibiotics present in plant materials ingested by humans may provide resistance to human pathogens thus resulting in illnesses that may be difficult to cure with presently available antibiotics. It has been shown that resistance of gut bacteria to antibiotics increased when fed with increasing concentrations of penicillin in contaminated waste milk. Small amounts of tetracycline can act as a catalyst in triggering the horizontal gene transfer between different bacteria. Thus increasing resistance may be of concern both for human and animal health if antibiotics are present in food crops. Our knowledge regarding the implications of manure-based antibiotics on the terrestrial environment and human health is limited. There is an urgent need to study:

i. Effect of cooking of food obtained from antibiotic fed animals / fish / poultry

ii. Fate of different antibiotics present in manure,

iii. Antibiotics and their degradation products taken up by plants grown on antibiotic-laden manure amended soils,

iv. Whether or not antibiotics or their degradation products are still bioactive to impart antibiotic resistance to gut and soil bacteria or cause adverse immunological reactions in humans.

Alternatives to Antibiotic Growth Promoters (AGPs)/Veterinary nutraceuticals

Use of antimicrobial agents as feed additives is a complex issue with implications for human and animal health, animal welfare, food safety, environmental aspects, development of production systems, feeding practices and management of the animals. A complete ban on the use of AGP’s will necessitate exploring the alternatives that can improve feed efficiency and general health status and enhance the immunity to fight against disease. Some of the alternative products have proved useful. Examples of such materials are zinc oxide, copper sulphate, plasma proteins, egg yolk antibodies, organic acids, probiotics, prebiotics, enzymes, bioactive peptides, botanicals (herbs/spices), nutraceuticals, essential oils and fermented liquid feeds. Several foods besides containing nutrients also contain certain compounds that enhance the production by providing either the nutritional balance, improving the metabolism or preventing the disease. Moreover at the same time there is increased interest over the food safety, environmental contamination and the general health risks that have made *natural* the norm, promoting the trend towards alternative strategies to manage and feed the animals and birds without reliance on antibiotics. Such types of foods are labeled as adaptogens, dietetics, nutracines, nutraceuticals or multifunctional additives.

Nutraceuticals are a combination of nutrients and pharmaceuticals. Nutraceuticals must improve the performance effectively and economically, with little therapeutic use, without causing cross resistance to other antibiotics at actual use level, without involving transferable drug resistance and causing any deleterious disturbance to the normal gut flora and creating environmental pollution. Moreover these must be non toxic to the animals and its handlers. Some of the nutraceuticals are briefly discussed :

Herbs/Botanicals

Vegetative parts of the plants (leaves, bark, fruit, roots, seed and their extract) are called herbs. The herbs contain a variety of chemical compounds that are used as body restoratives. These chemical compounds are active in altering the physiological and biochemical processes in the body. Herbs and spices have compounds with antibacterial effects. For example garlic contains allicin and ajoene which exhibit broad spectrum anti microbial properties and are effective in reducing cholesterol of liver, breast and thigh muscle. Another example is of *Yucca Schidiger,* which improves growth and feed efficiency. Botanicals / herbs help in improving the performance by reducing the stress associated with handling, transport and poor health by providing nutrients and or active principles, which act as anti stress agents. These improve egg production in birds by ensuring normal gut functioning and improving digestion by activating digestive secretions and improve growth rate and animal production by increasing feed efficiency.

These properties of various herbs are due to the active secondary metabolites that belong to class of isoprene derivatives, flavonoides and glucosinolates. Interaction between different active components within and between extract may have either cumulative or antagonistic effects. Use of herbs in poultry and pig feeds is now gaining momentum as it claims to have no side effect, and is safe and eco-friendly. Botanicals have proved to be equally beneficial as antibiotic growth promoters. Some botanical extracts have both positive and negative effect on the gut micro flora. This nature of botanicals can be useful in the stabilization of gut environment. Reports indicated the reduced northern fowl mite infestation with the topical application of garlic in laying hens. The desired activity of herbs may vary due to variability of composition of plant secondary metabolites, environmental conditions, harvesting time, stage of maturity, method of extraction and conservation, anti nutritional factor and nature of diet in which it is supplemented.

Prebiotics

Prebiotics are short chain non-digestible oligosaccharides with 2-10 units of monosaccharide used as feed ingredients. Prebiotics are commonly found in soybean and rapeseed meal. Legumes, cereals and yeast cell walls contain a-galactooligosaccharides (GOS), fructooligosaccharides (FOS) and mannanoligosaccharides (MOS), respectively. Lactobacilli, Bifidobacteria and Eubacteria selectively ferment some prebiotics. Prebiotics modify the gut microbial population balance by promoting the growth of beneficial flora in the intestines, thereby providing a healthier intestinal environment. These are not easily digestible and provide competitive advantage to favourable bacteria, inhibiting the colonization of harmful microbes by lowering intestinal pH and promoting the beneficial ones. Through a variety of mechanisms prebiotics are thought to increase resistance to infection. Prebiotics enhance the physical barrier (modulation of paracellular permeability, mucosal trophic action), improve functional barrier (mucosal immunity) and have a competitive adhesion to epithelial receptors. They increase SCFA production along the gastro-intestinal tract, and induce a shift to a more saccharolytic (carbohydrate fermenting) flora.

Galacto-oligosaccherides (GOS), Mannanoligosaccharides (MOS), Fructo- oligosaccharides (FOS) are frequently used in poultry diets. Harmful bacteria attach themselves to both the FOS and MOS and are excreted. Fructo- oligosaccharides (FOS), a derivative of inulin, inhibit the growth of pathogenic microorganism such as clostridia and salmonella. Increase in egg production and feed efficiency of layer with the use of dietary oligofructose and inulin has been widely reported. Oligosaccharides stimulate the secretion of cytokine and enhance the immune system of the pig to resist pathogenic bacterial challenges.

Probiotics

The live microbial food supplement i.e. probiotics or Direct Fed Microbials (DFM's), which when fed, improve the intestinal microbial balance of the host. Lactobacilli, Streptococci, Bifido bacteria, *Bacillus, Bacteroides, Pediococcus, leuconostoc, Propionibacterium,* and some yeast (*Saccharomyces cerevesiae*) and fungi (*Asperzillus oryzae*) are commonly used DFM's. *B Subtilis* and *B licheniformis* are commonly used in nursery pig rations as they are spore forming and are able to resist the environmental conditions of high temperature and moisture occurring during the pelleting process. Probiotics improve the survival with better growth, better-feed conversion and inhibition of diarrhea in piglets.

Probiotics can be administered through drinking water and by mixing in the feed. Probiotics should be given once or twice daily, after which the bacteria should establish itself in the alimentary canal and replace disease-promoting microorganisms. These must be added to the feed on a daily basis. Use of Probiotics bacterial cultures have greater effect during the early stages of growth, when, the gut is sterile especially in pigs and when the alimentary flora are unstable. Probiotics improve health and growth by modifying intestinal microbial balance by competitive exclusion thereby increasing uptake of nutrients due to improved gut permeability.

Some bacterial cultures when fed in single or multiple (few doses) to newly hatched birds, quickly establish as intestinal flora and prevent colonization of pathogenic bacteria. For example, *Lactobacilli acidophilus* produce lactocidin that has antibacterial effects on *Escherchia coli.* Some preparations are proven effective in protecting chicks from *Salmonella* infections and improving weight gain and feed efficiency in chicks and broilers. Probiotics appear to have a more pronounced effect on farms where housing and hygiene are not optimal. Supplementation of probiotics containing *Lactobaccilus acidophilus, Streptococcus faecium* and yeast @ 0.025% in the diets of broilers were found to be beneficial in early growth stage. In broilers supplementation of yeast culture at 0.1 % level increased the body weight and performance due to quantitative and qualitative alteration in the digestive tract flora with better nutrient utilization.

Dietary supplementation of probiotics has better growth performance with improvement in feed efficiency and low mortality during finishing. In layers, improvement in egg production and feed efficiency has also been reported. In pigs, the intestinal microflora is capable of resisting the establishment of certain intestinal pathogens.

Organic acids/acidifiers

Organic acids (C-1 to C-7) are widely distributed in nature as normal constituents of plants or animal tissues. Organic acids posses both the antibacterial and anti mould activities. They have long been used as preservative to prevent spoilage by checking microbial growth and maintain proper gut health. These are very efficacious when their use is adapted to the physiology and anatomy of birds.

Generally two types of acidifiers are used in the feed industry; (i) Feed acidifiers and (ii) Gut acidifiers. Feed acidifiers lower the pH of the feed and inhibit the growth of pathogenic micro flora. This inhibition reduces the micro flora competing for the host nutrients and prevents the occurrence of diseases and this results in better growth and performance. On the other hand gut acidifiers acidify the intestinal tract and modulate the intestine bacterial population in a positive and natural way. Maintenance of healthy gut for proper productivity is of utmost importance. Amongst various options available to poultry and pig feed industry, short chain fatty acids have shown tremendous promise in maintaining gut health through their varied modes of action. Antimicrobial activity of organic acids is related to reduction in pH. Acidifiers maintain an optimum pH in stomach, stimulate feed consumption, improve growth rates, improve feed conversion ratio, inhibit the growth and colonization of pathogenic bacteria, prevent damage to epithelial cells of intestines, and reduce microbial competition with host for nutrients. In poultry diets, organic acids are mainly used in order to sanitize the feed to avoid the problems related with salmonella.

State of the organic acids whether un-dissociated or dissociated is extremely important to define their capacity to inhibit the growth of bacteria. As a general rule, more than ten to twenty times the level of dissociated acids to reach the same inhibition of bacteria are required as compared to un-dissociated acids. At a pH below 3.0-3.5, almost all organic acids are very efficacious in controlling bacterial growth. The key basic principle on the mode of action of organic acids on bacteria is that non-dissociated (non-ionized) organic acids can penetrate the cell wall and disrupt the normal physiology of certain bacteria (*E. coli, Salmonella spp., C. perfringens, Listeria monocytogenes, and Campylobacter spp*).

Antioxidants

Auto oxidation of nutrients in the body results in the production of free radicals, which damage the cellular tissue and cause many disorders. To prevent auto oxidation, antioxidants are frequently used. Nutritional antioxidants are very helpful in reducing physiological stress both at an organ and cellular level. Feed antioxidants protect nutrients during storage, help the absorption of the oxidation sensible substances in the GIT, reduce aging by keeping the membrane intact, while the level of these enzymes decrease.

ß-Carotene, vitamins A, E and C and its calcium and sodium salts, ethoxyquin, lecithin, butylated hydroxytoulene (BHT), propyl gallate, chelated metal ions are commonly used antioxidants in poultry diets. Beneficial effects of antioxidants are due to their scavenging nature for free radicals; maintain the potency of dietary vitamins and stimulating bird's immune- responsiveness to infections. Antioxidant defence system includes the enzymes superoxide dismutase, catalase, and glutathione per oxidase. Many studies have shown that supplementation of Vitamin A, C & E can attenuate the side effects of extreme environmental stress.

Enzymes

Non starch polysaccharides (NSP, cellulose, glucans and xylans etc.) of the cereal grains (wheat, rye, oats) possess antinutritive activity which leads to the formation of viscous gel in the gut that interferes with proper absorption of nutrients and also produces sticky droppings in poultry. Similarly phytic acid and its salts as phytates present in the feedstuffs also bind minerals, carbohydrates, proteins and form insoluble complexes. These make the nutrients especially minerals like phosphorus unavailable to the monogastrics and are excreted in feaces. Supplementation of exogenous enzymes in the diets decreases gut viscosity and improves the availability of nutrients from feed, lowers the feed cost and helps in reducing the environmental pollution by minimizing the waste excretion. Exogenous enzymes in the diets of young animals complement the endogenous enzymes. Use of amylase, arabinase, cellulase, glucanase, hemicellulase, pectinase, xylanase, acid and alkali protease, lipases, esterases, phytase and tannase in poultry and pig feed industry has become a routine. Enzymes in pig and poultry feeds are added to supplement the endogenous enzymes especially to young birds and pigs.

Phytase improves the availability of phytate phosphorus as well as other organic nutrients, performance and mineral retention. Similarly supplementation glycosidase has been found to increase the energy utilization in birds, diminishing digestive disturbance in weaner pigs. Impact of improvement is more in young pigs. Use of ß glucanase and xylanase are beneficial with high fiber grains like wheat, barley and their by-products. a - galactosidase is used to breakdown the galactose units in raffinose and stachyose found in soybean. The efficacy of enzyme supplementation depends upon types of diet, animals, chemical linkage in the substrate that needs to be cleaved etc.

Recommendations

1. There is an urgent need to gather precise information on the use of antibiotics in the animal husbandry in the country and its potential reservoir in soil and water.
2. There is need for constant monitoring by compound livestock feed manufacturers association (CLFMA), ICAR, Drug controlling agencies for production and distribution of antibiotics for non-therapeutic uses.
3. Withdrawal of antibiotics all of a sudden from feed will lead to significant reduction in the production performance of live stock, poultry and aquaculture. This can raise issues of food security. A workable strategy in this context will be to classify the available antibiotics into two categories.

   Category A: Chloramphenicol, nitrofurans including: furaltadone, furazolidone, furylfuramide, nifuratel, nifuroxime, nifurprazine, nitrofurantoin, nitrofurazone, neomycin, nalidixic acid, sulphamethoxazole, chlorpromazine, colchicines, dapsone, dimetridazole, metronidazole, ronidazole, ipronidazole, other nitroimidazoles, clenbuterol, sulfonamide drugs (except approved sulfadimethoxine, sulfabromomethazine and sulfaethoxypyridazine) and fluroquinolones which are widely used as therapeutic antibiotics in controlling human and animal bacterial infections, should be reserved for exclusive use in human and animal disease management only.

   Category B: All other antibiotics including tetracyclines can be listed in the second category of antibiotics, which can be permitted as antimicrobial growth promoters (AGPs). The use of these antibiotics as AGPs should be based on a clear package of practices indicating maximum permissible concentration in feed, withdrawal time as well as method of disposal of solid and liquid based including metabolic waste.
4. Alternative nutritional strategies should be developed for enhanced digestion and absorption of the ingested feed stuffs. The challenge for live-stock productivity is to find suitable, reliable and cost effective management routines and feed additives for a sustainable and successful production.
5. Once suitable alternatives to AGP's are developed, use of antibiotics as AGPs in animal husbandry can be completely banned.
6. Health and hygiene should be the key to success without AGP's. Cleaning and disinfection routines should also be reviewed and upgraded. A failure to replace AGP's will result in an increase in adverse intestine-bacteria interactions.

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