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Potential use of silver nanoparticles as an additive in animal feeding

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1. Abstract

Among other uses, metallic silver and silver salts have currently been applied as antimicrobial agents in many aspects of medical industries, such as coating of catheters, dental resin composites and burn wounds, as well as in homeopathic medicine, with a minimal risk of toxicity in humans. However, their use in animal feeding as prebiotics have remain minimised, mostly because of the low cost antibiotics used as growth promoters in the second half of the XX Century. However, after the ban of this practice in the European Community, silver compounds appear as a potential alternative to other already in use, such as organic acids, oligosaccharides, plant extracts, etc. The major concerns about the safe use of an additive in animal feeding are its effective role as antimicrobial, acting selectively over potential pathogens but not over symbiotic microbial communities; a low toxic effect over the animal and its human consumer; and a low risk of environmental pollution.

Metallic silver nanoparticles (up to 100 nm) allow for a higher antimicrobial effect than silver salts, are more resistant to deactivation by gastric acids and have a low absorption rate through the intestinal mucosa, thus minimising its potential risk of toxicity. Besides, it has been shown that the doses that promote animal physiological and productive effects are very low (20 to 40 ppm), especially compared to the 10 to 100-fold higher concentration used with other metallic compounds such as copper and zinc, thus precluding a harmful environmental effect. This chapter describes the reasons why silver nanoparticles could be applied to animal feeding, and provides with some available data in this regard. In any case, its registration as feed additive is a previous requisite before being applied in practical conditions.

2. Introduction

From the second half of the XX Century, the modern application of technology on animal production has been associated to the intensification of the applied systems, looking for a higher economic profitability by reducing the time and increasing the total magnitude of production. The necessary shortening of the productive cycles and the earlier weaning of

animals leads to an increasing of sensitivity of animals, adapted to focus all their physiological resources to high growth performances and consequently making them more sensitive to the environmental conditions and the infection by different diseases, not necessarily of severe gravity, but that in any case produce considerable reductions in productivity. In terms of animal feeding and nutrition, this situation allowed to the transition from the concept of giving nutrients to meet the needs for improving growth as the basic rule to, once this has been assumed, the use of additives to improve productive performances over nutritive standards by reaching an optimum health status of animals.

Any substance is considered as a feed additive when, not having a direct utilisation as nutrient, is included at an optimum concentration in diet or in the drinking water to exert a positive action over the animal health status or the dietary nutrient utilisation. Because of their chemical nature as active principles, are generally included in very small proportions in diet. With the onset of the mentioned productive situation, the use of antibiotics as feed additives – or growth promoters – became predominant over other alternatives, because of their low cost and high and uniform response. It has to be considered that the use of antibiotics as growth promoters, given at sub therapeutical levels to all animals and for prolonged periods of time, is different to their use as therapeutics, administered at higher proportions to sick animals and only until recovery. Briefly, if a small amount of a substance selectively acts against some harmful microbial species occasionally established or transient in the digestive tract, thus controlling the microbial equilibrium of its microbiota, host animals would need to spend less metabolic effort in the immunological control of the situation. Then they would use the extra nutrients for other physiological purposes, thus reaching better productive performances. In this scenario, the magnitude of such growth promoter substances will be highest in young weaned animals, which low immune development and high growth requirements make them more exposed to pathological challenges. It has been reported that using antibiotics as growth promoters in diets increases weight gain and reduces feed to gain ratio (the amount of feed ingested to reach each unit of weight gain) in pigs by 0.16 and 0.07, respectively (Cromwell, 1991). It has to be noticed that, whereas this concept of host health improvement through microbial manipulation is generally applied for monogastric animals (pigs, poultry, rabbits, etc.), it is not totally so for ruminants, where the search of the digestive health interacts with the presence in former sites of the tract of a large fermentation chamber of extreme importance for the ruminant physiology.

As it has been shown that the continuous use of antibiotics as growth promoters provoke the retention in animal tissues and that the human consumption of such animal products would potentially increase processes of antibiotic resistance, movements of social pressure towards food security were claiming for a strict control and against their use in animal feeding, reaching the banning of using antibiotics as growth promoters from 2006 in the European Community (CE 1831/2003). In other way, the use of some trace elements such as zinc and copper, that have been systematically included as growth promoters in diets for weaned piglets because of their beneficial role in pig health status (Hahn & Baker 1993; Smith et al., 1997) have been also restricted to those levels that satisfy the metabolic needs of animals because of both their retention in animal tissues and environmental hazard. The addition of high doses of zinc (from 2500 to 3500 ppm, as zinc oxide) or copper (from 150 to 250 ppm, as copper sulphate) modulates the microbial status of the digestive tract and reduce the incidence of post-weaning diarrhoea (Jensen-Waern et al., 1998; Broom et al., 2006), generally promoting increases in productive performances (Hill et al., 2000; Case &

Carlson, 2002). However, it remains unclear to what extent the response is associated with its role over the digestive microbial ecosystem (Hogberg et al., 2005) or directly over the piglet metabolism (Zhou et al., 1994), by affecting the secretion and activity of pancreatic and intestinal digestive enzymes or the maintenance of the morphology of the intestinal mucosa (Li et al., 2001; Hedemann et al., 2006).

Considerable efforts have been made to look for alternatives to antibiotics growth promoters in animal feeding during the last three decades. Among the most widely used products in pig and poultry production can be cited the organic acids (Partanen & Mroz, 1999; Ravindran & Kornegay, 1993), plant extracts (Cowan 1999; Burt 2004), oligosaccharides (Mull & Perry 2004) or probiotics (Gardiner et al., 2004).

3. Silver as antimicrobial

Silver compounds have been historically used to control microbial proliferation (Wadhera & Fung, 2005). The antifungal and antibacterial effect of silver nanoparticles, even against antibiotic-resistant bacteria (Wright et al., 1994; 1999) has been demonstrated in *in vitro* conditions. Nowadays, silver compounds are routinely applied in a wide array of industrial and sanitary fields, such as coating of catheters and surgery material, the production of synthetic compounds for odontology, treatment of burn injuries, homeopathic medicine or water purification (Spencer, 1999; Klasen, 2000; Wadhera & Fung, 2005; Atiyeh et al., 2007; Hwang et al., 2007).

Traditionally, silver has been used as salts (ionic form), mainly nitrate, sulphate or chloride. However, silver cation is converted into the less effective silver chloride in the stomach or bloodstream, and can form complexes with various ligands. Silver nitrate is unstable, and can be toxic to tissues (Atiyeh et al., 2007). In contrast, metallic silver in form of colloidal solution or as 5 to 100 nm nanoparticles is more stable to hydrochloric acid, is absorbed at a much lower extent by eukaryotic cells and therefore is minimally toxic, and at the same time exert a higher antimicrobial effect (Choi et al., 2008), which explains why its use has been promoted in the last decades (Atiyeh et al., 2007). Lok et al. (2006) showed that, even though silver nanoparticles and silver ions in form of silver nitrate have a similar mechanism of action, their effective concentrations are at nanomolar and micromolar levels, respectively.

Silver exerts its antimicrobial activity through different mechanisms. It has been reported to uncouple the respiratory electron transport from oxidative phosphorylation and to inhibit respiratory chain enzymes (Schreurs & Rosemberg, 1982; Bard & Holt, 2005). Silver also adheres to bacterial surface, thus altering membrane functions, leading to a dissipation of the proton motive force (Percival et al., 2005; Lok et al., 2006), and interacts with nucleic acid bases, inhibiting cell replication (Wright et al., 1994; Yang et al., 2009). Some authors have demonstrated its toxic effect over different serovars of *Escherichia coli* (Zhao & Stevens, 1998; Sondi & Salopek-Sondi, 2004; Jung et al., 2008) and *Streptococcus faecalis* (Zhao & Stevens, 1998), but its observed effect over *Staphylococcus aureus* has been variable (Li et al., 2006; Kim et al., 2007; Jung et al., 2008). Yoon et al. (2007) observed a higher effect of silver nanoparticles on *Bacillus subtilis* than on *Escherichia coli*, suggesting a selective antimicrobial effect, possibly related to the structure of the bacterial membrane, although Singh et al. (2008) assume higher sensitivity of Gram-negative bacteria to treatment with nanoparticles.

The possible effects of metallic silver and silver ions over microorganisms from the digestive tract are scarcely documented. The selective response of silver in such ecosystem, with a

wide diversity of species that can exert either symbiotic (positive) or pathogen (negative) effects, deserves further attention.

4. Other effects of silver

Despite its potential effect on digestive microbial biodiversity and function, other effects of metallic silver related with host physiological status, such as the immunological status, the digestive enzymatic activity and intestinal structure can be expected. This can be assumed considering the chemical similarity of silver with other metals such as zinc and copper and the characteristics of their antimicrobial response. The capability of zinc and copper to minimise the negative effect of weaning on the height of intestinal villi, thus ensuring its absorbing potential (Li et al., 2001) and the enhancement of the metabolic pancreatic activity (Zhou et al., 1994) could also be potentially expected with the use of silver. Besides, studies related with the role of silver nanoparticles on wound treatment show its role on metalloproteinases regulation, reducing inflammation and favouring cellular apoptosis and cicatrization (Wright et al., 2002; Warriner & Burrell, 2005). Lansdown (2002) indicates that the topical use of silver promotes an increase of zinc and copper concentration over epithelial tissue, thus indirectly stimulating its positive effects.

A cytotoxic effect of silver on the host animal must also be considered. This has been occasionally observed in human medicine when chronic (extended in time) treatments with high doses of silver have been used, often related with the use of silver compounds for wound healing or in dental implants (Abe et al., 2003; Lam et al., 2004). Chronic ingestion of silver compounds may lead to its retention in skin, eyes and other organs such as liver, but it has been generally considered as a cosmetic problem, with minor or nil pathological symptoms (Lansdown, 2006). Wadhera & Fung (2005) state that no physiological alterations or damage of organs of patients with argyria (subcutaneous accumulation of silver associated with silver salts treatment), even with daily intake of 650 mg ionic silver for 10 months (corresponding to a total of 200 g silver intake). The minimal dose causing generalised argyria in humans has been fixed in 4 to 5 g (Brandt et al., 2005). According to Ricketts et al. (1970), the minimal dose of silver nitrate to cause inhibition of cell respiration in tissues is about 25-fold higher to that inhibits growth of *Pseudomonas aeruginosa*, and Gopinath et al. (2008) concluded that a necrotic effect on human cells of silver nanoparticles occur at concentrations above 44 µg/ml (44 ppm). However, no limiting concentration of silver intake has been fixed for humans, although the US Environmental Protecting Agency (EPA) recommends a maximum silver dose in drinking water for chronic or short term (1 to 10 days) intake of 0.05 and 1.14 ppm, respectively (ATSDS 1990).

5. Potential use of silver in animal feeding

In the 50's, colloidal silver was used as zootechnical additive in poultry diets, but its high cost at that time avoided its possibility to compete with the lower cost of antibiotics. Nowadays, the development of industrial processes of silver nanoparticles allows for its consideration as a potential feed additive, once the banning of the use of antibiotics as growth promoters. However, the availability of results testing metallic silver nanoparticles in animal production experiments is very scarce. It has been observed *in vitro* that the proportion of coliforms in pigs ileal contents was linearly reduced ($P < 0.05$), whereas no

effect was observed on lactobacilli proportion, when the concentration of colloidal silver in the medium increased from 0 to 25, 50 or 100 ppm (Fondevila et al., 2009). According to these results, metallic silver nanoparticles would reduce the viability of organisms with a potentially harmful effect, such as coliforms, whereas it does not affect lactobacilli, which positively compete against pathogens proliferation and reduce their virulence (Blomberg et al., 1993). A trend ($P = 0.07$) to a coliform reduction in ileal contents was also observed *in vivo* by Fondevila et al. (2009) when 20 and 40 ppm of metallic silver nanoparticles were given to weaned piglets as metallic silver adsorbed in a sepiolite matrix (ARGENTA, Laboratorios Argenol S.L., Spain) as antimicrobial and growth promoter for weaned pigs during their transition phase (from 5 to 20 kg weight). Besides, although concentration of major bacterial groups in the ileum of pigs were not markedly affected, the concentration of the pathogen *Clostridium perfringens*/ *Cl. histolyticum* group was reduced with 20 ppm silver ($P = 0.012$). In the same way, Sawosz et al. (2007) did not observe a major effect of colloidal silver on bacterial concentration in the digestive tract of quails, but only a significant increase in lactic acid bacteria was observed with 25 ppm.

Results on productive performances in several experiments with pigs and poultry carried out by our group were variable (Table 1): a numerical increase in daily growth was generally observed when 20 ppm silver were added compared with the control (no silver), but this effect was not generally significant. As the productive responses to an additive that improves the sanitary status of animals are in general inversely proportional to the environmental quality of the productive site (Cromwell 1995), it is likely that under the stress conditions of commercial farms the concentration of pathogenic bacteria increased and thus the effect of silver would be more manifested. In the same way, a lack of effect of adding zinc oxide had also been sometimes reported (Jensen-Waern et al., 1998; Broom et al., 2006), which would partly explain this lack of significant results. Studies in animals as models for humans have shown that high silver concentrations (between 95 and 300 ppm, corresponding to 2.4 and 7.5-fold the concentrations used in these experiments) in form of silver salts and given as chronic dose (for more than 18 weeks) reduce weight of mice (Rungby & Danscher 1984) and turkeys (Jensen et al., 1974). However, these dosing conditions are considered of much higher toxic potential than low concentration metallic silver given for short periods of time (Wadhera & Fung 2005). In an experiment (E. Gonzalo, M.A. Latorre & M. Fondevila, unpublished) where pigs were given 0, 20 and 40 ppm silver from weaning to slaughter weight (91 kg), the feed to gain ratio (amount of feed per unit of increased weight) was reduced ($P = 0.03$) by silver addition, indicating a higher growth efficiency and showing a reduction in overall production cost.

Another important aspect to verify when an additive is promoted to use is to what extent it does not challenge the health of the potential consumer. Inclusion of 2500 to 3000 ppm zinc in diets for post-weaning pig leads to tissue retention from 220 $\mu\text{g/g}$ (Jensen-Waern et al., 1998; Carlson et al., 1999) to 445 $\mu\text{g/g}$ (Zhang & Guo, 2007) in liver, and retentions up to 3020 $\mu\text{g/g}$ have been reported (Case and Carlson, 2002). In a study carried out with metallic silver, no silver retention was detected in renal or muscular (semimembranous) tissue in weaned piglets given 20 or 40 ppm silver for 35 days ($n=18$), and only 0.435 and 0.837 μg per g were recorded in liver (Fondevila et al., 2009). Another experiment repeated in the same conditions (Gonzalo, Latorre & Fondevila, unpublished) showed minimal silver retention in muscles (0.036 and 0.033 $\mu\text{g/g}$ with 20 and 40 ppm silver in diet) and kidney (0.034 and 0.039 $\mu\text{g/g}$, respectively) that was observed in 6 out of 8 animals, whereas silver was

detected in liver of all animals at 0.400 and 0.557 $\mu\text{g/g}$ for 20 and 40 ppm, respectively. It has to be considered that these concentrations are more than 3000-fold lower than in the case of zinc and the range is below the EPA recommendation, as it has been commented above. Further, pigs are not given silver additive during their growth and finishing phases (from 20 to 90-100 kg, commercial slaughter weight), and our group did not detect any traces of silver in muscles, kidneys or liver of 90 kg pigs receiving the additive up to 20 kg weight, thus showing the detoxifying capacity of liver to excrete silver (Lansdown, 2006).

In an experiment with broiler chicks as another animal productive species, dosage of metallic silver nanoparticles (ARGENTA) for 5 weeks was continued by 7 days of non-supplemented period (Prieto & Fondevila, unpublished). Silver retention was 0.035, 0.031 and 0.045 $\mu\text{g/g}$ in muscular tissue and 0.113, 0.086 and 0.185 $\mu\text{g/g}$ for the same treatments in liver tissue for 20, 30 and 40 silver ppm in diet, respectively (n=10). Only 5 out of 10 animals given 20 and 30 ppm silver showed detectable concentration in muscles, while 6 and 7 out of 10 animals with the same treatments showed silver concentration in the liver.

Experimental conditions	Ag dose (mg/kg)	Intake (g/d)	Growth (g/d)	F:G (kg/kg)	Reference
weaned pigs, n=5, 28 to 35 d age	0	162	107		Fondevila et al. (2009)
	20	143	122		
	40	177	157		
	s.e.m.	--	41.3		
weaned pigs, n=5, 35 to 42 d	0	253	314b		Fondevila et al. (2009)
	20	313	393ab		
	40	365	461 a		
	s.e.m.	--	36.4		
weaned pigs, n=6 pens of 4 pigs, 21 to 35 d	0	154b	66	2.13	Fondevila et al. (2009)
	20	189a	102	1.95	
	40	148 b	93	1.70	
	s.e.m.	8.5	11.0	0.196	
weaned pigs, n=6 pens of 4 pigs, 35 to 56 d	0	527b	337	1.56b	Fondevila et al. (2009)
	20	670a	375	1.80a	
	40	630a	347	1.82a	
	s.e.m.	32.3	21.2	0.050	
weaned pigs, n=6 pens of 2 pigs, 21 to 147 d; silver was dosed from 21 to 56 d of age	0	1737	684	2.53a	Gonzalo, Latorre & Fondevila (unpublished)
	20	1638	677	2.42b	
	40	1734	693	2.50ab	
	s.e.m.	46.9	16.8	0.029	
broilers, n=8 pens of 28 chicks, 1 to 42 d; silver was dosed from 1 to 35 d of age	0	99.7	54.6	1.83	Prieto & Fondevila (unpublished)
	20	97.3	55.3	1.76	
	30	96.6	53.9	1.79	
	40	99.0	54.1	1.83	
	s.e.m.	0.74	1.28	0.030	

Table 1. Effect of inclusion of metallic silver nanoparticles (ARGENTA) on productive performances of animals

F:G, feed to gain ratio, a,b, letters show differences among means (P<0.05)

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Nanotechnology will be soon required in most engineering and science curricula. It cannot be questioned that cutting-edge applications based on nanoscience are having a considerable impact in nearly all fields of research, from basic to more problem-solving scientific enterprises. In this sense, books like “Silver Nanoparticles” aim at filling the gaps for comprehensive information to help both newcomers and experts, in a particular fast-growing area of research. Besides, one of the key features of this book is that it could serve both academia and industry. “Silver nanoparticles” is a collection of eighteen chapters written by experts in their respective fields. These reviews are representative of the current research areas within silver nanoparticle nanoscience and nanotechnology.

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