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Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 6: *Macrolides: tilmicosin, tylosin and tylvalosin*

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Abstract

The specific concentrations of tilmicosin, tylosin and tylvalosin in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in bacteria relevant for human and animal health, as well as the specific antimicrobial concentrations in feed which have an effect in terms of growth promotion/increased yield, were assessed by EFSA in collaboration with EMA. Details of the methodology used for this assessment, associated data gaps and uncertainties, are presented in a separate document. To address antimicrobial resistance, the Feed Antimicrobial Resistance Selection Concentration (FARSC) model developed specifically for the assessment was applied. However, due to the lack of data on the parameters required to calculate the FARSC, it was not possible to conclude the assessment until further experimental data become available. To address growth promotion, data from scientific publications obtained from an extensive literature review were used. Levels in feed that showed to have an effect on growth promotion/increased yield were reported for tilmicosin and tylosin, whilst for tylvalosin no suitable data for the assessment were available. It was recommended to carry out studies to generate the data that are required to fill the gaps which prevented the calculation of the FARSC for these three antimicrobials.

Keywords: tilmicosin, tylosin, tylvalosin, antimicrobial resistance, growth promotion, yield increase, food-producing animals

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1. Introduction

The European Commission requested EFSA to assess, in collaboration with the European Medicines Agency (EMA), (i) the specific concentrations of antimicrobials resulting from cross-contamination in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health (term of reference 1, ToR1), and (ii) the levels of the antimicrobials which have a growth promotion/increase yield effect (ToR2). The assessment was requested to be conducted for 24 antimicrobial active substances specified in the mandate.[1]

For the different substances (grouped by class if applicable)[1], separate scientific opinions included within the 'Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed' series (Scientific Opinions Part 2–Part 13, EFSA BIOHAZ Panel, 2021b–l – see the Virtual Issue; for practical reasons, they will be referred as 'scientific opinion Part X' throughout the current document) were drafted. They present the results of the assessments performed to answer the following questions: *Assessment Question 1 (AQ1),* which are the specific antimicrobial concentrations in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/rumen, and *AQ2*, which are the specific antimicrobial concentrations in feed of food-producing animals that have an effect in terms of growth promotion/increased yield. The assessments were performed following the methodology described in Section 2 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (EFSA BIOHAZ Panel, 2021a, see also the Virtual Issue). The present document reports the results of the assessment for the macrolides: tilmicosin, tylosin and tylvalosin.

1.1. Background and Terms of Reference as provided by the requestor

The background and ToRs provided by the European Commission for the present document are reported in Section 1.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).

1.2. Interpretation of the Terms of Reference

The interpretation of the ToRs, to be followed for the assessment is in Section 1.2 of the Scientific Opinion "Part 1: Methodology, general data gaps and uncertainties" (see also the Virtual Issue).

1.3. Additional information

1.3.1. Short description of the class/substance

Macrolide antimicrobials are active mainly against Gram-positive bacteria and mycoplasmas, and show only limited activity against many Gram-negative bacteria. The main chemical characteristic common to all macrolides is the presence of a macrocyclic lactone ring and they are classified as either 12-, 14-, 15- or 16-membered ring macrolides. In addition, the majority of macrolides contain amino sugar moieties linked to the lactone ring. The best-known member of the 14-membered ring macrolides is erythromycin. Of relevance for the present scientific opinion are tylosin, tilmicosin and tylvalosin, which are exclusively used in veterinary settings. All three antimicrobials are 16-membered ring macrolides and tilmicosin and tylvalosin are semisynthetic derivatives of the natural product tylosin. Macrolides all act by binding to the nascent peptide exit tunnel near the peptidyl transferase centre of the 50S ribosomal subunit of the bacterial ribosome. In all macrolides, the macrolactone ring is similarly orientated in the ribosomal tunnel and a key hydrogen bond occurs with A2058 of the 23S rRNA (as well as other nucleotides). Macrolides were initially thought to block the ribosomal exit tunnel and cause dissociation of the peptidyl-tRNA from the ribosome and thereby act as general translational inhibitors, but recent work has shown that this blocking is sequence-dependent and that a subset of peptides can be synthesised even with the macrolide bound. Thus, in specific cases, the peptide interacts with the exit tunnel to bypass the blocking effect of the macrolide and allow synthesis of

[1] Aminoglycosides: apramycin, paromomycin, neomycin, spectinomycin; Amprolium; Beta-lactams: amoxicillin, penicillin V; Amphenicols: florfenicol, thiamphenicol; Lincosamides: lincomycin; Macrolides: tilmicosin, tylosin, tylvalosin; Pleuromutilins: tiamulin, valnemulin; Sulfonamides; Polymyxins: colistin; Quinolones: flumequine, oxolinic acid; Tetracyclines: tetracycline, chlortetracycline, oxytetracycline, doxycycline; Diaminopyrimidines: trimethoprim.

longer peptides or a delayed interruption of protein synthesis (Dinos, 2017; Vázquez-Laslop and Mankin, 2018).

Macrolides' spectrum of activity includes Gram-positive cocci, notably staphylococci, haemolytic streptococci, pneumococci and Gram-negative cocci. Gram-negative bacilli are generally intrinsically resistant with the exception of some clinically important genera, i.e. *Bordetella* spp, *Pasteurella* spp., *Campylobacter* spp., Chlamydia, *Helicobacter* spp. and *Legionella* spp.

The activity (minimum inhibitory concentration, MIC) values are different for the three substances, depending on the target bacteria (Rosales et al., 2020). Also taken into account the different pharmacokinetics (see Section 1.3.3), the substances will be analysed separately.

1.3.2. Main use[2]

The macrolides tilmicosin, tylosin and tylvalosin are widely used for treatment of diseases that are common in food-producing animals. The most common indications in all food animals are treatment of gastro-intestinal infections and treatment and metaphylaxis of respiratory infections (EMA/CVMP, 2011).

The main indications in swine are pneumonia, enteritis and arthritis, in cattle all common infections such as respiratory and genital infections, foot lesions and mastitis, and in poultry respiratory infections and necrotic enteritis. The indications for the recently approved macrolide products are restricted to a higher extent (EMA/CVMP, 2011).

In swine, tylvalosin is centrally authorised for oral administration and indicated in swine for treatment of porcine proliferative enteropathy caused by *Lawsonia intracellularis*, treatment and metaphylaxis of swine dysentery caused by *Brachyspira hyodysenteriae* and of swine enzootic pneumonia by *Mycoplasma hyopneumoniae*. Injectable products containing tylosin are also indicated for treatment and prevention of swine enzootic pneumonia and respiratory infections caused by *Actinobacillus pleuropneumoniae*, *Pasteurella multocida* and *Haemophilus parasuis* (EMA/CVMP, 2011).

In poultry, oral products containing macrolides (tylosin, tilmicosin or tylvalosin) are approved for the treatment and prevention of respiratory disease associated with *Mycoplasma gallisepticum and M. synoviae.*

In rabbits, oral products containing tilmicosin are approved for treatment and metaphylaxis of respiratory infections due to susceptible *Pasteurella multocida* and *Bordetella bronchiseptica*.

In cattle, oral administration occurs with tylosin and tilmicosin for metaphylaxis and treatment of pneumonia in calves. Furthermore, detailed indications for the injectable macrolides on centralised authorisation are, depending on the product, treatment and prevention of bovine respiratory infections caused by *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*, treatment and prevention of bovine respiratory disease associated with *Mannheimia haemolytica*, and *Mycoplasma bovis*, and infectious bovine keratoconjunctivitis associated with *Moraxella bovis* (EMA/CVMP, 2011).

1.3.3. Main pharmacokinetic data

Tilmicosin

The absolute bioavailability of tilmicosin after oral administration cannot been determined in swine because tilmicosin cannot be administered by intravenous route to swine.

Tilmicosin is excreted mainly via the bile into the faeces, but a small proportion is excreted via the urine.

Following oral administration of ^{14}C-tilmicosin to pigs, approximately 80% of the administered radioactivity is excreted via faeces and about half of the faecal radioactivity appeared to be the parent compound. According to the report by EMA, about 40% of an oral dose will be available as tilmicosin to microorganism (EMEA/CVMP, 1996).

In sheep and cattle, after a subcutaneous injection, around 70% of the radioactivity was excreted in faeces (EMEA/CVMP, 1996, 1999). The parent antimicrobial was estimated as 20% of the radioactivity in faeces, but the low recovery suggests that the percentage could be higher than 20% (EMEA/CVMP, 1996).

[2] Antimicrobials are currently used in food-producing animal production for treatment, prevention and/or metaphylaxis of a large number of infections, and also for growth promotion in non-EU countries. In the EU, in future, use of antimicrobials for prophylaxis or for metaphylaxis is to be restricted as addressed by Regulation (EU) 2019/6 and use in medicated feed for prophylaxis is to be prohibited under Regulation (EU) 2019/4.

Tylosin

The oral bioavailability of tylosin is greater than 22.5% in pigs (EMEA/CVMP, 1996) and ranges from 5.76% to 21.59% in fasted turkeys (Poźniak et al., 2020). In fasted broiler chickens, one short communication reported bioavailability ranging from 13.74% to 27.0% (Ji et al., 2014). Three other studies published in journals with no or low impact factor provided far different values: 35.4–40.6% (Abu-Basha et al., 2017), 89.2% (Aboubakr and Elbadawy, 2017) and 90.3% (Soliman and Sedeik, 2016).

Tylosin, mainly composed of tylosin A, is extensively metabolised. Tylosin factors B, C and D and dihydrodesmycosin have around 50%, 70%, 30% and 15% of the activity of tylosin A, respectively (EMEA/CVMP, 1997).

In pigs, 94–99% of the radioactivity after oral administration of ^{14}C-tylosin is excreted in faeces. The majority of the excreted residues is tylosin factor D (33%), tylosin factor A (6%) and dihydrodesmycosin. Other percentages of metabolites in faeces were described for other pigs: in one animal, tylosin D (43%) and dihydrodesmycosin (44%) were found and for another, tylosin D (6%) and seco acid of tylosin factor D (resulting from hydrolysis in the macrolide ring) (56%) were found (EMEA/CVMP, 1997). None of them had high amount of tylosin A. The low percentage of tylosin A in faeces after oral administration associated with a low bioavailability suggests that its low bioavailability is the consequence of a high hepatic first-pass metabolism and not of a low absorption through intestinal epithelium.

Among metabolites found in faeces of calves after intramuscular administration, there were tylosin A (29.8%), tylosin D (11.4%), tylosin C (25.2%) and desmethyl tylosin D (10.8%) (EMEA/CVMP, 1997).

Tylvalosin

The oral bioavailability of tylvalosin ranged from 33% to 53% in turkeys (Radi, 2016; Elbadawy et al., 2019).

In pigs, no value for the bioavailability is available even though tylvalosin is described as being rapidly absorbed after oral administration. However, plasma concentrations remain below the limit of quantification after oral administration of the recommended dose (EMA/CVMP, 2009). This observation suggests, as for tylosin, that there can be a high hepatic first-pass metabolism.

Tylvalosin is extensively metabolised with the parent drug accounting for less than 7% of the radioactivity in both urine and faeces. The main metabolite, 3-O-acetyltylosin (3-AT), possesses equivalent microbiological activity to the parent compound. It is not known to what extent other metabolites contribute to the overall effect of the antimicrobial (EMA/CVMP, 2009). However, since high concentrations of microbiological activity are detectable in bile, it is assumed that excreted products are broken down in the gastrointestinal tract (EMA/CVMP, 2009).

A human faecal binding from 0 to 98% was described from a study conducted with faeces from three individuals. The value of 50% for the binding was selected by EMA for the calculation of the acceptable daily intake (ADI) (EMA/CVMP, 2007).

1.3.4. Main resistance mechanisms

Two major resistance mechanisms to macrolides are known: (i) reduced binding affinity of the drug either due to modification of the (a) ribosome or the (b) antimicrobial and (ii) efflux of macrolides from the bacteria (for reviews, see Vester and Douthwaite, 2001; Roberts, 2004, 2008; Fyfe et al., 2016; Dinos, 2017; Arsic et al., 2018). Some of the mechanisms described below are not relevant for the tilmicosin, tylosin and tylvalosin (16-membered ring) since they affect only 14- to 15-membered rings, but they are included for completeness.

i) Ribosome modification.

23S rRNA modification and mutations. Both Gram-positive and -negative bacteria can acquire genes (*erm* genes) that encode enzymes that either mono- or di-methylate A2058 of the 23S rRNA and confer resistance to macrolides (as well as lincosamides and streptogramin B drugs). The *erm* genes have been found on high- and low-copy plasmids and transposons, and they can either be constitutively expressed or inducible by the antimicrobial. The inducible macrolide resistance genes are silent in the absence of the antimicrobial but activated in its presence, and this induction is regulated by ribosome stalling at a defined site of a regulatory open-reading frame upstream of the resistance gene. Apart from modification of A2058, several other 23S rRNA mutations can also confer macrolide resistance.

Mutations in ribosomal proteins. Mutations in genes encoding ribosomal proteins L4 and L22 can confer macrolide resistance or reduced susceptibility. For example, in *E. coli, S. pneumoniae, S. pyogenes, S. aureus* and several other species amino acid substitutions, deletion and duplication in L4/ L22 can result in decreased susceptibility to macrolides.

ii) Macrolide modification.

Macrolide phosphotransferases. These enzymes are macrolide-inactivating enzymes encoded by the *mph* genes that are common in Gram-negative and Gram-positive bacteria from different origins and usually found on mobile genetic elements.

Macrolide esterases. Enzymes that act as esterases and hydrolytically inactivate (specifically the 14- and 15-membered) macrolides have been found in several bacterial species (e.g. *E. coli, S. aureus*, *Pseudomonas* spp). These enzymes are encoded by the *ereA* and *ereB* genes which are present on mobile genetic elements.

Macrolide glycosyltransferases. Glycosylation is not a common mechanism of resistance and is mainly found as a self-protection mechanism in antibiotic-producing bacteria.

iii) Efflux proteins.

Mef pumps. These proteins belong to the major facilitator superfamily and work as proton antiporters. The *mef* genes, consisting of two major subclasses, *mef*(A) and *mef*(E), are found in both Gram-positive and -negative bacteria and they confer resistance to only 14- and 15-membered macrolides. The *mef* genes are inducible and regulated by transcription attenuation where the inducing macrolide acts to anti-attenuate transcription.

Msr protection proteins. These proteins are thought to act similar to the Tet(M) and Tet(O) proteins that confer tetracycline resistance by displacing the drugs from the ribosome in an energy-requiring process. Four classes of Msr proteins exist, A, C, D and E, all having an ATP-binding motif, and conferring resistance to 14- and 15-membered macrolides. The *msr* genes have been found in several bacterial genus, including *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Pseudomonas*.

Cross-resistance is commonly observed between the MLS_B antibiotics (macrolides, lincosamides and streptogramins).

2. Data and methodologies

The data sources and methodology used for this opinion are described in a dedicated document, the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).

3. Assessment

3.1. Introduction

As indicated in the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), exposure to low concentrations of antimicrobials (including sub-minimum inhibitory concentrations, sub-MIC) may have different effects on bacterial antimicrobial resistance evolution, properties of bacteria and in animal growth promotion. Some examples including emergence of and selection for antimicrobial resistance, mutagenesis, virulence and/or horizontal gene transfer (HGT), etc., for the antimicrobials under assessment are shown below.

3.1.1. Resistance development/spread due to sub-MIC concentrations of macrolides including tilmicosin, tylosin and tylvalosin: examples

A few studies have shown that sub-MIC levels of macrolides (in particular the ones used in human medicine) can cause resistance selection of both pre-existing resistant mutants as well as by *de novo* selection. Similarly, several studies have shown that subinhibitory levels of macrolides can stimulate HGT as well as reducing expression of virulence-associated functions including exoproteins and biofilm formation. Very limited data are available for the three antimicrobials, tylosin, tilmicosin and tylvalosin, that are evaluated here but the general similarities between the different drugs within the class of macrolides with regard to structure, mode of action and resistance mechanisms motivate the inclusion also of reports of other macrolides to evaluate the effects of sub-MIC levels on selection, *de novo* evolution and transmission.

3.1.1.1. Effects of Sub-MIC concentrations of other macrolides on selection for resistance and mutagenesis

- In *E. coli*, the minimal selective concentration (MSC) has been determined by competition assays for erythromycin and resistance conferred by the *mph* resistance operon (which inactivates the drug by phosphorylation). When present on a multiresistance plasmid and the chromosome, the MSCs were 3 mg/L and 0.3 mg/L, respectively, which are 1/6 and 1/60 below the MIC (Gullberg et al., 2014).
- Similarly, significant enrichment for the *ermF* gene (which confers erythromycin resistance by methylating 23S rRNA) was observed at 0.75 mg/L in a complex microbial community (Stanton et al., 2020). It is notable that these two very different experimental set-ups (single species vs complex community and *mph* vs *ermF* resistance mechanisms) gave similar MSC values.
- *De novo* selection for erythromycin resistance has also been observed in *Campylobacter coli* and *Enterococcus faecium* strains at 0.1 mg/L of erythromycin which is 2.5- and 10-fold below the MIC of the respective species (Ge et al., 2017). The mutations that conferred resistance were not identified in this study.

3.1.1.2. Effects of sub-MIC concentrations of other macrolides on horizontal gene transfer and virulence

- With regard to sub-MIC antimicrobial stimulation of HGT, it has been shown that 30 mg/L of erythromycin (> 8-fold below the MIC of the used *S. aureus* isolate) generates transducing phage capable of transferring the *ermC* gene from the donor to a recipient bacterium (Stanczak-Mrozek et al., 2017).
- Conjugative transfer of the plasmid-borne *erm*(B) gene from *L. plantarum* and *E. faecalis* was stimulated approximately 20-fold by 0.5 mg/L of erythromycin in *in vitro* experiments (which is > 500-fold below the MIC of the resistant donor strain) (Feld et al., 2008).
- Similarly, macrolides (azithromycin, erythromycin and clarithromycin, oleandomycin and spiramycin) at subinhibitory concentrations stimulate expression of transfer functions in Tn*916* which is likely to increase HGT (Scornec et al., 2017).
- Regarding effects on virulence-associated factors, it has long been known that the synthesis of many exoproteins (including virulence factors) and biofilm formation can be inhibited by sub-MIC concentrations of antimicrobials that block protein synthesis (e.g. macrolides and lincosamides). For example, in *P. aeruginosa*, subinhibitory macrolide (azithromycin, erythromycin and clarithromycin) levels can inhibit expression of many virulence factors (Molinari et al., 1993; Sofer et al., 1999; Wozniak and Keyser, 2004).
- Similarly, the macrolides erythromycin (Zhao et al., 2015) and tylosin (Wang et al., 2016) inhibit biofilm formation in *S. suis,* but notably the opposite effect has also been observed with macrolides (azithromycin, erythromycin and clarithromycin) in *S. epidermidis* (Wang et al., 2010), indicating that the effects on virulence-associated characteristics also depend on the species and/or isolate studied.

In summary, no data were available for the macrolides under assessment. However, data available for other macrolides show that sub-MIC concentrations (i.e. erythromycin) in both defined single species and complex microbial communities can have a number of effects, including selection for *de novo* resistance, enrichment of pre-existing resistance, increased horizontal gene transfer and decreased bacterial virulence. With regard to the concentrations of erythromycin, the biological effects are observed, the concentration for resistance selection appears to be the lowest (0.3–3 mg/L depending on experimental set-up) whereas effects on horizontal gene transfer and virulence effects are seen at even higher levels.

3.2. ToR1. Estimation of the antimicrobial levels in non-target feed that would not result in the selection of resistance: Feed Antimicrobial Resistance Selection Concentration (FARSC)

As explained in the Methodology Section (2.2.1.3) of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), the estimation of FARSC for these three macrolides for different animal species, if suitable data were available, would follow a two-step approach as described below:

The first step would be the calculation of the predicted minimal selective concentration (PMSC) for tilmicosin, tylosin and tylvalosin as indicated in Table 1. However, no MSC data required to do the calculations is available for tilmicosin, tylosin nor tylvalosin.

Table 1: Calculation of tilmicosin, tylosin and tylvalosin under assessment predicted minimal selective concentration (PMSC)

Antimicrobial (all values in mg/L)	MIC_{test}	MSC_{test}	MIC_{test}/ MSC_{test} ratio	MIC_{lowest}	Predicted MSC (PMSC) for most susceptible species (MIC_{lowest}/MIC_{test}/MSC_{test})
Tylosin	NA	NA	NA	0.5	NA
Tilmicosin	NA	NA	NA	0.5	NA
Tylvalosin	NA	NA	NA	NA	NA

MIC: minimum inhibitory concentration. MSC: minimal selective concentration. MSC_{test}: MSC experimentally determined. MIC_{lowest}: lowest MIC data for tylosin and tilmicosin calculated based on data from the EUCAST database as described in Bengtsson-Palme and Larsson (2016), see Methodology Section 2.2.1.3.1.1 in the Scientific Opinion Part 1. No MIC data for tylvalosin in the EUCAST database (EUCAST database https://mic.eucast.org/search/ last accessed 15 May 2021); NA: not available.

Due to the lack of PMSC, no FARSC could be calculated. If PMSC was available, FARSC ($FARSC_{intestine}$ and $FARSC_{rumen}$) corresponding to the maximal concentrations in feed could be calculated for each species from the equations below (for details, see Section 2.2.1.3.2 of the Scientific Opinion Part 1; see also the Virtual Issue) by including specific values for tilmicosin, tylosin and tylvalosin:

$$FARSC_{intestine}\ (\text{mg/kg feed}) = \frac{\text{PMSC} \times \text{daily faeces}}{(1-I) \times (1-F+F \times GE) \times \text{daily feed intake}}$$

$$FARSC_{rumen}\ (\text{mg/kg feed}) = \frac{\text{PMSC} \times \text{volume of rumen}}{(1-I) \times \text{daily feed intake}}$$

With daily faeces being the daily fresh faecal output in kg, I the inactive fraction, F the fraction available, GE the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream, and daily feed intake being the daily dry-matter feed intake expressed in kg.

Tilmicosin

Since 40% of an oral dose was recovered in faeces after oral administration to pigs, the factor ($1 - F + F \times GE$) was considered equal to 0.4 in pigs (Table 2).

No data on the fate of tilmicosin in other species are available.

The potential inactivation of tilmicosin by binding to intestinal contents is not described.

Table 2: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of tilmicosin in pigs

Tilmicosin data	Scenario #1
Inactive fraction (I)	NA
Fraction of the dose available for intestinal microorganisms corresponding to ($1 - F + F \times GE$) in pigs	0.4

Inactive fraction (I) is the fraction of antimicrobial that would not have any activity on bacteria. Bioavailability (F) is the fraction of antimicrobial that is absorbed from the digestive tract to the blood. Gastrointestinal elimination (GE) is the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to ($1 - F + F \times GE$). NA: not available.

Due to the absence of MSC and other PK data, the estimation of the FARSC for tilmicosin was not possible.

Tylosin

The oral bioavailability of tylosin ranges from 5% to 22% in fasted turkeys. No data are available for fed turkeys and the fate of tylosin in turkeys after absorption is unknown. The bioavailability for broilers is uncertain. Due to the lack of information, no value of pharmacokinetic (PK) parameters was selected for tylosin in turkeys or broilers.

From the different published percentages of excreted parent drug and metabolites after oral administration to pigs and their antimicrobial activities, the maximum activity on intestinal microorganisms would correspond to 25% of the oral dose. Thus, the factor $(1 - F + F \times GE)$ was considered equal to 0.25 in pigs (Table 3).

In calves, the published percentages of excreted parent drug and metabolites were obtained after intramuscular administration and could not be used for the calculation of FARSC.

Table 3: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of tylosin in pigs

Tylosin data	Scenario #1
Inactive fraction (I)	NA
Fraction of the dose available for intestinal microorganisms corresponding to $(1 - F + F \times GE)$ in pigs	0.25

Inactive fraction (I) is the fraction of antimicrobial that would not have any activity on bacteria. Bioavailability (F) is the fraction of antimicrobial that is absorbed from the digestive tract to the blood. Gastrointestinal elimination (GE) is the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to $(1 - F + F \times GE)$. NA: not available.

No data on the fate of tylosin were available after oral route in species other than pigs, and the potential inactivation of tylosin by binding to intestinal contents is not described.

Due to the absence of MSC and other PK data, the estimation of the FARSC for tylosin was not possible.

Tylvalosin

The oral bioavailability of tylvalosin ranged from 33% to 53% in turkeys. However, there are no data on the fate of tylvalosin in turkeys after absorption and especially on the metabolism before gut elimination.

There is no information on the bioavailability for other species nor on the metabolism to predict the percentage of active antimicrobial in contact with intestinal microorganisms.

Faecal binding in humans was described for tylvalosin, but there is no information for faeces from animal species.

Due to the lack of information for tylvalosin, no value of PK parameters was selected for the calculation of FARSC.

Due to the absence of MSC and other PK data, the estimation of the FARSC for tylvalosin was not possible.

3.2.1. Associated data gaps and uncertainties

With regard to the uncertainties and data gaps described in the Scientific Opinion Part 1 (Sections 3.1 and 3.3; see also the Virtual Issue), we identified the following for macrolides under assessment:

i) MSC data: no data for MSCs are available.
ii) MIC data: MIC data for only five species for tylosin, three species for tilmicosin and no data are available for tylvalosin in EUCAST database.
iii) Impact of complexity on determined MSCs: Although there are no data available for the substances under assessment, previous studies with erythromycin showed using two different experimental set-ups (single species competition vs enrichment of resistance genes in a complex community) similar concentrations for when enrichment was observed. Thus, in a competition experiment, the MSC was 0.3 mg/L (Gullberg et al., 2014) and in a complex microbial community enrichment was observed at 0.75 mg/L (Stanton et al., 2020).

iv) Bioavailability: for tilmicosin, no data are available. For tylosin, data were only available for turkeys and pigs. For tylvalosin, data were only available for turkeys.
v) Fraction eliminated in gut: Several studies suggest an elimination of macrolides as inactive metabolites. However, there are no quantitative data to consider this process except for tilmicosin and tylosin in pigs. Antimicrobial activity of the metabolites is insufficiently characterised.
vi) Inactive fraction: no data on the possible binding of macrolides in digestive tract of animal species are available.
vii) Ruminants: no data are available for macrolides administered to ruminants by oral route.

3.2.2. Concluding remarks

Due to the lack of data on the parameters required to calculate the FARSC, it is not possible to conclude the ToR1 assessment until further experimental data are available.

3.3. ToR2. Specific antimicrobial concentrations in feed which have an effect in terms of growth promotion/increased yield

3.3.1. Tilmicosin

3.3.1.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), resulted in 173 publications mentioning tilmicosin and any of the food-producing animal species considered[3] and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of tilmicosin.[4] After removing the reports not matching the eligibility criteria, 34 publications were identified.

3.3.1.2. Evaluation of the studies

The publications identified in the literature search were appraised for suitability for the assessment of the effects of tilmicosin on growth or yield of food-producing animals; this appraisal was performed by checking each study against a series of predefined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion Part 1; see also the Virtual Issue).[5] Thirty-three publications were not considered suitable for the assessment because of several shortcomings identified in the study design and/or reporting. The list of excluded publications and their shortcomings are presented in Appendix A.1 (Table A.1).

The publications considered suitable for the assessment are described and assessed in Section 3.3.1.5.

3.3.1.3. Assessment of the effects of tilmicosin on growth performance and yield

Only one publication was considered suitable for the assessment of the effects of tilmicosin on growth and yield performance in food-producing animals. The effects of the administration of the antimicrobial on the endpoints described in Section 2.2.2.2.2 of the Scientific Opinion Part 1 (see also the Virtual Issue) were evaluated. The selected publication and the effects on the relevant endpoints are described below. The summary of the study includes the description of the source of tilmicosin

[3] Ruminants: growing and dairy (cattle, sheep, goats, buffaloes); pigs: weaned, growing and reproductive; equines; rabbits; poultry: chickens and turkeys for fattening, laying hens, turkeys for breeding, minor avian species (ducks, guinea fowl, geese, quails, pheasants, ostrich); fish: salmon, trout, other farmed fish (seabass, seabream, carp); crustaceans; other animal species.

[4] (i) Intake-related parameters: feed intake, feed/gain ratio, feed efficiency, feed intake/milk yield, feed intake/egg mass; (ii) Weight-related parameters: body weight, body weight gain; (iii) Carcass-related parameters: carcass weight, carcass yield, carcass chemical composition, relative weight of the (different sections of) intestine; (iv) Milk or egg production/quality: milk yield, fat/protein yield, egg production/laying rate, egg weight, egg mass; (v) Digestibility/utilisation of nutrients: utilisation of some nutrients (e.g. DM, Ca, P), digestibility; (vi) Health-related parameters: reduction of morbidity and/or mortality; (vii) Herd/flock-related parameters; (viii) Other endpoints: e.g. intestinal morphological characteristics (*villi* height/width), changes in microbiota.

[5] The following exclusion criteria were applied: 'Combination of substances administered to the animals', 'Antimicrobial used different from the one under assessment', 'Administration via route different from oral', 'Use of the antimicrobial with a therapeutic scope', 'Animals subjected to challenges with pathogens', 'Animals in the study sick or not in good health, Zootechnical parameters not reported', 'Insufficient reporting/statistics', 'Other (indicate)'.

used – either as the base or as any specific form/commercial preparation – and the concentration(s) applied as reported in each study; where a specific compound has been used, the calculation of the concentration applied to the base substance is provided.

3.3.1.3.1. Studies in pigs

O'Sullivan et al. (2016) studied the effect of tilmicosin in pigs (Yorkshire, 20 kg initial body weight (BW)) exposed to a vaccine strain of porcine reproductive and respiratory syndrome virus (PRRSV). For the purpose of the current assessment, the pigs which had received the vaccine were not considered, and thus, only two treatments, in which a total of 58 animals were involved, were considered relevant: a control and a treatment consisting on tilmicosin (unspecified chemical form; Pulmotil Premix, Elanco Animal Health, Guelph, Ontario) supplemented at 400 mg/kg feed. Pigs were housed in six pens (8–10 pigs per pen, 3 pens per treatment). The study lasted 24 days. Average daily gain (ADG) was determined for each pig during the trial period. No clinical signs of disease were noted in any of the pigs throughout the experiment. Pigs from the group of 400 mg tilmicosin/kg feed showed an increased ADG compared to control (0.698 vs 0.637 kg/day). In summary, tilmicosin had a growth-promoting effect in pigs for fattening at 400 mg/kg feed.

3.3.1.4. Discussion

From the study examined, the test item has been described as 'tilmicosin' (two studies). Therefore, an uncertainty on the exact product used/concentration applied has been identified.

A detailed analysis of the uncertainties for tilmicosin is included in Appendix B (Table B.1) of this document, and in Section 3.3 of the Scientific Opinion Part 1 (see also the Virtual Issue).

From the literature search only one study reporting the effects of the oral administration of tilmicosin on growth promotion/increased yield in pigs was considered relevant. The study by O'Sullivan et al. (2016) showed enhanced ADG of tilmicosin at 400 mg/kg feed for 24 days in pigs for fattening.

3.3.1.5. Concluding remarks

It is judged 33–66% certain ('about as likely as not') that tilmicosin has growth-promoting/increase yield effects in pigs for fattening at the concentration of 400 mg/kg complete feed (one study).

No data are available in the scientific literature showing effects of tilmicosin on growth promotion/increased yield when added (i) to pigs for fattening feed at concentrations below 400 mg/kg, or (ii) to feed of any other food-producing animal species or categories.

3.3.2. Tylosin

3.3.2.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), resulted in 806 papers mentioning tylosin and any of the food-producing animal species considered[6] and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of tylosin.[4] After removing the reports not matching the eligibility criteria, 146 publications were identified.

3.3.2.2. Evaluation of the studies

The 146 publications identified in the literature search were appraised for suitability for the assessment of the effects of tylosin on growth or yield of food-producing animals; this appraisal was performed by checking each study against a series of predefined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties'; see also the Virtual Issue).[5] A total of 101 publications were not considered suitable for the assessment because of several shortcomings identified in their design or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.2 (Table A.2).

[6] Ruminants: growing and dairy (cattle, sheep, goats, buffaloes); pigs: weaned, growing and reproductive; equines; rabbits; poultry: chickens and turkeys for fattening, laying hens, turkeys for breeding, minor avian species (ducks, guinea fowl, geese, quails, pheasants, ostrich); fish: salmon, trout, other farmed fish (seabass, seabream, carp, other); crustaceans; other animal species.

The publications considered suitable for the assessment are described and assessed in Section 3.3.2.3.

3.3.2.3. Assessment of the effects of tylosin on growth performance and yield

Forty-five publications were considered suitable for the assessment of the effects of tylosin on growth and yield performance in food-producing animals. The effects of the administration of the antimicrobial on the endpoints described in Section 2.2.2.2.2 of the Scientific Opinion Part 1 (see also the Virtual Issue) were evaluated. The selected publications and the effects on the relevant endpoints are described below. The summary of the studies includes the description of the source of tylosin used – either as the base or as any specific form/commercial preparation – and the concentration(s) applied as reported in each study; where a specific compound has been used, the calculation of the concentration applied to the base substance is provided.

3.3.2.3.1. Studies in ruminants

In the study of Brown et al. (1973), the effects of tylosin phosphate (TP) (TYLAN® Premix, Elanco Products Company) and tylosin urea adduct (TUA) supplemented at 50, 75 and 100 mg per head per day (corresponding to ca. 7, 11 and 14.5 mg tylosin phosphate and tylosin urea adduct/kg dry matter (DM), and 6.3, 9.9 and 13.1 mg tylosin/kg DM) on performance and in the prevention of liver abscesses in fattening cattle fed high concentration rations (to increase the incidence of liver abscesses) were studied. A total of four feedlot experiments were performed. Experiment 1 included 40 crossbred heifers (BW 213 kg) per treatment and lasted 150 days; Experiment 2 included 166 Brahma crossbred steers (control 41 and tylosin treatments 19–22 animals, BW 259 kg) and lasted 151 days; in Experiment 3, a total of 160 mixed breed steers (control 40 and tylosin treatments 20 animals, BW 288 kg); and in Experiment 4, a total of 328 yearling crossbred heifers (BW 290 kg). In Experiment 2, 3 and 4, the effect of the tylosin activity from TP and TUA was evaluated at levels of 50, 75 and 100 mg tylosin per head per day, whereas in Experiment 1, only the treatments containing 100 mg tylosin per head per day of the two forms of tylosin were compared to the control diet. Average daily gain (ADG) and feed conversion ratio (F:G) were calculated by treatment group for the entire experiment. At slaughter, the number of livers condemned for abscesses was recorded for each treatment group in each experiment and was scored according to their severity (from 1 to 4). During the experiment, some animals presented health problems (including acidosis, enterotoxaemia) and the overall incidence of liver abscesses in control cattle in the four experiments averaged 23.1%. All the three levels studied and the two forms of tylosin reduced the incidence of abscesses. Regarding the effect on performance, no differences on ADG and F:G were observed between the two forms of tylosin or among the three levels of inclusion. Combining all the levels of tylosin (with an overall tylosin concentration of ca. 11 mg tylosin/kg DM), an increase of ADG (1.066 vs 1.012 kg/day) and improved F:G (7.412 vs 7.646) were observed when compared to the control. Dietary TP or TUA supplementation at 11 mg/kg DM (corresponding to 9.9 mg tylosin/kg DM) reduced the incidence of liver abscesses and had a growth-promoting effect in cattle for fattening.

In the study of Brown et al. (1975), the effect of tylosin on performance and prevention of liver abscesses in fattening cattle was evaluated. A total of four feedlot experiments were performed. The Experiment 1 included 50 crossbred steers (BW 268 kg) per treatment and lasted 153 days; Experiment 2 included 102/105 mixed crossbred cattle (BW 352 kg) per treatment and lasted 157 days; Experiment 3 included 26 crossbred steers (BW 288 kg) per treatment and lasted 168 days; and Experiment 4, 430 mixed steers (BW 286 kg) per treatment and lasted 154 days. Basal diets were either not supplemented or supplemented with 75 mg tylosin (unspecified form) per head per day (corresponding to ca. 10 mg tylosin/kg DM). ADG and F:G were calculated per treatment pen for the entire experiment. At slaughter, the number of livers condemned for abscesses was recorded for each treatment group in each feedlot and was scored using four levels of severity. The overall incidence of liver abscesses in the four feedlot experiments was 56.2 % for the control cattle and 18.6 % for animals treated with tylosin. Moreover, tylosin supplementation improved F:G compared to the control (7.87 vs 8.21). Dietary tylosin supplementation at 10 mg/kg DM reduced the incidence of liver abscesses and had a growth-promoting effect in cattle for fattening.

In the study of Lean et al. (2000), a total of 80 cows at 118–189 days in milk (Holstein-Friesian) were distributed in four groups of 20 animals and allocated to four dietary treatments. One basal diet was either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified form) at the concentration of 20 mg/kg feed (corresponding to ca. 14 mg tylosin/kg feed). The dairy cows were placed at some

degree of risk of ruminal and metabolic acidosis. The study lasted 24 days. Mortality and health status were checked every day. BW was recorded at the end of the trial and cumulative FI, daily milk production and milk components (protein, fat, lactose, somatic cells) were measured. Dietary tylosin supplementation at 14 mg/kg DM did not have an increase yield effect in dairy cows.

In the study of Mir (1989), two experiments were carried out and the overall outcomes analysed independently. The basal diet was either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified form) at the concentration of 10 mg/kg BW. In Experiment 1 (feeding trial, 85 days), a total of 60 lambs for fattening of 20 kg BW (Suffolk-crossbred) were distributed in 60 individual crates and allocated to six dietary treatments. Animal weight and cumulative FI were recorded biweekly and daily, respectively, and the G:F calculated at the end of trial. In Experiment 2 (digestibility trial, 28 days), a total of 30 mature wethers of 45 kg BW were distributed in individual pens and allocated to the same diets; at the end of the trial, faeces and rumen fluid were collected to measure apparent digestibility of DM, organic matter (OM), nitrogen (N), acid-detergent fibre (ADF), neutral-detergent fibre (NDF) and energy, and to measure volatile fatty acid (VFA), ammonia and pH, respectively. The lambs treated with tylosin showed, compared to the control group, a reduction in apparent digestibility of DM (55.0% vs 60.6%), OM (57.6% vs 62.3%), ADF (45.9% vs 53.9%), NDF (45.4% vs 51.8%) and energy (52.3% vs 56.0%), despite improved apparent digestibility for N (69.5% vs 66.3%). Dietary tylosin supplementation at 10 mg/kg DM had a negative effect on the performance of lambs for fattening.

In the study of Mir et al. (2008), a total of 48 finishing steers (Hereford × Angus × Charolais; BW 373 kg) were individually distributed and allocated to four dietary treatments. The basal diet (finisher) was either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of TP (Elanco, Clinton, MO, USA) at the concentration of 11 mg/kg DM (corresponding to 9.9 mg tylosin/kg DM). The study lasted 185 days. Cumulative FI and animal weight were recorded daily and at the end of trial, respectively. At the end of the trial, G:F was calculated and additionally animals were slaughtered, and liver abscess incidence and severity measured. Carcass characteristics including hot carcass weight, fat depth, *Longissimus dorsi* muscle area, marbling score and lean meat yield were also measured, and *pars costalis* diaphragmatic muscle and subcutaneous fat sampled to determine fatty acid composition. At the end of the trial, animals treated with tylosin showed, compared to the control group, lower DM intake (8.9 vs 10.1 kg/day) and final weights (576 vs 608 kg BW). In addition, *cis*-monounsaturated fatty acids contents in the *pars costalis* diaphragmatic muscle and subcutaneous fat were lower than in the control group (41.8% vs 45.2% and 47.1% vs 51.4%, respectively). Total trans-fatty acids and total conjugated-linoleic acid contents were higher in the *pars costalis* diaphragmatic muscle of the tylosin-treated animals than in the controls, as well as fatty acid proportion in tissue. Dietary TP supplementation at 11 mg/kg DM (corresponding to 9.9 mg tylosin/kg DM) had a negative effect on the performance of cattle for fattening.

In the study of Potter et al. (1985), a total of 14 experiments were carried out in feedlot cattle and overall outcomes pooled. A total of ca. 1,648 animals of 253–465 kg BW, mainly steers (Hereford, Hereford × Angus, Holstein, other crosses), were distributed in 163 pens (8–24 pens) in groups of 5–24 animals and allocated to four dietary treatments (under a 2 × 2 factorial design). The basal diets were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and the treatment consisted of tylosin (unspecified form) supplemented at a concentration of 11 mg tylosin/kg DM. The studies lasted from 84 to 223 days. At the end of each trial, animals were weighed, cumulative FI was recorded and F:G calculated; animals were slaughtered, and liver abscesses were enumerated. At the end of the trial, animals treated with tylosin showed, compared to the control group, improved ADG (1.334 vs 1.315 kg/day). In addition, tylosin-treated animals showed a lower incidence of liver abscesses compared to the controls (8.7% vs 27.2%). Dietary tylosin supplementation at 11 mg/kg DM had a growth-promoting effect in cattle for fattening.

In the study of Pukrop et al. (2019), a total of 72 crossbred steers (Angus × Simmental; 358 ± 7.6 kg initial BW) were distributed in 12 pens in groups of six animals and allocated to three dietary treatments. The diet TMR was either not supplemented or supplemented (top-dressed) with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified chemical form, Tylan®; Elanco Animal Health, Indianapolis, IN, USA) supplemented at a concentration of 90 mg tylosin/steer per day (equivalent to 9 mg tylosin/kg DM). The study lasted 167 days. During the experiment, steers were weighed monthly prior to feeding to determine BW and

ADG. FI was recorded daily and F:G calculated. Steers were slaughtered at two different time points when a target weight of 624 kg was achieved. *Longissimus lumborum* muscle samples were taken caudal from the last rib on the right side of each carcass for pH, tenderness, purge and cook loss and lipid oxidation analysis. Dietary tylosin supplementation at 9 mg/kg DM did not have a growth-promoting effect in cattle for fattening.

In the study of Stanford et al. (2015) consisting of two trials conducted over two consecutive years, 240 (per year) predominantly Angus mixed-breed steer calves (251 ± 25.7 kg, 273 ± 25.5 kg; year 1 and 2, respectively) were allocated to five dietary treatments and distributed in five pens per treatment (in one treatment, the pens were only four) in groups of 10 animals per each pen. Two basal diets (grower and finisher) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of TP (TYL, Elanco Division of Eli Lily Canada Inc., Guelph, ON, USA) supplemented at a concentration of 11 mg/kg DM (corresponding to 9.9 mg tylosin/kg DM). The study lasted 233 days in the first year and 187 days in the second year. Health status was checked twice daily. Animals were weighted at the start of the experiment and at slaughtering. Growth performance (DM intake, ADG, gain to feed ratio (G:F)), health status and carcass characteristics from steers were evaluated. Dietary TP supplementation at 11 mg/kg DM (corresponding to 9.9 mg tylosin/kg DM) did not have a growth-promoting effect in cattle for fattening.

3.3.2.3.2. Studies in pigs

Amachawadi et al. (2011) studied the effect of feed grade antimicrobials and copper on performance in pigs. A total of 240 weaned piglets (unspecified breed/genotype; 34 days of age with an average BW 7.7 kg) were used in a 35-day growth trial to compare the effects of copper (Cu, from copper sulfate) and feed grade antimicrobials in a 2 × 3 factorial design (the factors being copper level 16.5 and 141.5 mg Cu/kg feed and antimicrobial level 0-control or chlortetracycline or tylosin supplementation). Pigs were allocated to eight pens (each with five pigs) per treatment. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with tylosin (unspecified chemical form; Tylan, Elanco Animal Health, Greenfield, IN, USA) at the concentration of 100 mg/kg feed. Following 13 days of acclimatisation period, pigs were fed dietary treatments for 21 days followed by another 14 days on the control diet to examine for any carryover effects. Pig weights and FI were recorded every week to calculate BW gain, FI and F:G. No copper × antimicrobial interactions were observed for any pig performance indicators. At the end of the experiment (day 35), dietary supplementation with tylosin did not affect performance (final BW, BW gain, FI and F:G), while for days 21–35 of experiment, tylosin-fed pigs showed a reduced BW gain (0.79 vs 0.74 kg/day). Tylosin dietary supplementation at 100 mg/kg feed did not affect the overall weaned piglets' performance parameters.

In another study, Brumm and Peo (1985) studied the effect of receiving diets containing alfalfa meal and certain feed additives on performance of pigs for fattening previously transported long distances in three different experiments. In one of the three experiments (Experiment 2), two treatment groups were relevant. In these groups, 80 crossbred pigs were distributed in eight pens, and received two dietary treatments (four pens per treatment with 10 pigs per pen) consisting on a basal diet either not supplemented (control) or supplemented with 44 mg tylosin/kg feed (unspecified form). In each tylosin level (0 and 44 mg/kg), two diets were used, a basal diet and a basal diet plus 10% dehydrated alfalfa meal, corresponding to two pens per diet and tylosin level. The study involved two periods, a 14-day tylosin supplementation period and a period from 15 days of experiment to BW of 95 kg, in which all pigs were switched to the control diet. The effect of additive on BW gain, daily FI and F:G, was determined at 14 days and for the whole experimental period. Faecal score was rated daily for the severity of diarrhoea using a scale ranging from 1 (normal) to 5 (severe diarrhoea). Dietary tylosin supplementation at 44 mg/kg feed did not have a growth-promoting effect in pigs for fattening.

In the study of Edwards et al. (2014), a total of 1,008 male pigs of 28–30 kg BW (PIC commercial progeny) were allocated to one of four dietary treatments and distributed in 12 pens per treatment in groups of 21 animals. Two were the relevant treatments obtained from two basal diets (grower and finisher) which were either not supplemented (control) or supplemented with tylosin (unspecified chemical form; Tylan 250) at concentrations of 40 and 20 mg/kg feed for grower and finisher diets, respectively. The study lasted 80 days. Mortality and health status were checked every day. Animals' weight was recorded on days 0, 38 (grower phase) and 80 (finisher phase); FI was recorded daily and F:G calculated at the end of the experiment. All pigs were slaughtered and the weights of the carcass

and backfat thickness were measured. Dietary tylosin supplementation at 40 and 20 mg/kg grower (30–58 kg BW) and finisher (59–97 kg BW) diet, respectively, did not have a growth-promoting effect in pigs for fattening.

In another study (NCR-89 Committee on Confinement Management of Swine, 1986), a total of 23 experiment replications were carried out and overall outcomes pooled. A total of 1,352 mixed growing pigs (unspecified breed/genotype) of 70–98 days of age (20–36 kg BW, average 25 kg BW) were distributed in 46–138 pens in groups of 10–20 animals and allocated to four dietary treatments (under a 2 × 2 factorial design), the factors being space allowance and tylosin dietary supplementation, i.e. adequate vs limited space allowance and none vs tylosin feeding. Floor space allowances per pig were 0.32 m^2 changed to 0.56 m^2 at 57 kg BW vs 0.46 m^2 changed to 0.74 m^2 at 57 kg BW. The two basal diets (grower and finisher) were either not supplemented (control) or supplemented with tylosin (unspecified form) at concentrations of 44 mg/kg feed for grower diets (until 57 kg BW) and 22 mg/kg feed for finisher diets (until 96 kg BW). The studies lasted for the entire grower–finisher period (from 25 kg BW to 96 kg BW). Animals' BW and FI were recorded at the end of each phase and F:G calculated at the end of the experiment. At the end of the trial, and irrespectively of space allowance, animals treated with tylosin showed, compared to the control group, improved F:G (3.09 vs 3.14) and a tendency to improved BW gain (0.723 vs 0.712 kg/day). Dietary tylosin supplementation at 44 (25–57 kg BW) and 22 (57–96 kg BW) mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Hagsten et al. (1980), a total of five experiment replications were carried out and overall outcomes pooled. A total of 1,230 mixed pigs of 20 (Experiment 1) or 35 kg BW (Experiment 2–5) (crossbred) were distributed in 135 pens in groups of 6–10 animals and allocated to three dietary treatments. Two basal diets (grower and finisher) were either not supplemented (control) or supplemented with different treatments. One of the treatments consisted of tylosin (unspecified form) at concentrations of 22 mg/kg feed for both grower/finisher diets (< 55 kg BW and > 55 kg BW, respectively) in three experiments (Experiment 1, 2, 4) or 44 mg/kg feed for grower diets (< 55 kg BW) and 22 mg/kg feed for finisher diets (> 55 kg BW) in two experiments (Experiment 3, 5). The studies lasted an entire grower–finisher period (from 20 to 86 kg BW or from 35 to 102 kg BW). Animals' BW and FI were recorded at the end of each phase (55 kg BW for grower phase and 86–102 kg BW for finisher phase) and F:G calculated at the end of the experiment and each experimental phase. At the end of the trial, the pooled data from the five experiments showed that animals treated with tylosin had, compared to the control group, improved F:G (3.40 vs 3.48). Dietary tylosin supplementation
at 22–44 (20 or 35–55 kg BW) and 22 (55–86 or 102 kg BW) mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Hansen and Larsen (1994), a total of 93 male pigs (unspecified breed/genotype; initial BW of 60 kg) were used. The pigs were individually housed and allocated to three dietary treatments (31 pigs/replicates per treatment). Two were the relevant treatments obtained from a basal diet (grower) which was either not supplemented (control) or supplemented with tylosin (unspecified form) at the concentration of 20 mg/kg feed. The duration of the study was not indicated in days, but it was from 60 to 100 kg BW. Animals' BW was recorded at the beginning and at the end of the study. During the fattening phase, pigs were weighed every 2 weeks. FI was recorded and F:G calculated at the end of the experiment. All the animals were slaughtered when they reached 100 kg BW. Dietary tylosin supplementation at 20 mg/kg feed did not have growth-promoting effects in pigs for fattening.

In the study of Harvey et al. (1995), a total of 36 barrows (Yorkshire × American Landrace × Hampshire, 42 days of age) were allocated to six dietary treatments and distributed in three pens (replicates) per treatment, in groups of two animals. Two were the relevant treatments obtained from a basal diet (starter) which was either not supplemented (control) or supplemented with tylosin (unspecified form) at a concentration of 110 mg/kg feed. The study lasted 28 days. Mortality and health status were checked daily. Animals' weight and FI were recorded weekly. At the end of the trial, animals were bled for hematologic (red blood cells, mean cell volume, haematocrit, haemoglobin, mean cell haemoglobin concentration (MHC) and leukocytes), immunologic (lymphoblastogenesis stimulation index and blastogenic response to phytohaemagglutinin) and serum biochemical (alkaline phosphatase (ALP), gamma glutamil transferase (GGT), albumin, calcium, cholesterol, glucose, phosphorus, iron, triglycerides, urea, N and iron-binding capacity) measurements; additionally, 24 animals (four animals per treatment) were slaughtered and the weight of liver, left kidney, spleen and heart was recorded, and specimens from each organ were examined microscopically. Dietary tylosin supplementation at 110 mg/kg feed did not have growth-promoting effects in weaned piglets.

In the study of Hawe et al. (1992), a total of eight boars and eight gilts of 40 kg BW (Landrace × Large White) were individually distributed in 16 pens and allocated to eight dietary treatments (under a 2 × 2 × 2 factorial design), with the factors being fibre, lactose and tylosin. Four diets differing in lactose/fibre level (control-wheat and soybean meal, fibre only, lactose only, and fibre-lactose diets) were either not supplemented (control) or supplemented with tylosin phosphate (Tylamix, Elanco Products Ltd., Basingstoke) at a concentration of 200 mg/kg feed (corresponding to 180 mg tylosin/kg feed). The study lasted ca. 74 days. Animals' BW and FI were recorded weekly. On days 32 and 53, faeces were collected to determine dry matter and skatole/indole concentrations. At the end of the trial, all animals were slaughtered and the carcass yield was measured. Also, subcutaneous fat from the third and fourth cervical vertebrate was sampled to determine skatole/indole concentrations. Dietary tylosin phosphate supplementation at 200 mg/kg (corresponding to 180 mg tylosin/kg feed) feed did not have growth-promoting effects in pigs for fattening.

In the study of Holman and Chénier (2013), a total of 12 male and 12 female piglets (from Landrace × Yorkshire sows, weaned at 24 days of age) were distributed in six pens in groups of four animals (three pens with males and three pens with females) and allocated to three dietary treatments (corresponding to one pen with males and one pen with females in each treatment). Two were the relevant treatments obtained from three basal diets – weaner, starter, finisher – which were either not supplemented (control) or supplemented with tylosin (unspecified form) at concentrations of 44, 22 and 11 mg/kg feed in weaner (for 21 days), starter (for 21 days) and finisher (for 70 days) diets, respectively. The study lasted ca. 112 days. Animals' BW was recorded on days 28, 42, 84 and 133 of age. On days 21, 42, 63, 84, 133 and 147 (after antimicrobial withdrawal) of age, faeces were sampled to enumerate total anaerobic bacteria. Tylosin did not increase the growth rate of the pigs in the study. Dietary tylosin supplementation at 44, 22 and 11 mg/kg weaner, grower and finisher diet, respectively, did not have growth-promoting effects in pigs for fattening.

In the study of Kim et al. (2016), a total of six pigs of 100 days of age (Landrace) were distributed in six pens in groups of one animal and allocated to two dietary treatments (three pigs per treatment). One basal diet (grower) was either not supplemented (control) or supplemented with tylosin (unspecified chemical form; Sigma Inc., USA) at a concentration of 45 mg/kg feed. The study lasted 70 days. Animals' BW was recorded weekly. The study showed no growth-promoting effects of tylosin at 45 mg/kg feed in pigs for fattening.

In the study of Langlois et al. (1978), a total of five trials were carried out and overall outcomes were provided both separately and pooled. The five trials shared common experimental design, and, in each trial, a total of 60 mixed sex pigs (Specific-Pathogen-Free Yorkshire, 5−7 weeks of age, with initial BW of 14 kg) were allocated to five dietary treatments with three replicate pens per treatment and four pigs each. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified form) at a concentration of 44 mg/kg feed during weeks 6, 11, and 16. All five trials lasted 16 weeks (until approx. BW of 98 kg). Animal's BW and FI were recorded at weeks 6, 11 and 16 and F:G ratio calculated. In addition, at same dates, faeces were sampled to enumerate coliforms and lactobacilli, as well as coliform chlortetracycline-resistant isolates and intestinal isolates resistant to other antimicrobials. At the end of the experiment, dietary tylosin supplementation increased BW gain (810 vs 734 g/day) and improved F:G (2.81 vs 3.00) of pigs. At week 6, the pigs treated with tylosin for 6 weeks showed, compared to the control group, lower faecal counts of lactobacilli (9.58 vs 9.82 $\log_{10}$ CFU/g faeces). Moreover, at week 11, a greater number of faecal isolates resistant to ampicillin were detected in tylosin-treated animals than in the control ones (31% vs 14%). Dietary tylosin supplementation at 44 mg/kg diet had growth-promoting effects in pigs for fattening.

In the study of Lindemann et al. (2010), a total of 24 barrows of 63.0−78.7 kg BW ((Yorkshire × Duroc) × Chester White; (Yorkshire × Landrace) × Duroc; and (Yorkshire × Landrace × Duroc) × Chester) were distributed in single animal metabolism crates and allocated to two dietary treatments. One basal diet (grower) was either not supplemented (control) or supplemented with tylosin (unspecified chemical form; 0.5 g Tylan 40/kg feed, Tylan 40, Elanco Animal Health, Indianapolis, IN, USA) at a concentration of 44.1 mg/kg feed. The study lasted 17 days. Mortality and health status were checked daily. Animal FI was recorded from days 13 to 17. Faeces and urine were also collected the same days and DM, energy, phosphorus, nitrogen and calcium were measured to calculate nutrient apparent digestibility and retention. Dietary tylosin at 44.1 mg/kg feed did not have growth-promoting effects in pigs for fattening.

In the study of Livingstone and Livingston (1968), Experiment 2, a total of 96 mixed large white weaned piglets of 4.5 kg BW were allocated to eight dietary treatments (under a 2 × 4 factorial design), the factors being copper and antimicrobial dietary supplementation, i.e. none vs copper

feeding and none vs antimicrobial feeding, with 12 pigs (six males and six females) per treatment. Four were the relevant treatments obtained from four basal diets which were either not supplemented (control: no copper or antimicrobial) or supplemented with tylosin (unspecified chemical form; Elanco Products, London, UK) at the concentration of 40 mg/kg feed (and also no copper supplementation). The study lasted an entire fattening period (from 4.5 to 90 kg BW). Treatments were given from 4.5 to 20 kg BW, and from 21 to 90 kg BW the carry-over effect was studied. For period of 4.5–20 kg BW, pigs were pen housed in pairs (one male and one female) and for period 20–90 kg BW pigs were housed in groups of 12 (i.e. one pen per treatment). In both periods, pigs were fed individually. Mortality and health status were checked daily. Animals' BW and FI were recorded when animals reached designated BW of 12, 20, 45 and 90 kg. At the end of the trial, all the animals were slaughtered and the carcass characteristics (carcass-specific gravity and length, *Longissimus dorsi* muscle area, fat depth) were measured. There was no interaction between copper and antimicrobial dietary supplementation. At the end of the trial, pigs treated with tylosin, from 4.5 to 20 kg BW, showed, compared to the control group, improved daily live weight gain (DLWG) (381 vs 319 g/day) and improved F:G (2.03 vs 2.26). Dietary tylosin supplementation at 40 mg/kg feed had growth-promoting effects in weaned piglets.

In the study of Lowell et al. (2018), a total of 192 pigs (half barrows and half gilts, Fertilis-25 × PIC 359, 70 days of age) were distributed in 48 pens in groups of four animals and allocated to three dietary treatments. Two were the relevant treatments obtained from three basal diets (grower, early finisher and late finisher) which were either not supplemented (control) or supplemented with tylosin phosphate (Tylan 40 premix, Elanco Animal Health, Greenfield, IN, USA) at the concentration of 40 mg/kg feed (corresponding to 36 mg tylosin/kg feed). The study lasted 98 days. Animals' BW and feed intake (FI) were recorded at the end of each phase (days 35, 70 and 98). At the end of the trial, all animals were slaughtered, and the carcasses were weighed to determine the hot carcass and chilled carcass weights, as well as other carcass characteristics. This study showed no effect of tylosin phosphate on performance of pigs for fattening at 40 mg/kg feed (corresponding to 36 mg tylosin/kg feed).

In the study of Mazutti et al. (2016), a total of 72 weaned piglets (unspecified breed/genotype) at 28 days of age were allocated to three dietary treatments and distributed in eight pens per treatment, in groups of three animals. Two were the relevant treatments obtained from two basal diets – pre-starter and starter – which were either not supplemented (control) or supplemented with tylosin (unspecified form) at the concentration of 22 mg/kg feed. The study lasted 35 days. Mortality and health status, including faecal score, were checked daily. Animals' BW and FI were recorded weekly and G:F calculated for each phase (days 1–14 and days 15–35) and at the end of the trial. At the end of the trial, animals treated with tylosin showed, compared to the control group, increased FI (0.880 vs 0.794 kg/day) and final BW (24.3 vs 22.6 kg). Dietary tylosin at 22 mg/kg feed showed growth-promoting effects in weaned piglets.

In the study of McCormick et al. (2017), Experiment 1, a total of 24 barrows (Yorkshire × Landrace × Duroc, initial BW 17.5 kg) were allocated to four dietary treatments and distributed in six individual metabolism crates per treatment. One basal diet (grower) was either not supplemented (control) or supplemented with different treatments. One of the treatments consisted of tylosin (unspecified chemical form; Tylan 40, Elanco Animal Health, Greenfield, IN, USA) at the concentration of 44 mg/kg feed. The study lasted 10 days. Faeces and urine were collected daily from days 5 to 7, and phosphorus, calcium, N and gross energy measured to calculate nutrient apparent total tract digestibility and retention. At the end of the trial, animals treated with tylosin showed, compared to the control group, lower N retention (56.3% vs 63.3%). Dietary tylosin supplementation at 44 mg/kg feed showed a negative effect on feed utilisation by pigs for fattening.

In the study of Pilcher et al. (2013), a total of 18 growing barrows (PIC 337 sires × C22 or C29 dams; initial BW 32.6 kg) fitted with a T-canula in the distal ileum were allotted to a Youden square design with six dietary treatments, and three replicate periods. Animals were individually housed, and the individual pig was the experimental unit, with nine replicates per treatment, considering all three periods. The experimental design was a 2 × 2 factorial design (four treatments), with factors being dried distillers' grains with solubles – DDGS (0 or 250 g/kg feed) and TP (0 or 44 mg tylosin phosphate/kg feed, corresponding to 39.6 mg tylosin/kg feed; Tylan 40, Elanco Animal Health, Greenfield, IN, USA); there were also two other treatments consisting of a basal N-free diet not supplemented (control) or supplemented with TP at a concentration of 44 mg/kg feed, to estimate basal ileal endogenous amino acid losses. The study lasted 14 days. Mortality and health status were checked every day. On days 5–6 and 11–12, faeces were collected and on days 7–8 and 13–14 ileal digesta was collected and DM, gross energy, neutral detergent fibre, nitrogen, crude protein (CP) and

amino acids measured to calculate apparent total tract (ATTD) and ileal digestibility, as well as to calculate CP and amino acids standardised ileal digestibilities (SID). There was no effect of tylosin on basal ileal endogenous losses of CP and amino acids. Animals treated with tylosin did not show, compared to the control group, differences in any of the endpoints measured. Dietary TP supplementation at 44 mg/kg feed (corresponding to 39.6 mg tylosin/kg feed) did not have a growth-promoting effect in pigs for fattening.

In the study of Pilcher et al. (2015), a total of 72 finishing gilts (PIC 337 sires × C22 or C29 dams; initial BW 107 kg), individually housed in metabolic crates, were allocated to eight dietary treatments (under a 2 × 2 × 2 factorial design, with factors being dried distillers grains with solubles (DDGS) (0 or 300 g/kg feed), ractopamine HLC (0 or 5 mg/kg feed) and tylosin (0 or 44 mg/kg feed)). The individual pig was the experimental unit, with nine replicates per treatment. The basal diet (finisher, based on maize–soybean meal) was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control (0 mg tylosin/kg feed, no ractopamine) and a treatment consisting of TP (0.5 g Tylan 50/kg feed, Tylan 40, Elanco Animal Health) supplemented at a concentration of 44 mg/kg feed (corresponding to 39.6 mg tylosin/kg feed, with no ractopamine supplementation). The study lasted 17 days. Mortality and health status were checked every day. Cumulative feed and water intakes were recorded daily. BW was recorded at the end of the trial and G:F calculated. In addition, faecal and urine samples were collected on days 7–8 and 15–16 and N, dry matter and gross energy measured to calculate apparent total tract digestibility. Dietary tylosin supplementation at 44 mg/kg feed (corresponding to 39.6 mg tylosin/kg feed) did not have growth-promoting effects in pigs for fattening.

In the study of Roth and Kirchgessner (1993), a total of two trials were carried out and overall outcomes pooled. A total of 24 castrated male pigs of 67.9 kg BW at the half-way point of the study (German Landrace × Pietrain) were distributed in 24 metabolism cages in groups of one animal and allocated to six dietary treatments (under a 2 × 3 factorial design, with factors being CP concentration (165 or 140 g/kg feed) and antimicrobial feeding (0, 20 mg avilamycin/kg feed or 20 mg tylosin/kg feed)), into two trial runs. The basal diet (grower) was either not supplemented (control) or supplemented with different treatments. Four were the relevant treatments: two controls (0 mg tylosin/kg feed in both 165 or 140 g CP/kg feed) and two treatments consisting of tylosin (unspecified chemical form; Tylan^{R}, Eli Lilly GmbH, Dept. Elanco, Bad Homburg, Germany) supplemented at a concentration of 20 mg/kg feed (in both 165 or 140 g CP/kg feed). The trials lasted 14 days. From days 8 to 14, faeces and urine were collected daily, and N measured to calculate N intake, retention and excretion. For both protein concentrations, animals treated with tylosin did not show, compared to the control group, differences in any of the variables measured. Dietary tylosin supplementation at 20 mg/kg feed did not have growth-promoting effects in pigs for fattening.

In the study of Van Lunen (2003), a total of 384 growing pigs (unspecified breed/genotype; initial BW 20.8 kg) were allocated to two dietary treatments and distributed in eight pens per treatment, in groups of 24 animals (half castrated males and half females) per each pen. Four barley-based basal diets (starter, starter/grower, grower and finisher) were either not supplemented (control) or supplemented with TP at a concentration of 44, 22, 22 and 11 mg/kg feed (corresponding to 39.6, 19.8, 19.8 and 9.9 mg tylosin/kg feed) for the starter, starter/grower, grower and finisher diets, respectively. The study lasted up to the day when pigs attained a mean BW of 110 kg (corresponding to 94.1 days of trial). Mortality and health status were checked every day. BW and cumulative FI were recorded at the end of the trial and ADG and F:G calculated. At slaughtering, carcass weight, lean yield, fat thickness, lean muscle depth and carcass grading were assessed. At the end of the trial, the pigs treated with tylosin phosphate showed, compared to the control group, higher lean muscle depth (61.5 vs 60.3 mm). Mortality, BW, ADG, FI, FCR, dressed weight and lean yield were unaffected. Dietary TP supplementation at 44, 22, 22 and 11 mg/kg feed (corresponding to 39.6, 19.8, 19.8 and 9.9 mg tylosin/kg feed, respectively) for the starter, starter/grower, grower and finisher diets, respectively, did not have growth-promoting effects in pigs for fattening.

In the study of Wang et al. (2009), a total of 144 growing male/female pigs (Duroc × (Landrace × Yorkshire), initial BW 23.6 kg) were allocated to four dietary treatments and distributed in nine pens per treatment, in groups of four animals per each pen. Two were the relevant treatments obtained from a basal diet (based on maize and soybean) which was either not supplemented (control) or supplemented with tylosin (unspecified form) at the concentration of 44 mg/kg feed. The study lasted 42 days. Individual weights and FI were recorded and G:F, ADG and average daily feed intake (ADFI) calculated at the end of the experiment. From two pigs per pen, faeces were collected at the end of the experiment to calculate total tract apparent digestibility for DM, N and GE by using chromic

oxide as an indigestible marker. At the beginning and at the end of the experiment, two pigs from each pen were bled for haematological and biochemical parameters. At the end of the trial, the pigs treated with tylosin showed, compared to the control group, higher apparent digestibility coefficients for DM (0.754 vs 0.731), N (0.759 vs 0.750) and GE (0.760 vs 0.719). A higher immunoglobulin G (IgG) content in blood was also observed in the pigs receiving tylosin (748 vs 649 mg/dL for tylosin and control groups, respectively). Dietary tylosin supplementation at 44 mg/kg feed had growth-promoting effects in pigs for fattening.

In the study of Wang et al. (2011), Experiment 1, a total of 120 barrows (Duroc × (Landrace × Yorkshire), initial BW of 23.5 kg) were allocated to five dietary treatments and distributed in six pens per treatment, in groups of four animals per pen. The basal diet (based on maize and soybean) was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin supplementation (unspecified chemical form; CTC Bio Inc., Seoul, Republic of Korea) at a concentration of 1,000 mg/kg feed. The study lasted 35 days. BW and FI were recorded weekly and F:G calculated. On day 35 of trial, faeces were collected from two pigs per pen for bacterial counts. At the end of the trial, the pigs treated with tylosin showed, compared to the control group, higher final BW (45.1 vs 43.4 kg) and BW gain (0.771 vs 0.720 kg/day). Faecal microbial count for *E. coli* was lower in tylosin-treated pigs (6.65 vs 7.02 $\log_{10}$ CFU/g digesta). Dietary tylosin supplementation at 1,000 mg/kg feed showed growth-promoting effects in pigs for fattening.

In the study of Weber and Kerr (2008), a total of 180 weaned piglets (commercial hybrid PIC, initial BW 6.3 kg) were allocated to six dietary treatments and distributed in six pens per treatment, in groups of five animals per pen. Two were the relevant treatments obtained from two basal diets (maize and soybean-based; 1–14 and 14–28 d of trial) which were either not supplemented (control) or supplemented with tylosin (unspecified chemical form; Tylan, Elanco Animal Health, Greenfield, IN, USA) at the concentration of 110 mg/kg feed. The study lasted 28 days. BW and FI were recorded every 2 weeks and F:G calculated. Dietary tylosin supplementation at 110 mg/kg feed did not have growth-promoting effects in weaned piglets.

In the study of Wu et al. (2019), a total of 80 pigs for fattening (40 males and 40 females, hybrid Line 600 × 241 DNA Columbus, initial BW 93.9 kg) were individually housed and allocated to four treatments of 20 pigs (10 males and 10 females) each. Three were two relevant treatments, obtained from a basal diet which was either not supplemented (control) or supplemented with tylosin (unspecified chemical form; Tylan 100, Elanco Animal Health, Indianapolis, IN, USA) at a concentration of 110 mg/kg feed. Another treatment included tylosin supplementation (unspecified chemical form; Tylan Soluble, Elanco Animal Health, Indianapolis, IN, USA) in water for drinking at 66 mg/L for the first 3 days of each of 3 weeks. In the fourth treatment, the 20 pigs received 8.8 mg/kg weight tylosin (unspecified chemical form; Tylan 200, Elanco Animal Health, Indianapolis, IN, USA) through intramuscular injection twice daily for the first 3 days of each of 3 weeks. The duration of the study was 35 days, and, in all cases, the treatments were applied for the first 3 weeks of the experiment. BW and FI were recorded weekly and G:F calculated at 21 and 35 days. At day 21 of the study, there were no differences between the control pigs and those fed the diet supplemented with tylosin in FI, BW gain or G:F, while pigs receiving tylosin in water showed lower G:F than control pigs (0.322 vs 0.347). At the end (day 35) of the study, no differences were found among treatments in performance parameters. This study did not provide evidence on growth-promoting effects from tylosin supplementation at 110 mg/kg feed in pigs for fattening.

In the study of Yan et al. (2011), 140 weaned piglets (Landrace × Yorkshire, 21 days of age, initial BW 6.25 kg) were allocated to four dietary treatments and distributed in seven replicate pens (five piglets per pen) per treatment. Two were the relevant treatments obtained from three basal diets (days 1–7, 8–21 and 22–35 of trial) which were either not supplemented (control) or supplemented with tylosin (unspecified form) at a concentration of 44 mg/kg feed. The study lasted 35 days. Individual BW and feed consumption (per pen) were determined to calculate ADG, ADFI and G:F. Serum profiles were analysed from two piglets from each pen (randomly selected) on day 35. Chromic oxide was used as an indigestible marker to evaluate the nutrient digestibility. G:F was increased in the piglets receiving tylosin-supplemented diets compared to the control group (0.716 vs 0.644). N digestibility on day 7 (68.7% vs 62.8%) and on day 21 (77.1% vs 73.0%) was also higher when the diet was supplemented with tylosin. Thus, tylosin at 44 mg/kg feed had growth-promoting effects in weaned piglets.

In the study of Yan et al. (2012a), a total of 96 growing pigs (Duroc × (Landrace × Yorkshire), initial BW 26.6 kg) were allocated to four dietary treatments and distributed in six replicate pens (four

pigs per pen) per treatment. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with tylosin (unspecified form) at a concentration of 500 mg/kg feed. The study lasted 42 days. Body weight and FI were recorded at 1 and 42 days of the trial and G:F was calculated. Chromium oxide was used as a marker to determine feed digestibility. Fresh faecal samples were obtained from two pigs per pen on day 42 to enumerate *E. coli* and *Lactobacillus* colonies. On days 1 and 42, blood samples from two pigs per pen were collected for haematology. BW gain was greater in pigs fed supplemented tylosin diets than in the control group (662 vs 624 g/day). Feeding tylosin diets also increased dry matter digestibility (79.0% vs 76.0%) and reduced *E. coli* faecal counts (6.40 vs 6.79 $\log_{10}$ CFU/g digesta). Dietary tylosin supplementation at 500 mg/kg feed had growth-promoting effects in pigs for fattening.

In the study of Yan et al. (2012b), a total of 144 weaned piglets (Duroc × (Landrace × Yorkshire), initial BW of 8.45 kg) were allocated to four dietary treatments and distributed to nine replicate pens (four piglets per pen) per treatment. Two were the relevant treatments obtained from three basal diets (for weeks 0–1, 2–3 and 4–5) which were either not supplemented (control) or supplemented with tylosin (unspecified form) at a concentration of 1,000 mg/kg feed. The study lasted 35 days. BW and FI were recorded at 1 and 35 days of the trial and G:F was calculated. Chromium oxide was used as a marker to determine feed digestibility. Fresh faecal grab samples were obtained from two pigs per pen on day 35 to enumerate *E. coli* and *Lactobacillus* colonies. On day 35, blood samples from two pigs per pen were collected for haematology. Compared with those of the control group, the piglets fed tylosin supplemented diets showed greater BW gain (654 vs 495 g/day) and improved G:F (0.892 vs 0.691). Feeding tylosin diets also increased feed energy digestibility (78.3% vs 76.2%) and lymphocytes percentage in blood (67.2% vs 62.4%). Dietary tylosin supplementation at 1,000 mg/kg feed had growth-promoting effects in weaned piglets.

In the study of Zhang et al. (2012), a total of 140 piglets (Duroc × (Landrace × Yorkshire), weaned at 21 days of age with initial BW of 6.5 kg) were allocated to five dietary treatments and distributed in seven replicate pens (four piglets per pen) per treatment. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with tylosin (unspecified form) at a concentration of 500 mg/kg feed. The study lasted 35 days. BW and FI were recorded at 1, 14 and 35 days of the trial and G:F was calculated. Chromium oxide was used as a marker to determine feed digestibility. Fresh faecal grab samples were obtained from two pigs per pen on day 35 to enumerate *E. coli* and *Lactobacillus* colonies. On day 35, blood samples from two pigs per pen were collected for haematology. At the end (day 35) of the study, no differences were found among treatments in performance parameters, while feeding tylosin supplemented diets increased DM digestibility (83.8% vs 82.1%). Dietary tylosin supplementation at 500 mg/kg feed had growth-promoting effects in weaned piglets.

3.3.2.3.3. Studies in poultry

In the study of Da Silva et al. (2019), a total of 240 1-day-old male Cobb chickens for fattening were allocated to four dietary treatments and distributed in six cages per treatment, in groups of 10 birds per each cage. One basal diet based on maize and soybean meal was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified chemical form, supplied from Elanco) supplementation at a concentration of 500 mg tylosin/kg feed. Mortality and health status were checked every day. Individual zootechnical parameters were evaluated at 7 and 21 days. At the end of the experiment (21 days), final BW and FI were recorded, viability was measured and BW gain and F:G were calculated. No differences among control group and birds treated with tylosin at 500 mg/kg feed was observed on FI, final BW, weight gain, F:G or viability. Dietary tylosin supplementation at 500 mg/kg feed did not have growth-promoting effects in chickens for fattening.

In the study of Hughes et al. (2005), a total of 240 1-day-old male Cobb 500 chickens for fattening were allocated to six dietary treatments and distributed in four pens per treatment, in groups of 10 animals per each pen. Three diets (starter, grower and finisher) based on wheat, soybean meal, meat and bone meal, were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified form) supplementation at a concentration of 15 mg/kg feed. Mortality and health status were checked every day. Birds' weight and FI were recorded weekly. At the end of the trial (42 days), the birds treated with tylosin at 15 mg/kg feed compared to the control group did not show differences in any of the variables measured. Dietary tylosin supplementation at 15 mg/kg feed did not have growth-promoting effects in chickens for fattening.

In the study of Li et al. (2015), a total of 384 1-day-old Ross 308 chickens for fattening were allocated to four dietary treatments and distributed in six cages per treatment in groups of 16 animals per cage. Two basal diets (starter and finisher) based on maize and soybean meal were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified chemical form; feed grade tylosin from TYLOSIN 20 W.S.P; Shijiazhuang ZDHF Stock-Raising Co, Hebei, China) supplementation at a concentration of 200 mg/kg feed. Mortality and health status were checked daily. Animal weight and cumulative FI were recorded biweekly and G:F calculated at the end of each phase and at the end of the experiment (35 days). Eighteen animals from each treatment were slaughtered and breast muscle quality (colour, pH, water holding capacity and drip loss) was measured. At the end of the trial, the birds treated with tylosin at 200 mg/kg feed, compared to the control group, showed improved average total weight gain (1,661 vs 1,621 g). Dietary tylosin supplementation at 200 mg/kg feed showed growth-promoting effects in chickens for fattening.

In the study of Li et al. (2020), a total of 90 1-day-old mixed sex Arbor Acres chickens for fattening were allocated to three dietary treatments and distributed in 30 individual cages per treatment. Two basal diets (starter (0–3 weeks) and grower (4–6 weeks)) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting on tylosin (unspecified chemical form; tylosine (10%), Qilu Pharmaceutical Co., Ltd., Jinan, China) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were not specified. Chicken weight and cumulative FI were recorded at the start and the end of the experiment (day 42), and daily FI, daily weight gain and F:G were calculated. At 42 days of age, all the birds were bled, slaughtered and the carcass, breast muscle, leg muscle and abdominal fat were weighed. At the end of the trial (42 days), the birds treated with tylosine at 50 mg/kg feed, compared to the control group, showed higher BW (2,407 vs 2,107 g), higher daily FI (100 vs 85 g/day), higher carcass weight (2,325 vs 2,033 g) and higher blood triglycerides concentration (3.2 vs 2 mg/mL). Dietary supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Onifade and Babatunde (1997), two experiments were reported. In Experiment 1 (performance study), a total of 150 unsexed 7-day-old Hypeco chickens for fattening were allocated to five dietary treatments and distributed in three cages per treatment, in groups of 10 birds per each cage, until day 28 of age and then transferred to floor pens until day 35 of age. In Experiment 2, a nutrient retention study was conducted using six 28-day-old chickens of similar weight per treatment (three cages per treatment, two chickens per replicate cage). In both Experiment a basal high-fibre diet based on maize, soybean meal and palm kernel meal was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified form; tylosin 20) supplementation at the concentration of 150 mg/kg feed. The nutrient retention study lasted 7 days (from day 29 to day 35 of age) and feed was restricted at 75 g per chicken per day. Birds' BW and FI were recorded at start and end, and body weight gain and F:G calculated, and retention of dry matter, CP, ether extract, crude fibre, NDF, ADF, ADL, haemicellulose and cellulose was determined. The birds treated with tylosin at 150 mg/kg feed compared to the control group showed a higher daily FI (63.1 vs 60.2 g/day), an improved F:G (1.74 vs 1.81), a higher retention of haemicellulose (63.1% vs 60.5%). Dietary tylosin supplementation at 150 mg/kg feed had growth-promoting effects in chickens for fattening.

In the study of Onifade et al. (1999), Experiment 2, a total of 210 unsexed 7-day old Anak chickens were allocated to seven dietary treatments and distributed in three pens per treatment in groups of 10 animals in each pen. One basal diet (grower) based on maize, soybean meal and palm kernel meal was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control (designated as negative control diet with CP 18%) and a treatment consisting of tylosin (unspecified form) supplementation (in the negative control basal diet) at a concentration of d 75 mg/kg feed. The study lasted 28 days (until day 35 of age). Mortality and health status were not specified. Animal weight and cumulative FI were recorded on day 35 of age and F:G and protein efficiency ratio calculated. The birds were kept until 56 days of age to assess carcass characteristics. At the end of the experiment (day 56 of age), a total of 20 chickens (ten per treatment) were slaughtered and carcass, breast, and abdominal fat were weighed. At days 35 of age, the birds treated with tylosin at 75 mg/kg feed, compared to the control group, showed improved final weight (915 vs 816 g), cumulative weight gain (849 vs 750 g) and cumulative FI (1,548 vs 1,411 g). Dietary tylosin supplementation at 75 mg/kg feed had a promoting effect in chickens for fattening.

In the study of Stutz and Lawton (1984), Experiment 2, a total of 168 2-day-old male chickens for fattening (Hubbard) were allocated to six dietary treatments and distributed in six (control) or three

(experimental) pens per treatment, in groups of eight birds per pen. The basal diet based on maize and soybean meal was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified form) supplementation at a concentration of 55 mg/kg feed. The experiment lasted 80 days (from day 3 to day 11 of age). BW and cumulative FI were recorded and F:G calculated at the end of the experiment. At the end of the experiment, 32 chickens (control) or 16 chickens (tylosin treatment) were slaughtered for relative ileal weight determination, whereas ileal digesta from 12 chickens (control) or 6 chickens (tylosin treatment) were used for enumeration of *C. perfringens*. At the end of the experiment, the birds treated with tylosin at 55 mg/kg feed, compared to the control group, showed higher daily weight gain (124 vs 111 g/day), and an improved F:G (1.21 vs 1.26), and had decreased relative ileum weight (1.31% vs 1.62% BW) and lower *C. perfringens* count (2.5 vs 3.8 $\log_{10}$/g digesta). Dietary tylosin supplementation at 55 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Wang and Kim (2011), a total of 240 36-week-old, brown Hyline laying hens individually caged were allocated to five treatments and distributed in eight pens per treatment, in groups of six animals per each pen. The basal diet based on maize and soybean meal was either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified form) supplementation at a concentration of 110 mg/kg feed. Animals were weighed at the start and at the end of the trial. FI was recorded weekly. Egg production was recorded daily. Egg quality was assessed on 32 eggs per treatment (collected on a weekly basis). At the end of the trial (8 weeks), the birds treated with tylosin at 110 mg/kg feed, compared to the control group, showed improved F:G (1.76 vs 1.83) and egg weight (64.7 vs 62.4 g). Dietary tylosin supplementation at 110 mg/kg feed had a promoting effect on the performance of laying hens.

In the study of Wu et al. (2008), a total of 768 55-week-old white Bovans laying hens and 768 55-week-old white Dekalb laying hens were allocated to eight dietary treatments and distributed in 16 pens per treatment, in groups of 12 birds per each pen. Four basal diets based on maize and soybean meal with increasing dietary energy levels (2,776, 2,820, 2,864 and 2,908 kcal/kg) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tylosin (unspecified chemical form, supplied from Elanco Animal Health, Memphis, TN, USA) supplementation at a concentration of 33 mg/kg feed. Mortality and egg production were checked every day. BW was measured at the end of the experiment (10 weeks). FI was measured weekly. The following qualitative parameters of the eggs were assessed at different times during the study: egg weight, egg mass and whole egg solids. Dietary tylosin supplementation at 33 mg/kg feed did not have any effect on laying performance of hens or the characteristics of eggs. Dietary tylosin supplementation at 33 mg/kg feed did not have a promoting effect on the performance of laying hens.

3.3.2.4. Discussion

From the studies examined, the test item has been described as (i) 'tylosin phosphate' (seven studies) or (ii) 'tylosin phosphate' and 'tylosin urea adduct' (one study) or (iii) a tylosin commercial preparation (unspecified chemical form; 16 studies) or (iv) 'tylosin' (unspecified form; 21 studies). Therefore, for the cases (iii) and (iv), an uncertainty on the exact product used/concentration applied has been identified.

A detailed analysis of the uncertainties for tylosin is included in Appendix B (Table B.2) of this document, and the Section 3.3 of the Scientific Opinion Part 1 (see also the Virtual Issue).

3.3.2.4.1. Ruminants

The only eight studies considered as suitable for the assessment were mostly conducted in cattle for fattening (six studies) and only a single study was available in dairy cows and another in weaning lambs. Except for one study (Brown et al., 1973), treatments contained groups of animals treated with only one tylosin concentration and did not allow to assess any dose-related effects.

In three studies in cattle for fattening, dietary tylosin supplementation at 10 mg tylosin/kg DM (Brown et al., 1975), 11 mg TP or TUA/kg DM (corresponding to 9.9 mg tylosin/kg DM) (Brown et al., 1973) and 11 mg tylosin/kg DM (Potter et al., 1985) showed growth-promoting effects. Other three studies in cattle for fattening showed that dietary tylosin supplementation at the same range of concentration in feed either did not affect animal performance (Pukrop et al. (2019), 9 mg tylosin/kg DM; Stanford et al. (2015), 11 mg tylosin phosphate/kg DM, corresponding to 9.9 mg tylosin/kg DM)

or had a negative effect (Mir et al. (2008), 11 mg tylosin phosphate/kg DM, corresponding to 9.9 mg tylosin/kg DM). The only study available in dairy cows showed no effect of 14 mg/kg DM on growth performance/milk yield (Lean et al., 2000).

The only study in lambs for fattening (Mir, 1989) showed negative effects on performance of 10 mg tylosin/kg DM.

3.3.2.4.2. Pigs

The 28 studies considered as suitable for the assessment covered only two animal categories within pigs: weaned piglets (8) and pigs for fattening (20). In all studies, treatments included groups of animals treated with only one tylosin concentration and did not allow assessment of any dose-related effects.

In five studies in weaned piglets, dietary tylosin supplementation at 22–1,000 mg/kg feed had growth-promoting/increase yield effects in piglets (Mazutti et al. (2016), 22 mg tylosin/kg feed; Livingstone and Livingston (1968), 40 mg tylosin/kg feed; Yan et al. (2011), 44 mg tylosin/kg feed; Zhang et al. (2012), 500 mg tylosin/kg feed; Yan et al. (2012b), 1,000 mg tylosin/kg feed). Other three studies in weaned piglets showed that dietary tylosin supplementation at 100–110 mg/kg feed did not affect performance of piglets (Amachawadi et al. (2011), 100 mg tylosin/kg feed; Harvey et al. (1995) and Weber and Kerr (2008), 110 mg tylosin/kg feed).

In six studies in pigs for fattening, dietary tylosin supplementation at 22–1,000 mg/kg feed had growth-promoting/increase yield effects (NCR-89 Committee on Confinement Management of Swine (1986) and Hagsten et al. (1980), 22–44 mg tylosin/kg feed; Langlois et al. (1978) and Wang et al. (2009), 44 mg tylosin/kg feed; Yan et al. (2012a), 500 mg tylosin/kg feed; Wang et al. (2011), 1,000 mg tylosin/kg feed). In contrast, one study in pigs for fattening showed that dietary tylosin supplementation adversely affected feed utilisation of pigs (McCormick et al. (2017), 44 mg tylosin/kg feed).

The other 13 studies in pigs for fattening reported that dietary tylosin supplementation at 9.9–180 mg/kg feed did not affect performance of pigs (van Lunen (2003), 11–44 mg tylosin phosphate/kg feed, corresponding to 9.9–39.6 mg tylosin/kg feed; Holman and Chénier (2013), 11–44 mg tylosin/kg feed; Roth and Kirchgessner (1993) and Hansen and Larsen (1994), 20 mg tylosin/kg feed; Edwards et al. (2014), 20–40 mg tylosin/kg feed; Lowell et al. (2018), 40 mg tylosin phosphate/kg feed, corresponding to 36 mg tylosin/kg feed; Pilcher et al. (2013, 2015), 44 mg tylosin phosphate/kg feed, corresponding to 39.6 mg tylosin/kg feed; Brumm and Peo (1985), 44 mg tylosin/kg feed; Lindemann et al. (2010), 44.1 mg tylosin/kg feed; Kim et al. (2016), 45 mg tylosin/kg feed; Wu et al. (2019), 110 mg tylosin/kg feed; Hawe et al. (1992), 200 mg tylosin phosphate/kg feed, corresponding to 180 mg tylosin/kg feed).

3.3.2.4.3. Poultry

The nine studies considered as suitable for the assessment only covered two poultry production sectors: chicken for fattening (seven) and laying hens (two). In all studies, treatments contained groups of animals treated with only one tylosin concentration and did not allow assessment of any concentration-related effects.

In five studies in chickens, dietary tylosin supplementation at 50–200 mg/kg feed improved growth performance of chickens for fattening (Li et al. (2020), 50 mg tylosin/kg feed; Stutz and Lawton (1984), 55 mg tylosin/kg feed; Onifade et al. (1999), 75 mg tylosin/kg feed; Onifade and Babatunde (1997), 150 mg tylosin/kg feed; Li et al. (2015), 200 mg tylosin/kg feed). Two studies in chickens for fattening showed that dietary tylosin supplementation had no effect on the performance of chickens for fattening (Hughes et al. (2005), 15 mg tylosin/kg feed; Da Silva et al. (2019), 500 mg tylosin/kg feed).

In one study in laying hens, dietary tylosin supplementation improved performance of laying hens at 110 mg tylosin/kg feed (Wang and Kim (2011)). One study in laying hens showed that dietary tylosin supplementation had no effect on the performance of laying hens (Wu et al., 2008: 33 mg tylosin/kg feed).

3.3.2.5. Concluding remarks

It is judged 50–66% that tylosin has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 22 to 1,000 mg/kg feed (five studies) and in chickens for fattening at concentrations ranging from 50 to 200 mg/kg complete feed (five studies).

It is judged 33–66% certain ('about as likely as not') that tylosin has growth-promoting/increase yield effects in cattle for fattening at concentrations ranging from 9.9 to 11 mg/kg DM (three studies),

and in pigs for fattening at concentrations ranging from 22 to 1,000 mg/kg feed (six studies); and in laying hens at a concentration of 110 mg/kg complete feed (one study).

It is judged 33–66% certain ('about as likely as not') that tylosin has negative effects on performance of cattle for fattening at the concentration of 9.9 mg/kg DM (one study), on performance of lambs for fattening at the concentration of 10 mg/kg DM (one study) and on feed utilisation of pigs for fattening at the concentration of 44 mg/kg complete feed (one study).

No data are available in the scientific literature showing effects of tylosin on growth promotion/increased yield when added (i) to cattle for fattening feed at concentrations below 9.9 mg/kg DM, (ii) to weaned piglets feed at concentrations below 22 mg/kg (iii) to pigs for fattening feed at concentrations below 22 mg/kg, (iv) to chickens for fattening feed at concentrations below 50 mg/kg, (v) to laying hens at concentrations below 110 mg/kg or (vi) to feed of any other food-producing animal species or categories.

3.3.3. Tylvalosin

3.3.3.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the and the Virtual Issue), resulted in 22 publications mentioning tylvalosin and any of the food-producing animal species considered[3] and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of tylvalosin.[4] After removing the reports not matching the eligibility criteria, seven publications were identified.

3.3.3.2. Evaluation of the studies

The seven publications identified in the literature search were appraised for suitability for the assessment of the effects of tylvalosin on growth or yield of food-producing animals. This appraisal was performed by checking each study against a series of pre-defined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties', see also the Virtual Issue).[5] None of the publications were considered suitable for the assessment of the effects of tylvalosin on zootechnical performance because of several shortcomings identified in the design of the study or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.3 (Table A.3).

3.3.3.3. Concluding remark

Owing to the lack of suitable data, levels of tylvalosin in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

4. Conclusions

ToR1: to assess the specific concentrations of antimicrobials resulting from cross-contamination in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health.

AQ1. Which are the specific concentrations of tilmicosin, tylosin and tylvalosin in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/rumen?

- Due to the lack of data on the parameters required to calculate the Feed Antimicrobial Resistance Selection Concentration (FARSC) corresponding to the concentrations of those antimicrobials in non-target feed below which there would not be expected to be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health, it is not possible to conclude until further experimental data are available.

ToR2: **to assess which levels of the antimicrobials have a growth promotion/increase yield effect.**

AQ2: Which are the specific concentrations of tilmicosin, tylosin and tylvalosin in feed of food-producing animals that have an effect in terms of growth promotion/increased yield?

With regard to tilmicosin:

- It is judged 33–66% certain ('about as likely as not') that tilmicosin has growth-promoting/increase yield effects in pigs for fattening at the concentration of 400 mg/kg complete feed (one study).
- No data are available in the scientific literature showing effects of tilmicosin on growth promotion/increased yield when added (i) to pigs for fattening feed at concentrations below 400 mg/kg or (ii) to feed of any other food-producing animal species or categories.

With regard to tylosin:

- It is judged 33–66% certain that tylosin has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 22 to 1,000 mg/kg feed (five studies) and in chickens for fattening at concentrations ranging from 50 to 200 mg/kg complete feed (five studies).
- It is judged 33–66% certain ('about as likely as not') that tylosin has growth-promoting/increase yield effects in cattle for fattening at concentrations ranging from 9.9 to 11 mg/kg DM (three studies), and in pigs for fattening at concentrations ranging from 22 to 1,000 mg/kg feed (six studies) and in laying hens at a concentration of 110 mg/kg complete feed (one study).
- It is judged 33–66% certain ('about as likely as not') that tylosin has negative effects on performance of cattle for fattening at the concentration of 9.9 mg/kg DM (one study), on performance of lambs for fattening at the concentration of 10 mg/kg DM (one study), and on feed utilisation of pigs for fattening at the concentration of 44 mg/kg complete feed (one study).
- No data are available in the scientific literature showing the effect of tylosin on growth promotion/increased yield when added (i) to cattle for fattening feed at concentrations below 9.9 mg/kg DM, (ii) to weaned piglets feed at concentrations below 22 mg/kg, (iii) to pigs for fattening feed at concentrations below 22 mg/kg, (iv) to chickens for fattening feed at concentrations below 50 mg/kg, (v) to laying hens at concentrations below 110 mg/kg or (vi) to feed of any other food-producing animal species or categories.

With regard to tylvalosin:

- Owing to the lack of suitable data, levels of tylvalosin in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

The results from these assessments for the different animal species are summarised in Annex F (Tables F.1 and F.2) of EFSA BIOHAZ Panel, 2021a – Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).

5. Recommendation

To carry out studies to generate the data that are required to fill the gaps which have prevented calculation of the FARSC for tilmicosin, tylosin and tylvalosin.

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Yan L, Meng QW and Kim IH, 2012a. Effects of fermented garlic powder supplementation on growth performance, nutrient digestibility, blood characteristics and meat quality in growing-finishing pigs. Animal Science Journal, 83, 411–417. https://doi.org/10.1111/j.1740-0929.2011.00973.x

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Abbreviations

ADF	acid-detergent fibre
ADFI	average daily feed intake
ADG	average daily gain
ADI	acceptable daily intake
ALP	alkaline phosphatase
AQ	Assessment question
ATTD	calculate apparent total tract
bw	body weight used in toxicity studies
BW	body weight
CFU	colony forming unit
CP	crude protein
DDGS	dried distillers grains with solubles
DLWG	improved daily live weight gain
DM	dry matter
EUCAST	European Committee on Antimicrobial Susceptibility testing
F	fraction of the antimicrobial that is absorbed from the digestive tract to the blood
F:G	feed conversion ratio or feed to gain ratio
FARSC	Feed Antimicrobial Resistance Selection Concentration
FI	feed intake
G:F	Gain to feed ratio
GE	fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream
GGT	gamma glutamil transferase
HGT	horizontal gene transfer
I	fraction of the antimicrobial present in the digestive tracts that would be inactive on the microbiota
IgG	immunoglobulin G
MIC	minimum inhibitory concentration

MIC_{lowest}	minimum inhibitory concentration of the most susceptible species/strain included in the EUCAST database for a certain antimicrobial used to calculate the PMSC (see below)
MIC_{res}	minimum inhibitory concentration of the resistant strain
MIC_{susc}	minimum inhibitory concentration of the susceptible strain
MIC_{test}	minimum inhibitory concentration of the susceptible isolate used in the competition experiments to calculate the MSC
MSC	minimal selective concentration
N	nitrogen
NDF	neutral-detergent fibre
OM	organic matter
PK	pharmacokinetic
PMSC	predicted MSC
PRRSV	porcine reproductive and respiratory syndrome virus
rRNA	ribosomal ribonucleic acid
SID	standardised ileal digestibilities
TMR	total mixed ration
ToRs	Terms of Reference
TP	tylosin phosphate
TUA	tylosin urea adduct
VFA	volatile fatty acid

Appendix A – List of excluded publications and their shortcomings

A.1. Tilmicosin

The publications excluded from the assessment of the effects of tilmicosin growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) are summarised in Table A.1.

Table A.1: Publications not relevant for the assessment of the effects of tilmicosin on growth promotion/increased yield and excluding criteria

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Abell et al. (2017)	Ruminants									X(1)
Backstrom et al. (1994)	Pigs	X			X	X	X			
Bosi et al. (2011)	Pigs								X	
Booker et al. (2007)	Ruminants									X(2)
Charleston et al. (1998)	Poultry				X	X				
Chirase et al. (2004)	Ruminants			X	X					
Clark et al. (1998)	Pigs				X	X		X		
Dimitrova et al. (2019)	Pigs				X		X			
Dritz et al. (2002)	Pigs	X					X	X		
Duff et al. (2000)	Ruminants				X					X(3)

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Edrington et al. (2006)	Ruminants						X			
El-Ghany and Abd El-Gha (2009)	Poultry				X		X			
Fittipaldi et al. (2005)	Pigs				X		X			
Galyean et al. (1995)	Ruminants			X	X					
Garmyn et al. (2019)	Poultry				X		X		X	
Hoflack et al. (2001)	Pigs				X		X			
Kempf et al. (1997)	Poultry				X	X				
MacInnes et al. (2003)	Pigs				X	X				
Mateusen et al. (2001)	Pigs				X		X			
Moore et al. (1996a)	Pigs							X		
Moore et al. (1996b)	Pigs				X	X				
Nickell and White (2010)	Ruminants				X					X[4]
Nickell et al. (2008)	Ruminants			X	X					
Olson and Bäckström (2000)	Pigs				X	X	X			

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Pakpinyo et al. (2008)	Poultry				X	X		X		
Paradis et al. (2004b)	Pigs				X	X				
Rivera et al. (2018)	Ruminants						X			
Stipkovits et al. (2001)	Pigs	X			X	X		X		
van Donkersgoed and Merrill (2013a)	Ruminants			X	X					X(2)
van Donkersgoed and Merrill (2013b)	Ruminants			X	X					
Vandonkergoed (1992)	Ruminants									X(1)
Wallgren et al. (1999)	Pigs					X				
Weber et al. (2001)	Pigs	X								

(1): The article is a meta-analysis.
(2): Absence of a negative control.
(3): The design of this study was not appropriate to test performance/yield.
(4): The article is a review.

A.2. Tylosin

The publications excluded from the assessment of the effects of tylosin on growth/production yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) are summarised in Table A.2.

Table A.2: Publications not relevant for the assessment of the effects of tylosin on growth promotion/increased yield and excluding criteria

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Araujo et al. (2019)	Ruminants	X								
Backstrom et al. (1994)	Pigs	X			X	X	X			
Barbour et al. (2010)	Poultry	X								
Birkelo (2003)	Ruminants									X(1)
Bovera et al. (2009)	Rabbit	X			X		X			X(2)
Bovera et al. (2010)	Rabbit	X					X			X(2)
Bovera et al. (2012)	Rabbit	X								X(2)
Brennan et al. (2001)	Poultry				X	X				
Brumm et al. (2002)	Pigs	X								X(2)
Bruno et al. (2013)	Pigs	X				X				
Burrin et al. (1988)	Ruminants	X								
Catania et al. (2010)	Poultry				X		X			

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Cernicchiaro et al. (2016)	Ruminants	X								X(3)
Clary et al. (1993)	Ruminants	X								
Cooprider et al., 2011)	Ruminants	X								
Depenbusch et al. (2007)	Ruminants	X								
Depenbusch et al. (2008)	Ruminants	X								
Doornenbal and Frankham (1969)	Pigs	X								
Dritz et al. (1993)	Pigs	X							X	X(2)
Dritz et al. (2002)	Pigs	X					X	X		
Duff et al. (1994)	Ruminants	X								
Edmonds et al. (1985)	Pigs	X							X	
Elbayoumi et al. (2017)	Poultry						X		X(4)	
Elrefaey et al. (2013)	Poultry				X	X			X	
Evans et al. (2002	Poultry						X		X	
Faulkner et al. (2010)	Ruminants								X	X(5)

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Furusawa (2001)	Poultry				X				X	X(6)
Galyean et al. (1992)	Ruminants	X								
Garcés-Narro et al. (2013)	Poultry				X					
Garmyn et al. (2019)	Poultry				X		X		X	
Gates and Embry (1977)	Ruminants	X							X	
Gibb et al. (2008)	Ruminants	X								X(2)
Golder et al. (2014)	Ruminants	X					X			
Hamdy et al. (1982)	Poultry	X			X	X	X		X	
Harmon et al. (1987)	Ruminants	X								
Hilton et al. (2009)	Ruminants	X								
Hinz and Rottmann (1990)	Poultry				X	X			X	
Hong et al. (2012)	Pigs	X								
Hu and McDougald (2002)	Poultry				X	X				

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Ives et al. (2002)	Ruminants	X								
Jiao et al. (2017)	Ruminants	X								
Jones et al. (1976)	Poultry				X		X		X	
Jones et al. (2004)	Ruminants									
Jordan and Knight (1984)	Poultry				X	X			X	
Jordan et al. (1991)	Poultry				X	X				
Jordan et al. (1996)	Poultry				X	X			X	
Jordan et al. (1998)	Poultry				X	X				
Kempf et al. (1992)	Poultry				X	X			X	
Kirst et al. (1988)	Poultry			X(7)	X	X			X	
Koutoulis et al. (2013)	Poultry				X		X			
Lehel et al. (1995)	Poultry	X							X	
Maxwell et al. (2014)	Ruminants	X		X						X(5)
Maxwell et al. (2015)	Ruminants	X								X(8)

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Mercadante et al. (2015)	Ruminants	X								
Meyer et al. (2009)	Ruminants	X								
Meyer et al. (2013)	Ruminants	X								
Migaki et al. (1993)	Poultry				X	X			X	
Montgomery et al. (2009)	Ruminants	X								X(9)
Morris et al. (1990)	Ruminants	X								
Müller et al. (2018)	Ruminants	X								
Nagaraja and Chengappa (1998)	Ruminants									X(1)
Nerem et al. (2013)	Pigs					X	X			X(8)
Onifade (1997)	Poultry									X(10)
Ose and Tonkinson (1985)	Poultry				X	X			X	
Ose et al. (1979)	Poultry					X				
Paradis et al. (2004a)	Pigs				X	X				
Petrov (2006)	Pigs									

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Piccolo et al. (2009)	Rabbit	X							X	X(2)
Pommier et al. (2008)	Pigs						X		X	X(8)
Ran et al. (2018)	Ruminants	X								
Ridgway and Ryden (1966)	Poultry					X			X	
Rozeboom et al. (2005)	Pigs	X								
Rząsa et al. (2007)	Pigs	X							X	
Sacristán et al. (2012)	Pigs						X		X	
Salaheen et al. (2017)	Poultry	X								
Sandhu and Dean (1980)	Poultry	X				X		X		
Scott et al. (2017)	Ruminants	X								X(2)
Shen et al. (2018)	Ruminants	X								
Shryock et al. (1998)	Pigs					X				
Sides et al. (2009)	Ruminants	X								X(2)
Stackhouse-Lawson et al. (2013)	Ruminants	X								

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Stipkovits et al. (1977)	Poultry				X		X			X(2)
Stock et al. (1995)	Poultry	X								
Sullivan et al. (1965)	Poultry								X	
Tan et al. (1994)	Ruminants	X				X				
Tanner et al. (1993)	Poultry				X	X				
Thomas et al. (2017)	Ruminants	X								
Tsinas et al. (1998)	Pigs						X			
Tzika et al. (2009)	Pigs						X			
Ueda et al. (1994)	Pigs					X				
Veenhuizen et al. (1998)	Pigs				X		X			
Veum et al. (1980)	Pigs	X								
Vicca et al. (2005)	Pigs				X	X				
Visscher et al., 2018)	Pigs				X	X				
Wahlstrom (1970)	Pigs	X								

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Weber et al. (2001)	Pigs	X								
Wei et al. (2019)	Ruminants	X								
Wilson et al. (2018)	Ruminants								X	
Winterholler et al. (2008)	Ruminants	X								
Yan et al. (2012a,b,c)	Pigs								X	
Zinn (1987)	Ruminants	X								

(1): The publication is a literature review.
(2): Absence of a negative control.
(3): The study is a meta-analysis.
(4): Undefined concentration of tylosin in water.
(5): Additional tylosin-containing hormone implant.
(6): Designed to study the transfer of antibiotics to eggs.
(7): Administration by oral gavage.
(8): Additional therapeutic antibiotic administration.
(9): The adaptation period included tylosin in all experimental diets.
(10): For the purpose of this report, the study is considered a repetition of that of Onifade and Babatunde (1997).

A.3. Tylvalosin

The publications excluded from the assessment of the effects of tylvalosin on growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) are summarised in Table A.3.

Table A.3: Publications not relevant for the assessment of the effects of tylvalosin on growth promotion/increased yield and excluding criteria

Author (year)	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different from oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Forrester et al. (2011)	Poultry				X	X				
Guedes et al. (2009)	Pigs				X					
Garcés-Narro et al. (2013)	Poultry				X					X(1)
Pallarés et al. (2015)	Pigs				X		X		X	
Pommier et al. (2008)	Pigs						X		X	X(2)
Vyt et al. (2012)	Pigs				X				X	
Zhang et al. (2019)	Pigs						X		X	

(1): Tylvalosin was administered for only 2 days via water with the aim to decrease *Clostridium perfringens* in the intestinal tract
(2): Additional therapeutic antibiotic administration

Appendix B – Table of uncertainties

Uncertainties associated with the Growth promotion assessment

Table B.1: Potential sources of uncertainty identified in the levels of tilmicosin in feed which have growth promotion/increase yield effect and assessment of the impact that these uncertainties could have on the conclusion

Source of the uncertainty	Nature or cause of uncertainty	Impact of the uncertainty on the conclusion on the level(s) which have growth promotion/increase yield effect
Form(s) of antimicrobial used	The specific form of the antimicrobial used in the study (as the '(free) base' substance, its salts or specific products/formulations containing the base substance) has not been clearly described in several publications. In summarising the results, the concentrations have been reported as for 'base' substance when the form of the antimicrobial is not specified (conservative assumption).	Underestimation of the concentration which may have shown growth-promoting effect.
Evidence synthesis and integration	As described in Section 2.2.3 of the Scientific Opinion Part 1 (see also the Virtual Issue), the low number of studies retrieved prevented evidence synthesis.	Underestimation/Overestimation

Table B.2: Potential sources of uncertainty identified in the levels of tylosin in feed which have growth promotion/increase yield effect and assessment of the impact that these uncertainties could have on the conclusion

Source of the uncertainty	Nature or cause of uncertainty	Impact of the uncertainty on the conclusion on the level(s) which have growth promotion/increase yield effect
Form(s) of antimicrobial used	The specific form of the antimicrobial used in the study (as the '(free) base' substance, its salts or specific products/formulations containing the base substance) has not been clearly described in several publications. In summarising the results, the concentrations have been reported as for 'base' substance when the form of the antimicrobial is not specified (conservative assumption).	Underestimation of the concentration which may have shown growth-promoting effect.
Evidence synthesis and integration	As described in Section 2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue), although meta-analysis was not applicable to the studies retrieved, evidence synthesis was done, since: • 3 studies showing consistent (positive) results in a comparable range of concentrations were available in cattle for fattening. The uncertainty resulting in the process of evidence synthesis was based on 6 studies, 3 showing positive effect, 2 showing no effects and 1 showing negative effects; • 5 studies showing consistent (positive) results in a comparable range of concentrations were available in weaned piglets. The uncertainty resulting in the process of evidence synthesis was based	The extent of the underestimation or overestimation on the levels which shown growth-promoting effect is modulated by the consistency of the results.

Source of the uncertainty	Nature or cause of uncertainty	Impact of the uncertainty on the conclusion on the level(s) which have growth promotion/increase yield effect
	on 17 studies, 5 showing positive effect and 2 showing no effects; • 6 studies showing consistent (positive) results in a comparable range of concentrations were available in pigs for fattening. The uncertainty resulting in the process of evidence synthesis was based on 20 studies, 6 showing positive effect, 13 showing no effects and 1 showing negative effects; • 5 studies showing consistent (positive) results in a comparable range of concentrations were available in chickens for fattening. The uncertainty resulting in the process of evidence synthesis was based on 7 studies, 5 showing positive effect and 2 showing no effects. For laying hens, the low number of studies retrieved prevented evidence synthesis.	