SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 15 September 2021

doi: 10.2903/j.efsa.2021.6864

Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 12: *Tetracyclines: tetracycline, chlortetracycline, oxytetracycline, and doxycycline*

EFSA Panel on Biological Hazards (BIOHAZ),
Konstantinos Koutsoumanis, Ana Allende, Avelino Alvarez-Ordóñez, Declan Bolton, Sara Bover-Cid, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Roland Lindqvist, Maarten Nauta, Giuseppe Ru, Marion Simmons, Panagiotis Skandamis, Elisabetta Suffredini, Dan I Andersson, Vasileios Bampidis, Johan Bengtsson-Palme, Damien Bouchard, Aude Ferran, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Søren Saxmose Nielsen, Alena Pechová, Mariana Petkova, Sebastien Girault, Alessandro Broglia, Beatriz Guerra, Matteo Lorenzo Innocenti, Ernesto Liébana, Gloria López-Gálvez, Paola Manini, Pietro Stella and Luisa Peixe

Abstract

The specific concentrations of tetracycline, chlortetracycline, oxytetracycline and doxycycline 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. The FARSC for these four tetracyclines was estimated. 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 tetracycline, chlortetracycline, oxytetracycline, whilst for doxycycline no suitable data for the assessment were available. Uncertainties and data gaps associated with the levels reported were addressed. It was recommended to perform further studies to supply more diverse and complete data related to the requirements for calculation of the FARSC for these antimicrobials.

Keywords: tetracycline, chlortetracycline, oxytetracycline, doxycycline, antimicrobial resistance, growth promotion, food-producing animals

Requestor: European Commission

Question number: EFSA-Q-2021-00512

Correspondence: biohaz@efsa.europa.eu

Panel members: Ana Allende, Avelino Alvarez-Ordóñez, Declan Bolton, Sara Bover-Cid, Marianne Chemaly, Robert Davies, Alessandra De Cesare, Lieve Herman, Friederike Hilbert, Konstantinos Koutsoumanis, Roland Lindqvist, Maarten Nauta, Luisa Peixe, Giuseppe Ru, Marion Simmons, Panagiotis Skandamis and Elisabetta Suffredini.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The BIOHAZ Panel, leading Panel in charge of the adoption of the scientific opinion and assessment of Term of Reference 1 (ToR1, antimicrobial resistance) wishes to thank the following for the support provided to this scientific output: EFSA Panel on Animal Health and Welfare (AHAW Panel), who supported ToR1 assessments development and endorsement of those sections under their remit (animal production, main use of antimicrobials); EFSA Panel for Additives and Products or Substances used in Animal Feed (FEEDAP), in charge of the assessment and endorsement of ToR2 and providing advice and data needed for ToR1 assessments; European Medicines Agency (EMA), who was represented by an external expert and EMA secretariat as members of the Working Group (WG); Valeria Bortolaia, who was member of the WG until 17 April 2020; EFSA staff members: Angelica Amaduzzi, Gina Cioacata, Pilar García-Vello, Michaela Hempen, Rita Navarrete, Daniel Plaza and Anita Radovnikovic; EMA staff members: Barbara Freischem, Zoltan Kunsagi, Nicholas Jarrett, Jordi Torren and Julia Fábrega (currently EFSA staff). The BIOHAZ Panel wishes also to acknowledge the EMA Committee for Medicinal Products for Veterinary Use (CVMP) and their experts.

Suggested citation: EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021. Scientific Opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 12: *Tetracyclines: tetracycline, chlortetracycline, oxytetracycline, and doxycycline*. EFSA Journal 2021;19(10):6864, 116 pp. https://doi.org/10.2903/j.efsa.2021.6864

ISSN: 1831-4732

Table of contents

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 also 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 tetracyclines: tetracycline, chlortetracycline, oxytetracycline, and doxycycline assessments.

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 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

The tetracyclines are a class of antimicrobials first described in the late 1940s. Tetracycline, chlortetracycline and oxytetracycline were among the first substances described within this class. They are natural products of different *Streptomyces* spp. bacteria and are usually referred to as first-generation tetracyclines. The second-generation tetracyclines (e.g. doxycycline) are mainly the products of semisynthetic approaches with increased lipophilicity compared to the first-generation tetracyclines. The third- (tigecycline) and fourth- (eravacycline, omadacycline) generation tetracyclines are obtained from total synthesis and were specifically designed to overcome common mechanisms of tetracycline resistance (Agwuh and MacGowan, 2006; Greer, 2006; Fuoco, 2012; Grossman, 2016). Most tetracyclines target the ribosomal complex. Once inside the bacterial cell, they bind reversibly to the 16S rRNA of 30S ribosomal subunit, blocking protein synthesis by preventing the accommodation of incoming aminoacyl-tRNAs at the acceptor site (A-site) (Chopra and Roberts, 2001; Wilson, 2009). These tetracyclines are bacteriostatic when administered at therapeutic concentrations (Nelson and Levy, 2011). The most lipophilic tetracyclines have a bactericidal mechanism of action that relies on membrane perturbation (Nelson and Levy, 2011).

The spectrum of activity and minimum inhibitory concentration (MIC) values of tetracycline, chlortetracycline and oxytetracycline is similar (EMEA/CVMP, 1995). The third- and fourth-generation

[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.

products have higher activity (lower MIC). Tetracycline, chlortetracycline and oxytetracycline will be addressed jointly in the framework of this scientific opinion. Conversely, doxycycline is analysed separately due to its specific pharmacokinetics (PK) and antimicrobial activity.

1.3.2. Main use[2]

Tetracyclines are first-line drugs in food-producing animals, including aquatic animal species. They are broad-spectrum antimicrobials, acting against Gram-positive and Gram-negative bacteria, mycoplasma and several protozoans. The main indication for tetracycline in food-producing animals is the treatment of respiratory infections in cattle (*Pasteurella multocida, Mannheimia haemolytica, Mycoplasma* spp.), swine (*Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae, Mycoplasma* spp.) and poultry (*Pasteurella multocida, Mycoplasma gallisepticum, Mycoplasma synoviae* and secondary bacterial infections). Other treated infections include chlamydiosis, *Lawsonia* proliferative enteropathy, rickettsiosis and salmonellosis (Agunos et al., 2013; Giguère et al., 2013; Riviere and Papich, 2017). Tetracycline, chlortetracycline and oxytetracycline are commonly used notably as oral formulations (feed, water, oral doser, bolus medications) for all the food-producing species in Europe, as well as intramammary, intrauterine (mainly bovine) and topical applications.

Tetracycline is the representative molecule for first generation tetracyclines used in susceptibility testing assays, because it is more stable than chlortetracycline and oxytetracycline molecules in culture media.

The broad-spectrum, the low cost, the ease of administration *per os* and the general effectiveness led to the first-generation tetracyclines being widely used in food-producing animals. However, they had the drawback of rapid selection for, and emergence of (mainly) transferable resistance (Giguère et al., 2013; Riviere and Papich, 2017) in multiple pathogens and commensal organisms.

1.3.3. Main pharmacokinetic data

1.3.3.1. Main pharmacokinetic data for tetracycline, chlortetracycline and oxytetracycline

The bioavailability (i.e. the fraction of the antimicrobials absorbed from the digestive tract to the plasma) of the tetracyclines tetracycline, chlortetracycline and oxytetracycline after oral administration in non-fasted animals is generally very low.

The oral bioavailability of oxytetracycline in fed animals has been reported as 5% (Luthman and Jacobsson, 1983) to 46% (in milk replacer) in calves (Schifferli et al., 1982), 3–5% in pigs (Decundo et al., 2019), 3% in rainbow trout (Rogstad et al., 1991), 2% in Atlantic salmon (Elema et al., 1996), 6% in chickens (Ziółkowski et al., 2019) and 9% in turkeys (Dyer, 1989).

The oral bioavailability of chlortetracycline in non-fasted animals was reported as 37% in calves (Luthman and Jacobsson, 1983), 6% (Nielsen and Gyrd-Hansen, 1996), 13% (Wanner et al., 1991), 18% (Kilroy et al., 1990) or 25% in pigs (Riviere and Papich, 2017), 1% (Riviere and Papich, 2017) to 18% in chicken (Anadón et al., 2012) and 6% in turkeys (Pollet et al., 1985).

The oral bioavailability of tetracycline in fed animals ranged from 5% (Nielsen and Gyrd-Hansen, 1996) to 23% in pigs (Kniffen et al., 1989). In rabbits, the bioavailability was described as very low, but no value was provided (Percy and Black, 1988).

These percentages of bioavailability can be reduced by complexation with multivalent cations that precipitate with increasing pH, or by feed particles. In contrast, water and feed acidifiers improve the release and absorption of tetracyclines from medicated feeds in pigs, without leading to high bioavailability.

These results reveal a bioavailability ranging from 1% to 46% among animal species and suggest that the remaining fraction of the dose in the digestive tract (from around 50% to 99%) would pass through the distal part of the digestive tract, before being eliminated in faeces, and be available to microorganisms after consumption of contaminated feed.

For the fraction of the antimicrobial absorbed (1–46% of the dose depending on the antimicrobial and on the species), the elimination occurs mainly by glomerular filtration resulting in the excretion in urine and finally in the environment. Tetracyclines can also be partly excreted in bile and are recycled

[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.

back to the intestinal tract (especially for the most lipid soluble antimicrobials), but this intestinal elimination has, based on PK calculations, a very small influence on the already high intestinal concentrations resulting from the low level of systemic absorption of most tetracyclines after oral administration.

Chelation of tetracyclines by polyvalent metallic cations was described many years ago to explain decreased bioavailability of the antimicrobial after oral administration. In man, the plasma concentration obtained after the oral administration of 500 mg of tetracycline hydrochloride was 30% lower when administered simultaneously with zinc sulfate (45 mg Zn^{2+}) (Penttilä et al., 1975), and, similarly, the oxytetracycline plasma concentrations obtained after the administration of 500 mg oxytetracycline to human subjects were 50–60% lower when administered simultaneously with ferrous sulfate (200 mg) (Neuvonen et al., 1970). However, these results, although suggesting that chelates are not absorbable, did not directly prove that bound tetracyclines remained inactive in the gut.

A recent study from Ahn et al. (2018) provided detailed information on the activity of tetracycline in the human gut in the context of the determination of the microbiological acceptable daily intake (mADI). This study demonstrated by means of *in vitro* experiments that, in human faecal slurries proposed to be representative of colon content, only 41% of chelated tetracyclines would remain active because of extensive binding. No information was provided regarding the molecules to which tetracyclines were bound; therefore, there are uncertainties associated with extrapolating this finding to animals, as the animal gut content may show different binding properties than human material and may vary within groups of animals according to their age and diet.

For tetracycline, the optimal pH is around 6–7 (Maurin and Raoult, 2001) and, thus, the influence of the pH at intestinal concentrations was not considered in the calculations of FARSC.

1.3.3.2. Main pharmacokinetic data for doxycycline

Doxycycline is a semi-synthetic derivative of oxytetracycline and is more lipophilic than first-generation tetracyclines. There are several formulations containing doxycycline hyclate for food-producing animals that can be mixed to feed or drinking water. Doxycycline is not intended for use in dairy cows and laying hens, as no maximum residue limits (MRLs) are available (EMEA/CVMP, 1997).

The bioavailability of doxycycline after oral administration in fed animals is higher than chlortetracycline and oxytetracycline but remains low in most animal species.

The average oral bioavailability of doxycycline has been reported as 70% in fed veal calves (Meijer et al., 1993), 36% in fasted adult sheep (Castro et al., 2009), 21% in unfasted pigs (Baert et al., 2000), 41% in fasted chickens (Anadón et al., 1994), 25–63% in fasted turkeys (Santos et al., 1996), 6% after top dressing application in unfasted horses (Winther et al., 2011), 23.4% in tilapia (Yang et al., 2014) and 43.8% in channel catfish (Xu et al., 2020).

These results suggest that 30–94% of the dose would pass through the digestive tract and be available to microorganisms after consumption of contaminated feed. In a study of Peeters et al. (2016), in which six pigs were administered with feed containing doxycycline with 3% carry-over corresponding to 6.76 mg doxycycline/kg feed for 10 days, very high concentrations of doxycycline ranging from 1 to 6 mg/kg were found in caeca and colonic digesta. The mean concentration in faeces from day 4 to 10 was around 4 mg/kg faeces corresponding to 60% of the concentration in feed.

Doxycycline is distinguished from the other tetracyclines by its high rate of elimination through secretion through the intestinal wall (Riviere and Papich, 2017). In pigs and cattle, doxycycline was described as not transformed (Riond et al., 1989, Riond and Riviere, 1990). However, a report from EMA suggested that doxycycline may be metabolised by up to 40% and be largely excreted in faeces probably in a microbiologically inactive form (EMEA/CVMP, 1997). This assumption comes from an old article on the disposition of doxycycline in humans and dogs (Schach Von Wittenau and Twomey, 1971) demonstrating that oral doxycycline was well absorbed from the digestive tract, while the doxycycline excreted via intestines seemed not to be reabsorbed. They also observed that formic acid was needed to recover doxycycline from faeces, suggesting that doxycycline could be conjugated or included in a stable complex in faeces. The conclusion of the article was that the reasons for the apparent unavailability of much of the doxycycline eliminated with faeces was not clear at that time and we could not find other data published since then.

1.3.4. Main resistance mechanisms

Several mechanisms of tetracycline resistance have been described in bacteria, including increased efflux, reduced uptake, ribosomal protection and enzymatic inactivation (Chopra and Roberts, 2001;

Grossman, 2016). Tetracycline resistance can be mediated by acquisition of resistance genes and upregulation and/or mutation of intrinsic genes (Grossman, 2016). Tetracycline resistance is widespread in bacteria and has been described in at least 49 Gram-positive and 85 Gram-negative genera (https://faculty.washington.edu/marilynr/). Increased tetracycline efflux may be mediated by changes in the expression of intrinsic transcriptional activators and two-component systems and by horizontal acquisition of genes, as described both in Gram-negative and in Gram-positive bacteria. Various compounds including tetracyclines can modulate the expression of transcriptional regulators and two-component systems leading not only to tetracycline resistance, but to a multidrug resistance phenotype (Grossman, 2016). Regarding acquired genes, more than 30 genes carried on transposons or plasmids have been described. Intracellular accumulation of tetracyclines can also be controlled by reducing tetracycline uptake, which is a resistance mechanism described in association with modulation of expression of intrinsic genes in Gram-negative bacteria (Grossman, 2016). Ribosomal protection may be mediated by at least 14 genes and 11 mosaic genes, and such resistance has been described both in Gram-positive and in Gram-negative species. Most ribosomal protection genes mediating tetracycline resistance are carried on plasmids and a few have also been detected in the chromosome. Recently, a gene conferring resistance to tetracyclines, phenicols and oxazolidinones was described (Antonelli et al., 2018). Enzymatic inactivation may be mediated by at least 13 genes, which have been described in Gram-negative bacteria and, for the majority, in uncultured bacteria. These genes have been identified both in transposable elements, plasmids and in the chromosome, but for the majority the genomic location remains unidentified. The possibility of also inactivating the fourth-generation tetracyclines, as occurs with TetX, already described in several clinically relevant Gram-negative bacteria is of special concern (Yang et al., 2004). Finally, a gene mediating tetracycline resistance by an unknown mechanism has been described in Gram-positive bacteria, although its role in tetracycline resistance is controversial (Caryl et al., 2012; Roberts and Schwarz, 2016). Notably, new tetracycline resistance genes are continuously being discovered.

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 tetracyclines including tetracycline, chlortetracycline, oxytetracycline and doxycycline: examples

Several publications have demonstrated the development and selection of resistance to tetracyclines due to the use of these antimicrobials at low concentrations, especially in animal feed. Similarly, sub-MIC of tetracyclines may significantly increase the conjugation transfer frequency (*in vitro*, in the animal gut and in moist/wet feed or the environment), which could also indirectly cause an increase in tetracycline resistance. Finally, tetracyclines can also have effects on the virulence properties of bacteria, which potentially could increase spread of resistant clones. A subset of several relevant studies are briefly summarised below with regard to their main findings.

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

- In a competition experiment in defined laboratory growth medium between a tetracycline susceptible and resistant (due to the presence of a Tn*10* element) *S. enterica*, the minimal selective concentration (MSC) for tetracycline was determined to 15 μg/L, which is 100-fold below the MIC of the susceptible strain (Gullberg et al., 2011).

- Tetracycline (0.01 mg/L) increased the relative abundance of tetracycline-resistant bacteria in *in vitro* experiments representing biofilms of complex aquatic bacterial communities. In the same study, it was shown that tetracycline (0.001 and 0.01 mg/L) selected for *tet*(A) and *tet* (G). Furthermore, despite not affecting the overall taxonomic diversity, tetracycline (0.001 and 0.01 mg/L) had an effect on specific genera (Lundström et al., 2016).
- Exposure of *P. aeruginosa* to 1/10 MIC of tetracycline resulted in selection for resistance in a few hundred generations of serial passage (Chow et al., 2015).
- Tetracycline (1, 10 and 100 mg/L) administered via drinking water to human microbiota-associated mice selected for several tetracycline-resistant bacteria including Gram-positive anaerobes, *Bacteroides fragilis*, enterobacteria and enterococci, whereas it was not possible to conclude on the effect of tetracycline on selection of resistant lactobacilli, bifidobacteria and clostridia (Perrin-Guyomard et al., 2001).
- In *in vitro* experiments with a tetracycline susceptible (MIC = 4 μg/mL) *E. coli* strain, it was shown that at tetracycline concentration between 0.0075 and 0.06 μg/mL, there was an increase in bacterial counts as compared to the control without tetracycline (Migliore et al., 2013). This is an example of hormesis, i.e. a biphasic dose–response relationship that occurs when low concentrations of toxic agents elicit apparent improvements in growth.
- The effects of 0.15, 1.5, 15 and 150 mg/L of tetracycline, after 24 h and 40 days of exposure, in 3% human faecal suspensions, collected from three individuals were investigated using *in vitro* batch cultures. The evaluation of bacterial community changes at the genus level, from control to tetracycline-treated faecal samples, suggested that tetracycline (of 0.15 mg/L or above) under the conditions of the study could lead to slight differences in the composition of the intestinal microbiota. Twenty-three resistance genes were screened, being four *tet* genes (*tet*O, Q, W and X) major in control and tetracycline-dosed faecal samples. A variable or slight increase of copy number of *tet* genes was identified and appeared to be related to tetracycline treatment, inter-individual variability and duration of exposure (Jung et al., 2018).
- Benthic denitrification rates and bacterial communities were examined during continuous exposure to tetracycline at 0.5, 20 and 10,000 μg/L for 2 weeks in flow-through reactors. Denitrification rates were unaffected by exposure to tetracycline. In contrast, the bacterial community composition changed significantly during exposure from subinhibitory (ng-μg/L) to therapeutic (mg/L) concentrations (Roose-Amsaleg et al., 2013).
- Veal calves received therapeutic oral dosages of 1 g oxytetracycline, twice per day, during 5 days (referred to as oxytetracycline-high) or 100–200 μg per day during 7 weeks (referred to as oxytetracycline-low), mimicking animal exposure to environmental contamination. The temporal effects on the gut microbiota and antimicrobial resistance gene abundance were analysed by metagenomic sequencing. Oxytetracycline-high had a transient effect, significantly impacting gut microbiota composition between day 0 and day 2. Metagenomic sequence analysis showed that six antimicrobial resistance genes representing three gene classes (*tet* (M), *floR* and *mel*) were increased in relative abundance in the oxytetracycline-high group, but no increase was seen in oxytetracycline-low (Keijser et al., 2019).

3.1.1.2. Effects of Sub-MIC concentrations on horizontal gene transfer and virulence

- Tetracycline (0.01 mg/L) increased horizontal transfer of resistance (including ampicillin, chloramphenicol, ciprofloxacin, gentamicin, streptomycin and/or tetracycline resistance) from a complex donor community (treated effluent from a sewage treatment plant) to a recipient *E. coli* strain in *in vitro* experiments (Jutkina et al., 2016).
- Tetracycline (20 mg/L) increased the expression levels of genes involved in the conjugative transfer of a trimethoprim, sulfonamide and tetracycline resistance plasmid from *Aeromonas hydrophila*, which is expected to result in increased conjugation frequency in *Aeromonas hydrophila* (Cantas et al., 2012).
- In *in vivo* experiments to study the transfer of Tn*916* (a conjugative, tetracycline resistance transposon) from *E. faecalis*, administration of tetracycline (5, 10 and 50 mg/L) to mice resulted in significantly higher numbers of transconjugants (measured as colony forming unit (CFU)/g of faeces) compared to the untreated controls. No significant difference in number of transconjugants among the mice receiving 5, 10 or 50 mg/L of tetracycline was observed (Bahl et al., 2004).

- *E. coli* transconjugants acquiring a tetracycline resistance plasmids from an *E. coli* donor increased significantly in the gut of mice administered 0.1 g/L of tetracycline in drinking water as compared to the number of transconjugants in the gut of mice receiving 0, 0.01 or 0.2 g/L of tetracycline. This was likely due to a selective advantage of the transconjugants as colonisers in the presence of low but non-negligible amounts of tetracycline, which led the authors to hypothesise that there is an optimal 'window' between the highest and the lowest tetracycline doses tested that allows the establishment of transconjugants in the intestine (Licht et al., 2003).
- Addition of tetracycline (0.01 g/L) in drinking water of chickens increased the transfer of tetracycline resistance between chicken-origin *E. coli* in an *in vivo* chicken model (Hart et al., 2006).
- Stimulation (40- to 2,300-fold) of transfer of conjugative transposon Tn*916* in *E. faecalis* by subinhibitory levels of several antimicrobials, including tetracycline and doxycycline. For tetracycline, maximum transfer frequency at 20 mg/L and for doxycycline at 2 mg/L. However, stimulatory effects start at 0.1 mg/L and 0.01 mg/L, respectively (Scornec et al., 2017).
- Up to 10^6-fold stimulation of transfer of conjugative transposon CTnDOT in *Bacteroides* at 1 mg/L of tetracycline (Whittle et al., 2002).
- Sub-MIC levels of doxycycline and oxytetracycline (1 mg/L) induced *htp*G gene (encodes a virulence factor) in *S.* Typhimurium and increased virulence in mouse model (200 mg/L in drinking water, *in vivo* concentration unknown) (Verbrugghe et al., 2016).
- Sub-MIC levels of tetracycline (1 mg/L) induced the T3SS in cytotoxic *P. aeruginosa* and thus, enhanced the cytotoxic effect on macrophages (cell line) fourfold (Linares et al., 2006).

In summary, several different studies show that sub-MIC concentrations of tetracyclines 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, alterations in composition of bacterial communities, increased horizontal gene transfer and increased bacterial virulence, all of which could potentially contribute to an increase in both the number of resistance genes (fraction of bacteria that are resistant) and the level of resistance (MIC value of the resistant cells) in a microbial population. With regard to the concentrations of tetracyclines where the biological effects are observed, the concentration for resistance enrichment and selection appears to be the lowest (0.001 and 0.015 mg/L depending on experimental set-up), whereas effects on horizontal gene transfer occur in the 0.01–1 mg/L range and virulence effects are seen at even higher levels (mg/L).

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)

3.2.1. Tetracycline, chlortetracycline and oxytetracycline FARSC determination

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 this value for these three tetracyclines for different animal species followed a two-step approach as described below.

The first step was the calculation of the predicted minimal selective concentration (PMSC) for tetracycline (representative of the other substances) as indicated in Table 1.

In this case, for tetracycline, resistance selection was performed in defined environments with a single species (*S. enterica* var Typhimurium LT2). The minimal selective concentration (MSC) determined from competition experiment between wild type (wt) and a *tetA* (tetracycline resistance) mutant was 100-fold below MIC of wt (MIC_{test} = 1.5 mg/L and MSC_{test} = 0.015 mg/L (Gullberg et al., 2011). Accordingly, the ratio MIC_{test}/MSC_{test} was 100 (Table 1).

The PMSC for tetracycline, calculated using the lowest MIC value available in the EUCAST MIC distribution database (MIC_{lowest}), divided by the MIC_{test}/MSC_{test} factor (as described in 2.2.3.2 of the Scientific Opinion Part 1; see also the Virtual Issue), was 0.00016 mg/L (Table 1).

Table 1: Calculation of the tetracycline 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 ($MIC_{lowest}/MIC_{test}/MSC_{test}$)
Tetracycline	1.5 (*S. enterica*)	0.015 (*S. enterica*)	100	0.016	0.00016

MIC: minimum inhibitory concentration; MSC: minimal selective concentration; MSC_{test}: MSC experimentally determined; MIC_{lowest}: lowest MIC data for tetracycline 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). (EUCAST database https://mic.eucast.org/search/ last accessed 15 May 2021). NA: not available.

From the PMSC, the FARSC ($FARSC_{intestine}$ and $FARSC_{rumen}$) corresponding to the maximal concentrations in feed were 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 tetracycline:

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

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

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.

A recent publication of Ahn et al. (2018) estimated that the binding of tetracyclines was 56.9 ± 9.1% and 58.2 ± 10.8% in 25% (w/v) human faecal slurries spiked with 0.15 and 1.5 μg/mL tetracycline, respectively. Therefore, I for tetracyclines was set to 0.5. Due to the lack of information for animal species and to the possibility that tetracycline chelates to intestinal contents, other simulations were performed with I equal to 0.2 and 0.8.

From the publications cited above, F for tetracycline was set to 0.3 for young ruminants and 0.1 for pigs, poultry, horses and rabbits. No value of F was found for adult ruminants and so, no FARSC was provided for these species. Due to the large range of the bioavailability reported in the literature, other additional simulations (named 'scenario' in Table 2) were performed with values ranging from 0.01 to 0.5 depending on the species. Since GE is multiplied by F, its influence would systematically be very low when F is low. So, GE was set to 0.

The different selected values of F and I for the calculations of FARSC are summarised in Table 2. There is no value for the bioavailability in adult ruminants, horses and rabbits. The first set of values (scenario 1) corresponds to the average of published values, while scenario 2 corresponds to scenario that would lead to lower FARSC and scenario 3 to scenario that would lead to higher FARSC. The lowest FARSC (scenario 2) was obtained with lowest published values of I (lower inactivation of the drug resulting in higher activity on bacteria) and lowest published values of F (lower absorption resulting in more drug in the intestines). The estimated $FARSC_{intestine}$ values obtained with these three different set of values (= scenario) for the parameters are reported in Table 3.

Table 2: Predicted minimal selective concentration (PMSC) and pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration ($FARSC_{intestine}$) of tetracycline for the different animal species

Tetracycline data	Scenario #1	Scenario #2	Scenario #3
PMSC (mg/L)		0.00016	
Inactive fraction (*I*)	0.5	0.2	0.8
Bioavailability (*F*) veal calves	0.3	0.05	0.5
Bioavailability (*F*) pig	0.1	0.03	0.25
Bioavailability (*F*) poultry	0.1	0.01	0.2
Bioavailability (*F*) salmon	0.02	0.02	0.02
Gastrointestinal elimination (*GE*)	0	0	0

PMSC: Predicted minimal selective concentration (PMSC). 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. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to ($1 - F$). 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 blood stream.

Table 3: The Feed Antimicrobial Resistance Selection Concentration of tetracycline (representing chlortetracycline and oxytetracycline) corresponding to the maximum concentration of residues in non-target feed that would not develop resistance in the large intestine bacteria ($FARSC_{intestine}$)

Animal category[(a)]	Body weight (kg)[(a)]	Daily Feed Intake (kg DM/animal per day)[(a)]	Daily output of fresh faeces (kg FM/animal per day)[(b)]	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 1	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 2	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 3
Sow lactating	175	5.28	7.7	0.52	0.30	1.56
Piglet (weaned)	20	0.88	0.88	0.36	0.21	1.07
Pig for fattening	60	2.2	2.64	0.43	0.25	1.28
Veal calf (milk replacer)	100	1.89	2.36	0.57	0.26	**2.00**
Dairy cows	650	20	55.71	–	–	–
Cattle for fattening	400	8	18.89	–	–	–
Goat (adult)	60	1.2	1.73	–	–	–
Sheep (adult)	60	1.2	1.47	–	–	–
Chicken for fattening	2	0.158	0.133	0.30	0.17	0.84
Laying hen	2	0.106	0.16	0.54	0.30	1.51
Turkey for fattening	3	0.176	0.109	0.22	**0.13**	0.62
Horse	400	8	8.33	–	–	–
Rabbit	2	0.1	0.053	–	–	–
Salmon	0.12	0.0021	0.00238	0.37	0.23	0.93

DM: dry matter: FM: faecal matter; FARSC: Feed Antimicrobial Resistance Selection Concentration.
(a): EFSA FEEDAP Panel (2017), as indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.
(b): Estimated data, obtained as indicated in Section 2.1.1.3.1 of the Scientific Opinion Part 1.

The values of $FARSC_{intestine}$, for the species with available data, ranged in the scenario 1 using averaged published values from 0.22×10^{-3} mg/kg feed in turkeys for fattening to 0.57×10^{-3} mg/kg feed in veal calves. From other simulations (scenario 2 and scenario 3) made with a wider range of values for the data used in the calculation, FARSC could range from 0.13×10^{-3} mg to 0.62×10^{-3} mg /kg feed for turkeys for fattening and from 0.26×10^{-3} mg to 2×10^{-3} mg/kg feed in veal calves. In general, for the different animal species, it ranged from 0.13 to 2.00×10^{-3} mg/kg feed.

For the estimation of $FARSC_{rumen}$, no data were available concerning the activity of tetracycline so, I was set to 0. The estimated $FARSC_{rumen}$ values are reported in Table 4.

The values of $FARSC_{rumen}$ ranged, for the different species, from 0.72 to 2.40 $\times$ 10^{-3} mg /kg feed.

Table 4: The Feed Antimicrobial Resistance Selection Concentration of tetracycline (representing chlortetracycline and oxytetracycline) corresponding to the maximum concentration of tetracycline residues in non-target feed that would not develop resistance in the rumen bacteria ($FARSC_{rumen}$)

Animal category[(a)]	Body weight (kg)[(a)]	Daily Feed Intake (kg DM/animal per day)[(a)]	Volume of rumen content (L)[(b)]	$FARSC_{rumen}$ ($\times$ 10^{-3} mg drug/kg feed)
Dairy cows	650	20	90–180	**0.72**–1.44
Cattle for fattening	400	8	60–120	1.20–**2.40**
Sheep/Goat	60	1.2	9–18	1.20–**2.40**

DM: dry matter; FARSC: Feed Antimicrobial Resistance Selection Concentration.
(a): EFSA FEEDAP Panel (2017), as indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.
(b): Source of data indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.

3.2.2. Doxycycline FARSC determination

The PMSC value used was the same as for all other tetracyclines (see Section 3.2.1).

From the PMSC, the FARSC was calculated for each species from the equations shown above (see Section 3.2.1) by including specific values for doxycycline.

Due to the lack of information for the inactive fraction in the digestive tract, the I value was set to 0. However, since one old study suggested that the inactivation of doxycycline in the distal part of the intestines could be high, other simulations were done with I set to 0.7.

From the publications cited above, F for doxycycline was set to 0.7 for calves, 0.3 for adult ruminants, 0.2 for pigs, 0.06 for horses and 0.03 for rabbits. Due to the wider range of reported individual bioavailability in the literature, other additional simulations were performed with F values from 0 to 0.8 depending on the species.

Since the EMA reported that doxycycline was excreted in faeces, mostly in a microbiologically inactive form, GE was set to 0.

The different values of the parameters used for the calculations are summarised in Table 5 and the estimated FARSC values are reported in Table 6. There is no value for the bioavailability in rabbits. The first set of values (scenario 1) corresponds to the average of published values while scenario 2 corresponds to scenario that would lead to lower FARSC and scenario 3 to scenario that would lead to higher FARSC. The lowest FARSC (scenario 2) were obtained with lowest published values of I (lower inactivation of the drug resulting in higher activity on bacteria) and lowest published values of F (lower absorption resulting in more drug in the intestines). The estimated $FARSC_{intestine}$ values obtained with these three different set of values (= scenario) for the parameters are reported in Table 6.

The values of FARSC, for the species with available data, ranged in the scenario 1 using averaged published values from 0.17 $\times$ 10^{-3} mg/kg feed in turkeys for fattening to 0.67 $\times$ 10^{-3} mg/kg feed in veal calves. From other simulations (scenario 2 and scenario 3) made with a wider range of values for

Table 5: Predicted minimal selective concentration (PMSC) and pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of doxycycline (DOX) for the different animal species

Doxycycline data	Scenario #1	Scenario #2	Scenario #3
PMSC (mg/L)		0.00016	
Inactive fraction (I)	0	0	0.7
Bioavailability (F) veal calves	0.7	0.5	0.8
Bioavailability (F) adult ruminant	0.3	0.1	0.5
Bioavailability (F) pig	0.2	0.05	0.4
Bioavailability (F) poultry	0.4	0.2	0.6

Doxycycline data	Scenario #1	Scenario #2	Scenario #3
Bioavailability (*F*) horse	0.06	0	0.15
Bioavailability (*F*) salmon	0.3	0.2	0.5
Gastrointestinal elimination (*GE*)	0	0	0

PMSC: predicted minimal selective concentration. 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. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to (1 – *F*). 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.

Table 6: The Feed Antimicrobial Resistance Selection Concentration of doxycycline corresponding to the maximum concentration of doxycycline residues in non-target feed that would not develop resistance in the large intestinal bacteria ($FARSC_{intestine}$)

Animal category[(a)]	Body weight (kg)[(a)]	Daily Feed Intake (kg DM/animal per day)[(a)]	Daily output of fresh faeces (kg FM/animal per day)[(b)]	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 1	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 2	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 3
Sow lactating	175	5.28	7.7	0.29	0.25	1.30
Piglet (weaned)	20	0.88	0.88	0.20	0.17	0.89
Pig for fattening	60	2.2	2.64	0.24	0.20	1.07
Veal calf (milk replacer)	100	1.89	2.36	0.67	0.40	**3.33**
Dairy cows	650	20	55.71	0.64	0.50	2.97
Cattle for fattening	400	8	18.89	0.54	0.42	2.52
Goat (adult)	60	1.2	1.73	0.33	0.26	1.54
Sheep (adult)	60	1.2	1.47	0.28	0.22	1.31
Chicken for fattening	2	0.158	0.133	0.22	0.17	1.12
Laying hen	2	0.106	0.16	0.40	0.30	2.01
Turkey for fattening	3	0.176	0.109	0.17	**0.12**	0.83
Horse	400	8	8.33	0.18	0.17	0.65
Rabbit	2	0.1	0.053	–	–	–
Salmon	0.12	0.0021	0.00238	0.26	0.23	1.21

DM: dry matter; FM: faecal matter; FARSC: Feed Antimicrobial Resistance Selection Concentration.
(a): EFSA FEEDAP Panel (2017), as indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.
(b): Estimated data, obtained as indicated in Section 2.1.1.3.1 of the Scientific Opinion Part 1.

the data used in the calculation, FARSC could range from 0.12 to 0.83 $\times$ 10^{-3} mg/kg feed for turkeys for fattening and from 0.40 to 3.33 $\times$ 10^{-3} mg/kg feed in veal calves. In general, for the different animal species, it ranged from 0.12 to 3.33 $\times$ 10^{-3} mg/kg feed.

The estimation of $FARS_{Crumen}$ for doxycycline was identical as $FARSC_{rumen}$ for tetracycline since the PMSC was the same and *I* was also set to 0 (absence of data). $FARSC_{rumen}$ values are shown in Table 7.

Table 7: The Feed Antimicrobial Resistance Selection Concentration of doxycycline corresponding to the maximum concentration of doxycycline residues in non-target feed that would not develop resistance in the rumen bacteria ($FARSC_{rumen}$)

Animal category[(a)]	Body weight (kg)[(a)]	Daily Feed Intake (kg DM/animal per day)[(a)]	Volume of rumen content (L)[(b)]	FARSC ($\times 10^{-3}$ mg drug/kg feed)
Dairy cows	650	20	90–180	**0.72**–1.44
Cattle for fattening	400	8	60–120	1.20–**2.40**
Sheep/Goat	60	1.2	9–18	1.20–**2.40**

DM: dry matter; FARSC: Feed Antimicrobial Resistance Selection Concentration.
(a): EFSA FEEDAP Panel (2017), as indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.
(b): Source of data indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.

The values of $FARSC_{rumen}$ ranged, for the different species, from 0.72×10^{-3} to 2.40×10^{-3} mg/kg feed.

3.2.3. 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 the tetracyclines under assessment:

i) MSC data: data for MSCs are only available for tetracycline for *S. enterica* (Gullberg et al., 2011) but not for other tetracyclines.
ii) Extrapolation from one antimicrobial to another within an antimicrobial class: we suggest that this uncertainty is limited with the reasonable assumption that MSCs are similar if the different antimicrobials within a class share similar MICs, mechanism of action and resistance mechanisms. The PK properties are also very similar for tetracycline, chlortetracycline and oxytetracycline within animal species/categories and thus FARSC calculations for tetracycline were extrapolated to the other two substances. For doxycycline, only the same MSC values as those from tetracycline were used.
iii) Impact of complexity on determined MSCs: no data determining the community effect on the MSC of chlortetracycline, oxytetracycline or doxycycline. For tetracycline, a single study shows that tetracycline may have a reduced MSC in the presence of a microbial community (Lundström et al., 2016) as compared to a competition experiment in defined laboratory growth medium (Gullberg et al., 2011). Those data suggest that the MSCs differ by 15-fold between communities and single species. Thus, for tetracycline the MSC is reported to be 15-fold lower in the community compared to the defined single species set-up (1 μg/L vs 15 μg/L) (Gullberg et al., 2011; Lundström et al., 2016). The conservative estimate would therefore be the community MSC value (1 μg/L).
iv) Inactive fraction: no data available for tetracycline and doxycycline in the rumen and the estimations were obtained with a value of 0 for the inactive fraction. As the chemical properties of doxycycline and other tetracyclines are different, the obtained values for the inactive fraction of tetracyclines in human faecal slurries were not applied for doxycycline. One publication on the disposition of doxycycline in dogs and humans in 1971 suggested that doxycycline could be inactivated in large intestines without any quantitative data.
v) Bioavailability: the bioavailability for tetracycline, chlortetracycline and oxytetracycline were considered as similar for a given species. No data was found for adult ruminants, horses and rabbits and no calculation of FARSC in large intestines were done for these species. For doxycycline, the value for adult sheep was applied to other adult ruminants. The value for tilapia and channel catfish was applied to Atlantic salmon. No data were found for rabbits and no calculation of FARSC were done for this species.
vi) Intestinal elimination: no data found for tetracycline. For doxycycline, the EMA reported that the drug is excreted in faeces mostly in microbiologically inactive form. So, the *GE* was set 0.

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

3.2.4. Concluding remarks

The **FARSC for chlortetracycline, oxytetracycline and tetracycline** (for large intestine and/or rumen in the case of adult ruminants after weaning) ranges, for the different animal species, from 0.13 to 2.4 $\times$ 10^{-3} mg/kg feed. No FARSC was determined for horses and rabbits.

–[0.30–1.56] $\times$ 10^{-3} mg/kg feed for lactating sows
–[0.21–1.07] $\times$ 10^{-3} mg/kg feed for piglets
–[0.25–1.28] $\times$ 10^{-3} mg/kg feed for pigs for fattening
–[0.26–2.00] $\times$ 10^{-3} mg/kg feed for veal calves
–[0.72–1.44] $\times$ 10^{-3} mg/kg feed for dairy cows ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined)
–[1.20–2.40] $\times$ 10^{-3} mg/kg feed for cattle for fattening, adult sheep and goats ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined)
–[0.17–0.84] $\times$ 10^{-3} mg/kg feed for chickens for fattening
–[0.30–1.51] $\times$ 10^{-3} mg/kg feed for laying hens
–[0.13–0.62] $\times$ 10^{-3} mg/kg feed for turkeys for fattening
–[0.23–0.93] $\times$ 10^{-3} mg/kg feed for salmons

The values for dairy cows, cattle for fattening, sheep and goats only correspond to $FARSC_{rumen}$, because the absence of data on bioavailability for ruminants after weaning prevents the calculation of $FARSC_{instestine}$.

The **FARSC for doxycycline** ranges, for the different species, from 0.12 to 3.33 μg/kg feed. No FARSC was determined for rabbits.

–[0.25–1.30] $\times$ 10^{-3} mg/kg feed for lactating sows
–[0.17–0.89] $\times$ 10^{-3} mg/kg feed for piglets
–[0.20–1.07] $\times$ 10^{-3} mg/kg feed for pigs for fattening
–[0.40–3.33] $\times$ 10^{-3} mg/kg feed for veal calves
–[0.50–2.97] $\times$ 10^{-3} mg/kg feed for dairy cows ($FARSC_{intestine}$ and $FARSC_{rumen}$)
–[0.42–2.52] $\times$ 10^{-3} mg/kg feed for cattle for fattening ($FARSC_{intestine}$ and $FARSC_{rumen}$)
–[0.26–2.40] $\times$ 10^{-3} mg/kg feed for goats ($FARSC_{intestine}$ and $FARSC_{rumen}$)
–[0.22–2.40] $\times$ 10^{-3} mg/kg feed for sheep ($FARSC_{intestine}$ and $FARSC_{rumen}$)
–[0.17–1.12] $\times$ 10^{-3} mg/kg feed for chickens for fattening
–[0.30–2.01] $\times$ 10^{-3} mg/kg feed for laying hens
–[0.12–0.83] $\times$ 10^{-3} mg/kg feed for turkeys for fattening
–[0.17–0.65] $\times$ 10^{-3} mg/kg feed for horses
–[0.23–1.21] $\times$ 10^{-3} mg/kg feed for salmons

The probability that tetracycline, chlortetracycline, oxytetracycline and/or doxycycline concentrations below the lowest FARSC value for an animal species will confer any enrichment of, and/or selection for, resistant bacteria in the intestine and/or rumen is estimated to be 1–5% (extremely unlikely).

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

3.3.1. Tetracycline

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 (see also the Virtual Issue), resulted in 3,145 publications mentioning tetracycline and any of the food-producing animal species considered[3] and any of the performance

[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, other); crustaceans; other animal species.

parameters identified as relevant for the assessment of the possible growth-promoting effects of tetracycline.[4] After removing the reports not matching the eligibility criteria, 34 publications were identified.

3.3.1.2. Evaluation of the studies

The 34 publications identified in the literature search were appraised for suitability for the assessment of the effects of tetracycline 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; see also the Virtual Issue).[5] A total of 32 publications were not considered suitable for the assessment because of several shortcomings identified in the design of the study or the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix B.1 (Table B.1).

The publications considered suitable for the assessment are presented in Section 3.3.1.3.

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

Two publications were considered suitable for the assessment of the effects of tetracycline 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 papers and the effects on the relevant endpoints are described below. The summary of the studies includes the description of the source of tetracycline 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. Study in pigs

In the study reported by Wenner et al. (2013) a total of 360 pigs (progeny of successive farrowings of one Landrace breeding female population) were divided into six experimental groups, following a 3 × 2 factorial design (three diets × two housing systems) with seven pen replicates of 12 pigs (indoors) or 6 replicates of 6 pigs (outdoors) per treatment. Four were the relevant treatments: two non-supplemented control diets (0 mg tetracycline/kg feed, indoors or outdoors) and two tetracycline (unspecified form) supplemented diets (55 mg tetracycline/kg feed, indoors or outdoors). For each treatment, three basal diets (starter, grower and finisher) based on maize and soybean meal were used. Body weight (BW) was recorded every two weeks. Average daily feed intake (ADFI) and feed to gain ratio (F:G) were calculated for each growing phase and at the end of the experiment for the overall period (84 days). Tenth rib backfat thickness and loin muscle area were measured via ultrasound at the end of the experiment. At the end of the trial, the pigs receiving tetracycline-supplemented diets showed lower average daily gain and required three more days to reach the final weight, compared to the control group. Dietary tetracycline supplementation (at 55 mg/kg feed) adversely affected the performance of pigs for fattening.

3.3.1.3.2. Study in poultry

In the study reported by Manafi et al. (2018) a total of 600 one-day-old male Ross 308 chickens for fattening were allocated to six dietary treatments and distributed in five pens per treatment (with 20 birds per pen). Three diets based on maize and soybean meal (starter, grower and finisher) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments, a control (0 mg tetracycline/kg feed) and a treatment consisting of tetracycline–supplemented (unspecified form) diet at a concentration of 500 mg/kg feed. Mortality and health status were checked every day. BW and cumulative feed intake (FI) were recorded weekly (per pen),

[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)'.

and F:G calculated. At the end of the experiment (42 days), five birds per treatment were slaughtered, and the weights of the liver, spleen, heart, abdominal fat, breast muscle, thigh muscle and eviscerated carcass, and length of the intestine were measured. In addition, the small intestine was dissected, and *villi* height, crypt depth and the number of goblet cells were determined at the ileum. The faecal digesta of ten birds per treatment were used to enumerate *Escherichia coli*, total coliform bacteria and *Salmonella*. At the end of the trial, the birds receiving tetracycline at 500 mg/kg feed showed higher final BW (2.58 vs 2.51 kg) and improved F:G (1.68 vs 1.73) than those in the control group. Some carcass traits were negatively affected by tetracycline supplementation which reduced the relative weight of thigh and breast muscle, liver and abdominal fat The faecal digesta of the treated animals showed a reduction in total coliform bacteria (1.73 vs 3.06 log_{10} CFU/g), *E. coli* (0.13 vs 3.56 log_{10} CFU/g) and *Salmonella* (0.56 vs 3.43 log_{10} CFU/g), compared to the control, and an increase in *villi* height (3.63 vs 3.13 μm) and crypt depth (0.96 vs 0.88 μm) and a decrease in Goblet cell numbers (8.33 vs 9.00). Dietary tetracycline supplementation (500 mg/kg feed) had beneficial effects on growth performance in chickens for fattening, but with conflicting results on carcass traits.

3.3.1.4. Discussion

From the studies examined, the test item has been described as tetracycline (unspecified form; two studies). Therefore, an uncertainty on the exact product used/concentration applied has been identified.

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

The two studies considered suitable for the assessment covered only two species/categories (chickens for fattening and pigs) and one concentration of tetracycline against control, precluding any assessment of dose-effect relationships.

3.3.1.4.1. Pigs

The study in grower-finisher pigs (Wenner et al., 2013) showed an adverse effect on productivity (reduced average daily gain) related to the inclusion of 55 mg tetracycline/kg feed.

3.3.1.4.2. Poultry

The study in chickens for fattening (Manafi et al., 2018) showed positive effects on growth performance as a consequence of the inclusion of 500 mg tetracycline /kg feed. However, contrasting results were reported regarding the chickens' carcass traits. A reduction in coliforms, *E. coli* and *Salmonella* in the gastrointestinal tract was also observed.

3.3.1.5. Concluding remarks

It is judged 33–66% certain ('about as likely as not') that tetracycline has growth-promoting/ increase yield effects in chickens for fattening at a concentration of 500 mg/kg complete feed (one study).

It is judged 33–66% certain ('about as likely as not') that tetracycline has negative effects at a concentration of 55 mg/kg complete feed on growth performance in pigs for fattening (one study).

No data are available in the scientific literature showing effect of tetracycline on growth promotion/ increased yield when added (i) to chickens for fattening feed at concentrations below 500 mg/kg, or (ii) to feed of any other food-producing animal species or categories.

3.3.2. Chlortetracycline

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 (see also the Virtual Issue), resulted in 2,225 papers mentioning chlortetracycline 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 chlortetracycline.[4] After removing the reports not matching the eligibility criteria, 234 publications were identified.

3.3.2.2. Evaluation of the studies

The 234 publications identified in the literature search were appraised for suitability for the assessment of the effects of chlortetracycline 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; see also the Virtual Issue).[5] A total of 144 publications were not considered suitable for the assessment 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 B.2 (Table B.2).

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 chlortetracycline on growth performance and yield

A total of 90 publications were considered suitable for the assessment of the effects of chlortetracycline 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 chlortetracycline 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 studies of Baldwin et al. (2000) and Rumsey et al. (2000) the results of an experiment to evaluate the effect of dietary protein level and chlortetracycline supplementation on animal performance, carcass merit characteristics and visceral organ mass in beef steers were presented. A total of 32 Angus steers (285 kg BW) were individually housed, and, after an adaptation period of six weeks, randomly allotted by weight to a 2 × 2 factorial design of dietary treatments consisting of either 10 or 13% crude protein (CP) with a maize meal carrier containing either 0 or 350 mg chlortetracycline/head and day (unspecified chemical form; Aureomycin Hoffmann-La Roche Inc., Paramus, NJ, USA) (corresponding to ca. 35 mg/kg DM). The study lasted 91 days. Animals were weighed at the beginning and end of the experiment and once weekly. Feed intake was measured daily and F:G calculated. On day 56, all animals were challenged with a combination of thyrotropin-releasing hormone (TRH) and growth hormone-releasing hormone (GHRH) to test responsiveness of the pituitary. At the end of the study, steers were slaughtered, visceral organs removed, and samples collected and prepared for histopathological and immunohistochemical analysis. Longissimus fat cover (cm) and marbling (scores: 2.00 = slight to 3.00 = small) were greater for steers fed chlortetracycline than for steers not fed chlortetracycline for both the 10% CP (0.36 vs 0.30 cm; 2.6 vs 2.3) and 13% CP diets (0.42 vs 0.29 cm; 2.8 vs 2.2). Dietary administration of chlortetracycline decreased small intestinal weight both on absolute (4.83 vs 5.39 kg) and percentage of empty BW bases (1.22 vs 1.36%) and increased the *villi* height in jejunum (351 vs 302 μm). Dietary chlortetracycline supplementation at 35 mg/kg DM had growth-promoting effects in cattle for fattening.

In the study of Beacom et al. (1988) a total of 209 Charolais-sired, three-way cross steers and heifers, classified in five groups according to their weight (light heifers (BW 354 kg); light steers (367 kg); medium weight heifers (420 kg); medium weight steers (420 kg) and a mixed group of heavy steers and heifers (470 kg)), were distributed in 20 pens (four animals per BW-sex-category) and allocated to four dietary treatments. Two basal diets (a high forage-based diet for 56 days and a rolled grain-based finishing diet until slaughter) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of 35 mg/kg DM chlortetracycline (unspecified form). The study lasted until animal's slaughter when they were judged to qualify for Canada's A1 or A2 grade. Cattle were weighed weekly. At the end of the trial, just prior to shipping to a commercial abattoir, ruminant fluid was sampled by stomach tube from five animals within each of the 20 pens and subsequently analysed for volatile fatty acids. All livers were visually inspected, and representative lesions were examined histologically. During the high-forage feeding period, animals supplemented with chlortetracycline had a higher average daily gain (1.52 vs 1.32 kg/day) and a higher daily FI (14.1 vs 13.1 kg) compared to controls; for the total feeding trial, feed:gain ratio was better for the animals supplemented with chlortetracycline (9.2 vs 9.9) compared to controls. Dietary chlortetracycline supplementation at 35 mg/kg DM had growth-promoting effects in cattle for fattening.

In the study of Brown et al. (1975) the effect of chlortetracycline on performance and prevention of liver abscesses in fattening cattle was evaluated. A total of four feedlot experiments were performed. The first experiment included 50 crossbred steers (BW 268 kg) per treatment and lasted 153 days; the second experiment included 102/104 mixed crossbred cattle (BW 352 kg) per treatment and lasted 157 days; the third experiment included 26 crossbred steers (BW 288 kg) per treatment and lasted 168 days; and the fourth experiment included 430 mixed steers (BW 286 kg) per treatment and lasted 154 days. Basal diets were either not supplemented or supplemented with 70 mg chlortetracycline (unspecified form) per head per day (corresponding to ca. 9 mg chlortetracycline/kg DM). Average daily gain and feed conversion ratio were calculated by treatment 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 for level of severity. The overall incidence of liver abscesses in the four feedlot experiments was 56% for the control cattle and 44% for animals treated with chlortetracycline. Dietary chlortetracycline supplementation at 9 mg/kg DM reduced the incidence of liver abscesses but did not have growth-promoting effects in cattle for fattening.

In the study of Brown et al. (1960) the effect of chlortetracycline supplementation on urea utilisation in young dairy calves fed different levels of protein was evaluated. Forty-eight 2-day-old male and female calves (Holstein and Jersey breeds) were distributed in eight pens in groups of six calves (three Jersey and three Holstein). Four experimental starters ranging from 6.5% to 15.3% protein equivalent were fed with and without chlortetracycline (unspecified chemical form; Aurofac D). Chlortetracycline was added to the starters at a rate of 33.3 mg/kg of feed. Animals on the chlortetracycline containing starters were fed additionally 50 mg of chlortetracycline daily in the milk, up to 42 days of age. Overall, the mean chlortetracycline concentration in the diet of treated animals was 45 mg/kg DM. The study lasted 12 weeks (from 2 to 86 days of age). All calves were weighed at two days of age and at weekly intervals thereafter throughout the 12 weeks experimental period. Skeletal measurements (height at withers and heart girth) were made at two and 86 days of age. Daily record was kept of milk and starter consumption. Digestion and nitrogen balance studies were conducted, using two male calves (one Jersey and one Holstein) from each experimental group at 5, 8 and 11 weeks of age. Blood samples were collected from two calves in each group at two days and at 4, 8 and 12 weeks of age. Calves receiving chlortetracycline compared to the control animals showed a higher average daily gain (0.499 vs 0.417 kg) and a reduced feed:gain ratio (3.02 vs 3.30). Dietary chlortetracycline supplementation at 45 mg/kg DM had growth-promoting effects in calves.

In the study of Bush et al. (1959) the effect of chlortetracycline on growth and nutrient utilisation was studied in dairy calves. Sixteen 4-day-old male dairy calves (12 Holsteins, two Ayrshires and two Brown Swiss) were distributed in two dietary treatments, supplemented or not with 80 mg chlortetracycline (unspecified chemical form; Aurofac D) per calf and day (corresponding to 45 mg chlortetracycline/kg DM) for 16 weeks. Growth performance was studied during the 16-week experiment. The incidence and severity of diarrhoea was observed daily. In each group, a three-day adjustment period preceded a six-day collection period, during which total collections of urine and faeces were made. Digestion trials were conducted during the 5th, 8th and 11th weeks of the experiment. At the beginning of the 12th week each calf was given Ca45 for studying calcium utilisation and bone growth. Blood, urine and faeces were assayed for Ca45 determination. Calves receiving chlortetracycline showed higher average weight gain (74.4 vs 65.8 kg) during the 16-week experiment compared to the control animals. Dietary chlortetracycline supplementation at 45 mg/kg DM had growth-promoting effects in calves.

In the study of Cabral et al. (2013) a total of 40 12-week-old Holstein heifers were allocated to four dietary treatments. Two were the relevant treatments: a control and a supplementation with 22 mg chlortetracycline (unspecified form)/kg BW (corresponding to 589 mg/kg DM). The study lasted 12 weeks. Heifers on the chlortetracycline group were treated Monday through Friday and carrier only on Saturday and Sunday; these heifers were provided their respective treatment during weeks 1 to 4, 6 and 10 (week 5, 7–9 and 11–12 heifers were provided the non-medicated carrier). DM intake was monitored for each heifer throughout the 12 weeks study and feed provided was adjusted according to individual intakes. Skeletal measurements (withers and hip height, body length and heart girth) were taken weekly throughout the study and blood samples were obtained every three days per week for analysis of thyroxine concentration. Dietary chlortetracycline supplementation at 589 mg/kg DM did not have growth-promoting effects in cattle for fattening.

In the study of Hibbs and Conrad (1958), in Experiment 2, the effect of rumen cud inoculations from older cattle and chlortetracycline on performance of calves fed high roughage pellets was examined. A total of ten Jersey calves were either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; Aurofac D) at 100 mg per day (corresponding to ca. 70 mg/kg DM). All calves in each group (i.e. 5) were rumen inoculated with cud from older cattle. The study lasted 16 weeks. All calves nursed their dams for approximately three days and then were fed whole milk to seven weeks of age. The pelleted ration was offered free choice after the third day and, from the end of the milk feeding period to 16 weeks. Chlortetracycline was fed in milk up to seven weeks and in warm water for weeks 8–16. Records were kept of daily feed consumption, weekly changes in BW and withers height at 1, 8, 12 and 16 weeks of age. A five-day digestion trial was conducted at week 13; pellets were fed in constant amounts based on consumption immediately prior to the trial and urine and faeces were collected separately using metabolic crates. Based on the average pellet consumption of the different pellet groups, and using the digestion percentages experimentally determined, the intake of total digestible nutrients and protein digested were calculated. Dietary chlortetracycline supplementation at 70 mg/kg DM did not have growth-promoting effects in cattle for fattening.

In the study of Kitts et al. (2006) a total of 96 crossbred steers of 400 kg BW (English-Continental) were distributed in 16 pens in groups of six animals and allocated to four dietary treatments (in a 2 × 2-factorial design). Two were the relevant treatments obtained from a basal diet which was either not supplemented or supplemented with chlortetracycline (unspecified chemical form; Aureomycin, Alpharma Animal Health, Fort Lee, NJ, USA) at a concentration of 40 mg/kg DM. The study lasted 139 days. Animal's weight was recorded monthly, cumulative FI weekly and the gain to feed ratio (G:F) calculated. In addition, on day 118, the subcutaneous fat of the animals from two pens per treatment (heaviest animals) was measured and then slaughtered (on day 125). At the end of the trial (day 139), the rest of animals were slaughtered and the carcass graded and quality measured (longissimus muscle area and fat, peri-organ fat, marbling and bone maturity). At the end of the trial, the steer treated with chlortetracycline showed, compared to the control group, reduced dry matter (DM) intake (8.82 vs 9.28 kg feed/day); however, since both ADG and G:F were numerically higher in the group treated with chlortetracycline, the reduced DM intake in this group does not seem to represent an adverse effect of the treatment. Dietary chlortetracycline supplementation at 40 mg/kg DM did not have growth-promoting effects in cattle for fattening.

In the study of Kitts et al. (2007) a total of 24 crossbred steers of ca. 365 kg BW (Simmental-Angus) were distributed in 24 individual pens and allocated to four dietary treatments (under a 2 × 2-factorial design). Two were the relevant treatments and a basal diet was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; Aureomycin, Alpharma Animal Health, Fort Lee, NJ, USA) at a concentration of 350 mg/animal/day (corresponding to ca. 38 mg/kg DM). The study lasted 112 days. Animal's weight and cumulative FI were recorded monthly and the G:F calculated. At days 30, 56 and 106 hormone challenges were performed with the injection of thyrotropin-releasing hormone (TRH) and growth hormone-releasing hormone (GHRH) to measure circulating growth hormone (GH), thyroid-stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3) responses. In addition, at the end of the trial all animals were slaughtered, and the carcass graded and its quality measured (hot carcass weight, dressing percentage, longissimus muscle area and fat, periorgan fat, marbling and bone maturity). Dietary chlortetracycline supplementation at 38 mg/kg DM did not have growth-promoting effects in cattle for fattening.

In the study of Mir (1989) two experiments were carried out and the overall outcomes analysed independently. In Experiment 1 (performance trial), a total of 60 weaned lambs of 20 kg BW (Suffolk-crossbred) were distributed in individual crates and allocated to six dietary treatments. The basal diet was either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplemented at the concentration of 11 mg/kg DM. The study lasted 85 days. Animal's weight and cumulative FI were recorded twice per week biweekly and daily, respectively, and the G:F calculated at the end of trial. The lambs treated with chlortetracycline showed, compared to the control group, an impaired F:G (6.17 vs 5.68). In Experiment 2 (digestibility trial), a total of 30 mature wethers (unspecified breed) of 45 kg BW were distributed in individual pens and allocated to the same diets. The study lasted 28 days. At the end of the trial, faeces and rumen fluid were collected to measure nutrients' apparent digestibility (DM), organic matter (OM), nitrogen (N), acid-detergent fibre (ADF), neutral-detergent fibre (NDF) and energy) and fermentation parameters (volatile fatty acid (VFA), ammonia and pH), respectively. The lambs treated with chlortetracycline showed, compared to the control group, a reduction in apparent digestibility of DM (54.8% vs 60.6%), OM (57.2% vs 62.3%), ADF (42.4% vs 53.9%) and NDF (42.5% vs 51.8%) despite of improved N digestibility (69.6% vs 66.3%). Dietary chlortetracycline supplementation at 11 mg/kg DM had a negative effect on the performance of lambs.

In the study of Murdock et al. (1961) a total of 40 mixed calves (Holstein, half male and half female) of three days of age were distributed in individual pens and allocated to four dietary treatments (in a 2 × 2 factorial design) to compare growth response and the incidence and severity of scours of calves fed limited whole milk or milk replacer, with or without chlortetracycline. Chlortetracycline (unspecified form) was supplemented at a concentration of 13.8 mg/kg whole milk or 110 mg/kg milk replacer (corresponding to 83 and 80 mg/kg DM, respectively). The study lasted 77 days. Mortality, scouring and health status were checked every day. Animal's weight, height at withers and heart girth and cumulative FI were recorded weekly. At the end of the trial, the calves treated with chlortetracycline showed, compared to the controls and regardless of the milk type, improved BW gain until weaning at day 46 (ca. 35.5 vs 29.8 kg BW/day for males and ca. 29.7 vs 27.0 kg BW/day for females) and heart girth gain (ca. 15.7 vs 13.5 cm in for males and ca. 14.5 vs 13.5 cm for females). Dietary chlortetracycline supplementation at 80 mg/kg DM had growth-promoting effects in calves.

In the study of Reid et al. (2014) a total of 40 Holstein heifers (BW 363 kg, 12-month-old) were allocated to one of two dietary treatments. The basal diet was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; Aureomycin 90, Alpharma Inc., Fort Lee, NJ, USA) at a concentration of 350 mg/head/day (corresponding to ca. 40 mg/kg DM). The study lasted 90 days. BW, health score (coughing, diarrhoea, coat condition, eyeball recession into the orbit) and body condition score (BCS, 1–5 scale) were recorded weekly. Heifers were bred by artificial insemination and reproductive data were collected. Blood samples from all heifers were collected every four days to determine serum glucose, serum thyroxine and plasma progesterone. Reproduction traits were also recorded (age at first breeding and conception rate at first breeding and during the experiment). Dietary chlortetracycline supplementation at 40 mg/kg DM did not have growth-promoting effects in cattle for fattening.

In the study of Rumsey et al. (1982) three trials were carried out and the overall outcomes analysed separately. In Trial 1, a total of 50 lambs (Morlan × Western), 21 kg BW, were allocated to five dietary treatments; the study lasted nine weeks. In Trial 2, a total of 80 wether lambs of mixed breeding, 25 kg BW, were allocated to four dietary treatments; the study lasted 13 weeks. In Trial 3, 80 crossbred lambs (crossbred ewes × Hampshire rams), 22 kg BW, were allocated to four dietary treatments; the study lasted 13 weeks. In all trials, the basal diets were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) at the concentration of 25 mg/kg DM. Feed intake was recorded daily and BW weekly. Blood samples were collected at 0, 4 and 9 weeks (Trial 1) and at 0, 7 and 13 weeks (Trials 2 and 3) to determine haematocrit, cell volume, haemoglobin, total lipids, cholesterol, T3 and T4. At the end of the trial, animals were slaughtered and ruminal fluid samples collected to analyse volatile fatty acids (VFA), pH and ammonia. Weights of liver, kidney, thyroid, adrenal, bile and carcass were recorded. Animals receiving chlortetracycline showed, compared to the control group, an improvement of ADG at the end of the trial (204.8 vs 163.8 g/day) and a higher liver weight (663 vs 630 g) in Trial 1; in Trial 3, the improvement of ADG was only significant for the period from 0 to 9 weeks (339 vs 294 g/day); and in Trial 2, a reduction of DMI (1,450.5 vs 1,479.4 g/day) and of ADG (220.8 vs 227.0 g/day) at the end of the trial was observed. Dietary chlortetracycline supplementation at 25 mg/kg DM showed growth-promoting effects in cattle for fattening in one of the three experiments conducted.

In the study Stanford et al. (2015) two trials were conducted over two consecutive years by using 240 (per year) predominantly Angus mixed-breed steer calves (BW 251 kg and 273 kg in year 1 and 2, respectively), allocated to five dietary treatments and distributed in five replicates per treatment in groups of ten animals. The basal diets were either not supplemented or supplemented with different treatments. Three were the relevant treatments: control and two treatments consisting of chlortetracycline (unspecified chemical form; Alpharma Canada Corp., Kirkland, QC, Canada) supplemented at a concentration of 350 mg/head/day (corresponding to ca. 39 and 36 mg/kg DM in year 1 and 2, respectively) and chlortetracycline (unspecified chemical form; Alpharma Canada Corp., Kirkland, QC, Canada) supplemented at a concentration of 11 mg/kg feed. The study lasted 233 days in the 1st year and 187 days in the 2nd year. Health status was checked twice daily. Animals were weighted at the start of the experiment and at slaughter. Growth performance (DMI, ADG, G:F), health status and carcass characteristics were assessed. In the first year of the study, chlortetracycline at 350 mg/head per day improved DMI in comparison with control group (8.95 vs 8.72 kg/day, respectively). In the second year, chlortetracycline at 350 mg/head per day reduced DMI in comparison with control group and chlortetracycline supplemented at a concentration of 11 mg/kg (9.84, 9.98 and 10.04 kg/day, respectively). Dietary chlortetracycline supplementation at 11 and 36–39 mg/kg DM did not have growth-promoting effects in cattle for fattening.

In the study of Ternus et al. (1971), Experiment 1, a total of 96 lambs (unspecified breed; BW 29.2 kg) were distributed in 16 pens in groups of six animals and allocated to four dietary treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplemented at a concentration of 55 mg/kg feed (corresponding to ca. 63 mg/kg DM). The study lasted 77 days. BW and FI were measured. Dietary chlortetracycline supplementation at 63 mg/kg DM did not have growth-promoting effects in lambs for fattening.

3.3.2.3.2. Studies in pigs

Ahmed et al. (2018) studied the effect of fermented bamboo vinegar liquid on growth performance, nutrient digestibility, faecal *Escherichia coli* concentration and ammonia emissions in growing pigs and included a positive control with 30 mg/kg of chlortetracycline. A total of 84 growing pigs (Landrace × Yorkshire, 68 days of age, BW 28 kg) were assigned to four dietary treatments, and each treatment had three replicate pens with seven pigs per replicate. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 30 mg/kg feed. The study lasted 42 days. The initial and final BW of pigs and feed consumption per group were recorded and F:G calculated. Fresh faecal samples were collected directly via rectal massage (from six pigs per treatment, two per replicate) and used to determine nutrient digestibility, faecal ammonia and *E. coli* counts. An increase on the final BW (62 vs 59.7 kg) and BW gain (808 vs 748 g/day) and a reduction on F:G (2.53 vs 2.75) were observed in pigs supplemented with chlortetracycline. Antimicrobial supplementation increased the faecal digestibility of DM and CP (79.8% vs 71.9% and 75.0% vs 70.3%, respectively) and reduced the faecal *E. coli* counts and ammonia emissions of growing pigs. Dietary chlortetracycline supplementation (at 30 mg/kg feed) had a growth-promoting effect in pigs for fattening.

Amachawadi et al. (2011) studied in pigs the effect of feeding grade antimicrobials and copper on performance. A total of 240 weaned piglets (unspecified breed/genotype; 34 days of age, 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 or chlortetracycline or tylosin supplementation). Pigs were allocated to eight pens (each with five pigs) per treatment. Two were the relevant treatments, and a basal diet was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; Alpharma, Fort Lee, NJ, USA) at the concentration of 500 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 feed disappearance were recorded every week to calculate BW gain, FI and F:G. No copper × antimicrobial interactions were observed for any of pig performance response. At the end of the experiment (day 35), dietary supplementation with chlortetracycline did not affect performance (final BW, BW gain, FI and F:G), while for days 1 to 21 of experiment, chlortetracycline fed pigs showed an increased BW at day 21 (18.6 vs 17.6 kg) and BW gain (0.52 vs 0.47 kg/day). Dietary chlortetracycline supplementation (at 500 mg/kg feed) had a growth-promoting effect in weaned piglets.

In another study (Brown et al., 1952) the effectiveness of dietary chlortetracycline (Aureomycin hydrochloride) supplementation (22 mg/kg feed) on the growth and metabolism of growing pigs was studied. Twenty-four Hampshire barrow pigs (eight weeks of age, BW 16.3 kg) were divided into four dietary treatments on the basis of litter and BW (under a 2 × 2 factorial design), the factors being feeding method and chlortetracycline hydrochloride dietary supplementation, i.e. equalised vs ad libitum FI and none vs chlortetracycline hydrochloride (Aureomycin hydrochloride) feeding at concentration of 22 mg/kg feed (corresponding to 20.5 mg chlortetracycline/kg feed). Pigs were penned individually with six replicate pens per treatment; the experimental diets were fed from 16.3 kg to 90 kg BW. Animals' BW and FI were recorded at the end of each phase (47 kg BW for grower phase and 90 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, animals fed ad libitum and treated with chlortetracycline had, compared to the control group (fed ad libitum and not treated with chlortetracycline), improved BW gain (0.69 vs 0.60 kg/day). Growth parameters were not affected by chlortetracycline hydrochloride dietary supplementation in animals with equalised FI. Dietary chlortetracycline hydrochloride supplementation (at 22 mg/kg feed) (corresponding to 20.5 mg chlortetracycline/kg feed) had a growth-promoting effect in pigs for fattening.

In another study, Brumm and Peo (1985) studied the effect of receiving diets containing alfalfa meal and certain antimicrobials on performance of comingled feeder pigs previously transported long distances in three different experiments. In two of the three experiments (Experiments 2 and 3), two treatment groups were relevant for the current assessment. In these groups, 80 crossbred pigs

(Experiment 2, BW 19.1 kg; Experiment 3, BW 16.8 kg) were distributed in eight pens, and received two dietary treatments consisting on a basal diet either not supplemented (control) or supplemented with 110 mg chlortetracycline (unspecified form)/kg feed; in each chlortetracycline level (0 and 110 mg/kg), two diets were used, a basal diet and a basal diet plus 10% dehydrated alfalfa meal, corresponding to two pens (ten pigs per pen) per diet and chlortetracycline level, thus a 2 × 2 factorial design was applied. Experiments 2 and 3 lasted two periods, a 14-day chlortetracycline 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 chlortetracycline on BW gain (kg), daily FI (kg) and F:G, was determined at 14 days and in 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. In Experiment 2, at the end of the 14-day supplementation period, dietary chlortetracycline supplementation improved BW gain (0.45 vs 0.37 kg/day) and F:G (2.05 vs 2.36) compared to the control group and, at the end of the experiment, improved BW gain (0.63 vs 0.60 kg/day) compared to the control group. In Experiment 3, at the end of the 14-day supplementation period, dietary chlortetracycline supplementation increased ADFI (0.83 vs 0.77 kg/day) compared to the control group, and, at the end of the experiment, improved BW gain (0.62 vs 0.58 kg/day) and F:G (3.11 vs 3.26) compared to the control group. In both Experiments 2 and 3, dietary chlortetracycline supplementation reduced faecal score. Dietary chlortetracycline supplementation at 110 mg/kg feed had a growth-promoting effect in pigs for fattening.

Another study (Capps et al., 2020) aimed to assess the impact of copper, alone or with chlortetracycline, on growth performance, transferable copper resistance gene and faecal enterococci in weaned piglets. A total of 320 barrow piglets (DNA 200 × 400, DNA Genetics; 21 days of age, BW 7.4 kg) in a 35-day study. Piglets were fed a common non-medicated diet for seven days of acclimation. Treatments were arranged in a 2 × 2 factorial design with main effects of added copper (0 vs 200 mg/kg feed from copper sulfate) and chlortetracycline (0 vs 440 mg/kg feed, unspecified chemical form; Aureomycin 50®, Zoetis Services LLC, NJ, USA), and distributed in 16 replicates/treatments with five pigs each for 28 days. Chlortetracycline was not administered on day 22 of the experiment. Animals' BW and FI were recorded on days 1, 14 and 28 and F:G calculated. At the end of the experiment, pigs fed chlortetracycline improved BW (20.1 vs 18.9 kg) and BW gain (457 vs 412 g/day) and increased ADFI (647 vs 602 g/day) and G:F (0.706 vs 0.684). Dietary chlortetracycline supplementation at 440 mg/kg feed had a growth-promoting effect in weaned piglets.

Cha et al. (2013) investigated the effects of an additive known to exert antimicrobial properties (GallaRhois) on growth performance and diarrhoea incidence of weaned piglets; the study included a chlortetracycline group as positive control. One hundred crossbred weaned piglets ((Yorkshire × Landrace) × Duroc; 28 days of age, BW 6.85 kg) were randomly assigned into five experimental groups (20 pigs per treatment). Two were the relevant treatments, and the basal diet was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 300 mg/kg feed. Treatments were administered for 28 days. BW and FI were measured weekly. At the end of the experiment, blood samples were collected. Faecal scoring and measurement of diarrhoea duration were conducted daily. The severity of diarrhoea was noted by visually scoring the consistency of the faeces using a scale of 0–3 (0 no diarrhoea to 3 severe diarrhoea). Supplementation with chlortetracycline increased the final BW (18.9 vs 17.2 kg), BW gain (432 vs 371 g/day) ADFI (713 vs 657 g/day) and reduced the F:G (1.65 vs 1.77). Chlortetracycline reduced the faecal score (1.86 vs 2.47), the duration of diarrhoea (2.13 vs 3.58 days), the rate of diarrhoea (22 vs 26%) and the incidence of diarrhoea (2.3 vs 3.5%) and had no effect on blood biochemical parameters. Dietary chlortetracycline supplementation at 300 mg/kg feed had a growth-promoting effect in weaned piglets.

Chen et al. (2005) conducted a study to investigate the effects of biotite (aluminosilicate mineral) supplementation on growth performance, nutrients digestibility and blood constituents and to finally evaluate if biotite could replace antimicrobials in growing pigs, including two chlortetracycline groups as positive control. One hundred twenty growing pigs ((Landrace × Yorkshire) × Duroc; BW 18.3 kg) were used in a 28-day growth trial. Pigs were allotted to four treatments (with six replicate pens per treatment with five pigs each) by sex and BW in a randomised complete block design. Two were the relevant treatments obtained from a basal diet (maize–soybean-based) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 1,000 mg/kg feed. BW and FI were measured at the end of the experiment to determine BW gain, ADFI and G:F. Chromic oxide was added to the diets as indigestible marker for nutrient digestibility study (DM, N, Ca and P). Blood samples were collected from pigs at the end of the experiment.

Chlortetracycline supplementation increased the G:F (0.529 vs 0.495), but had no effect on BW gain and ADFI or other determined parameters. Dietary chlortetracycline supplementation at 1,000 mg/kg feed had a growth-promoting effect in pigs for fattening.

The objective of the study by Chen et al. (2006) was to investigate the effects of feeding probiotics (*Enterococcus faecium*) on growth performance, nutrient digestibility, blood characteristics and faecal noxious gas in growing-finishing pigs. A positive control including 1,000 mg chlortetracycline/kg feed was used. A total of 80 growing-finishing pigs ((Landrace × Yorkshire) × Duroc; BW 50.5 kg) were used in an eight weeks growth trial. Pigs were allotted to four treatments (four replicate per treatment and five pigs per pen) according to randomised complete block design. Two were the relevant treatments obtained from a basal diet (maize–soybean-based) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 1,000 mg/kg feed. BW and FI were measured at four weeks intervals to determine BW gain, ADFI and G:F. One week before the end of the experiment, chromium oxide was added to calculate digestibility coefficients (DM and N). At the end of the experiment, faecal grab samples were taken randomly from at least two pigs per pen for analysis of faecal NH_3-N, H_2S and VFA concentrations. At the beginning of the experiment, two pigs were randomly chosen from each pen and blood samples were taken. The same pigs were bled at the end of the experiment for evaluation of white and red blood cells and lymphocyte levels. No effect of dietary supplementation of chlortetracycline on any of the endpoints measured was identified. Dietary chlortetracycline supplementation at 1,000 mg/kg feed did not have a growth-promoting effect in pigs for fattening.

Another study (Cheng et al., 2018) was performed to investigate the effect of the inclusion of oregano essential oil in a reduced protein, amino acid supplemented diet on growth performance, nutrient digestibility, gut health and antioxidative capacity of growing finishing pigs as an alternative to antimicrobials. Forty-eight growing barrows (Large White × Landrace; 75 days of age, BW 29.6 kg) were randomly allotted to four treatments (with 12 replicate pens with 1 pig per replicate). Treatments included a normal protein diet (CP 17%), a reduced protein amino acid supplemented diet and a reduced protein amino acid supplemented diet either supplemented with chlortetracycline (150 mg/kg feed, unspecified form) or oregano essential oil. Pigs were fed with the experimental diets for 98 days in two phases: growing (from 30 to 65 kg BW) and finishing (from 66 to 115 kg BW). The study lasted 98 days. Pigs were individually weighed on days 1, 49 and 98. Feed intake was recorded, and ADFI, BW gain and G:F were calculated per pig. Chromic oxide was supplemented to diets for determining the apparent total tract digestibility of DM, gross energy and CP from day 36 to 42 and day 85 to 91, respectively. On day 98, six barrows per treatment were sacrificed and used for carcass measurements. Blood samples (for determination of antioxidant capacity) and ileum content (for quantification of selected ileal bacteria) were collected. Jejunum and ileum samples were taken for intestinal morphological analysis. Antimicrobial supplementation increased the ADFI in the growing period (2.14 vs 1.79 kg/day) and overall period (2.63 vs 2.38 kg/day), and had no effect on BW and BW gain. Dietary chlortetracycline supplementation showed negative effect on G:F (0.345 vs 0.377) in the growing period, but no effect in the overall period. Chlortetracycline reduced the CP apparent digestibility during the growing and finishing period (negative effect). Chlortetracycline also increased the backfat thickness in 10th rib and reduced the *Lactobacillus* and *E. coli* intestinal content and did not affect the intestinal histology. No effect of the antimicrobial on plasma antioxidant enzymes was observed. Dietary chlortetracycline supplementation at 150 mg/kg feed adversely affected growth performance and CP digestibility in pigs for fattening.

In another study (Choi et al., 2011a), the effect of a potential multimicrobe probiotic was investigated in two experiments with weaned piglets; a positive control with chlortetracycline was also included in both experiments. In Experiment 1, a total of 288 weaned piglets (Landrace × Yorkshire × Duroc; mixed sex, BW 6.4 kg) were allotted to four treatments (four pens with 18 pigs per pen). Two were the relevant treatments obtained from a basal diet (maize–soybean-based) which was either not supplemented (control: 0 mg chlortetracycline/kg feed) or supplemented with chlortetracycline (unspecified chemical form; 1 g/kg Aurofac 200G, providing 100 g chlortetracycline/kg, CTC Bio Inc., Seoul, Republic of Korea) at a concentration of 100 mg/kg feed. In Experiment 2, a total of 288 weaned piglets (Landrace × Yorkshire × Duroc, mixed sex, with initial BW of 5.8 kg) were allotted to four treatments (four pens with 18 pigs per pen) in a 2 × 2 factorial arrangement of treatments to evaluate the effect of two levels of probiotic subjected to high temperature drying (3 and 6 g/kg feed) without or with antimicrobial (0 or 100 mg chlortetracycline/kg). Both experiments 1 and 2 lasted 28 days. In both experiments, individual weaned pig BW and feed disappearance from each pen was recorded at the end of every phase to calculate BW gain, ADFI and G:F. Chromium oxide was used as indigestible marker in diets to calculate the apparent total tract digestibility (DM, CP and gross energy) and faeces were collected over a three days period (from 25 to 28 days). On days 14 and 28, fresh faecal samples were collected from two pigs per pen and used to measure faecal bacteria counts. In Experiment 2, the effect of diets on small intestinal morphology and microflora of ileal and caecal digesta were performed in two pigs per pen. At the end of the Experiment 1, chlortetracycline increased the BW gain (346 vs 298 g/day), ADFI (476 vs 443 g/day) and G:F (0.728 vs 0.672). Chlortetracycline did not affect the apparent total tract digestibility of DM, CP and gross energy in weaned piglets (at day 28) and reduced the *Clostridium* spp. (7.38 vs 8.29 $\log_{10}$ CFU/g). At the end of the experiment 2, chlortetracycline supplementation increased the BW gain (343 vs 330 g/day) and ADFI (521 vs 510 g/day), but no effect on G:F was observed. An increase of DM digestibility (83.2 vs 82.3%) was found at day 14, but no effect was observed for other parameters on neither day 14 nor day 28. A reduction on *Clostridium* spp. (7.12 vs 7.37 $\log_{10}$ CFU/g) on day 28 and no effect on ileum and caecum microbiota was obtained with chlortetracycline. Chlortetracycline supplementation increased the *villus* height in jejunum (416 vs 411 μm) and ileum (368 vs 363 μm) and *villus* height/crypt depth (1.53 vs 1.47). Dietary chlortetracycline supplementation at 100 mg/kg feed had a growth-promoting effect in weaned piglets.

In another study (Choi et al., 2011b), the effect of a potential multimicrobe probiotic and different antimicrobials was investigated in weaned piglets. A total of 288 weaned piglets (Landrace × (Yorkshire × Duroc); male:female 1:1, BW 7.0 kg) were allotted to four treatments (four pens with 18 pigs per pen). Two were the relevant treatments, obtained from a basal diet (maize–soybean-based) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 100 mg/kg feed. The experiment lasted 28 days. Individual weaned pig BW and feed disappearance from each pen was recorded at the end of every phase to calculate BW gain, ADFI and G:F. Chromium oxide was used as indigestible marker in diets to calculate the apparent total tract digestibility (DM, CP and gross energy) and faeces were collected over a three days period (from 25 to 28 days). At day 14 and 28, fresh faecal samples were collected from two pigs per pen and used to measure faecal bacteria counts. At the end of the experiment, chlortetracycline increased the BW gain (363 vs 315 g/day) and G:F (0.720 vs 0.658). Chlortetracycline also increased the apparent total tract digestibility of CP (82.2 vs 77.2%) in weaned piglets (at day 28), but did not affect the apparent total tract digestibility of DM and gross energy, and reduced the *Clostridium* spp. (7.28 vs 7.90 $\log_{10}$ CFU/g at day 14 and 8.09 vs 8.63 $\log_{10}$ CFU/g at day 28) and coliforms (6.53 vs 7.14 $\log_{10}$ CFU/g at day 14 and 5.72 vs 6.37 $\log_{10}$ CFU/g at day 28). Dietary chlortetracycline supplementation at 100 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Feldpausch et al. (2018), two 47-day experiments were conducted with 21-day-old weaned piglets (PIC1050, 240 piglets in Experiment 1 and 350 piglets in Experiment 2; BW 6.1 kg in both experiments). The study aimed at determining the effects of feeding low and high concentrations of chlortetracycline and antimicrobial alternatives (copper, zinc and essential oil), alone or in combination, on growth performance. On day 5 post-weaning, pens of five pigs were allotted to diet treatment with eight (Experiment 1) or seven (Experiment 2) replicate pens per treatment. In Experiment 1, treatments were fed from day 5 to 26 post-weaning and arranged in a 2 × 3 factorial with main effects of added zinc oxide (0 vs 2,500 mg Zn/kg) and chlortetracycline (0, 55, 441 mg/kg feed; unspecified form). In Experiment 2, treatments were fed from day 5 to 33 and structured in a (2 × 2 × 2) + 2 factorial with main effects of added copper sulfate (0 vs 125 mg/kg Cu), added zinc oxide (0 vs 3,000 mg Zn/kg) with 3,000 mg Zn/kg from day 5 to 12 and 2,000 mg/kg Zn from day 13 to 33, and origanum oil (0 vs 0.1%). The additional treatments were performed with subtherapeutic (55 mg/kg feed) and therapeutic (441 mg/kg feed) levels of chlortetracycline (unspecified form). Following the treatment period (from 5 to 26 day in Experiment 1 and from 5 to 33 day in Experiment 2), a common diet without antimicrobial was fed until 47 days in both experiments. In both experiments, BW gain, ADFI and G:F were determined by weighing pigs and measuring feed disappearance on day 5, 26 (Experiment 1) or 33 (Experiment 2) and 47. In Experiment 1, the piglets treated with chlortetracycline (at both 55 and 441 mg/kg feed) showed, compared to the control group, higher BW (14.6 and 14.9 vs 14.3 kg at day 26), BW gain (0.39 and 0.41 vs 0.37 kg/day during days 5 to 26) and ADFI (0.53 and 0.55 vs 0.52 kg/day for days 5–26). In Experiment 2, the piglets treated with chlortetracycline (at 441 mg/kg feed) showed, compared to the control group, higher BW gain (0.46 vs 0.44 kg/day for days 5–33). Dietary chlortetracycline supplementation at 55 and 441 mg/kg feed had a growth-promoting effect in weaned piglets.

Another study (Han et al., 2018) was carried out to investigate the effect of dietary combinations of organic acids and medium chain fatty acids as replacement for chlortetracycline on the growth performance, serum immunity and faecal microbiota of weaned piglets. Over 28 days, 144 weaned piglets (Duroc × (Landrace × Yorkshire), 72 male and 72 female, BW of 8.1 kg) were allocated to four treatments (six replicates per treatment with six piglets each). Two were the relevant treatments obtained from a basal diet (maize–soybean-based) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 75 mg/kg feed. The study lasted 28 days (with two feeding periods from 1 to 14 and 15–28 days). BW was recorded at the beginning of the experiment and on days 14 and 28, and feed consumption per pen was recorded at the end of each phase (day 14 and 28) to calculate BW gain, ADFI and F:G. Faecal score was performed using a five-grade scoring system (from 1 indicating well-formed faeces to 5 as watery faeces). Piglets with scores higher than 3 were regarded as having diarrhoea. At 14- and 28-days samples were collected from two boars of each replication to analyse immunoglobulins (IgG, IgA and IgM), and antioxidant capacity. On day 24, fresh faecal samples were collected for bacterial characterisation. Piglets fed chlortetracycline had a higher BW (10.1 vs 9.7 kg at day 14 and 13.6 vs 12.5 at day 28), BW gain (for 1–14 days 153 vs 122 g/day and for 1–28 days 200 vs 164 g/day) and reduced the F:G (for days 1–14 2.0 vs 2.4 and for days 1–28 2.0 vs 2.3). The incidence of post-weaning diarrhoea during days 1–14 was lower in piglets fed chlortetracycline (12.5 vs 22.4%). Antimicrobial supplementation increased the concentration of IgG (20.8 vs 19.6 g/L at day 14) and IgA (1.31 vs 1.16 g/L at day 28). The antimicrobial supplementation modified the ratio of faecal Firmicutes to Bacteroidetes, decreasing the abundance of the Bacteroidetes phylum and *Escherichia-Shigella* but showed no increase of *Lactobacillus*. Dietary chlortetracycline supplementation at 75 mg/kg feed had a growth-promoting effect in weaned piglets.

Another study (Helm et al., 2019) was performed to investigate the mechanisms of action by which antimicrobials increase nursery pig performance. A total of 24 weaned female piglets (Genetiporc 6.0 × Genetiporc F25, PIC; 19–21 days of age, BW 6.75 kg) were randomly allotted to individually pens and assigned to one of two dietary treatments (12 piglets/treatment) consisting in either a control diet or subtherapeutic antimicrobial (40 mg chlortetracycline/kg feed, unspecified form, Zoetis, NJ, USA). The experiment lasted 35 days with a two-phase feeding program (1–14 days and 15–35 days). On days 1, 7, 14, 21, 28 and 35 post-weaning, individual pig BW and feed disappearance were recorded to calculate BW gain, ADFI and G:F ratio. On day 35, six pigs per treatment were killed and sections from the ileum, colon, Longissimus dorsi muscle, liver and content from caecum were taken and used for proteomics and measurement of caecal short-chain fatty acid (SCFA) concentrations. Fresh ileum and colon segments were also used to determine intestinal barrier integrity and ileal active nutrient transport (glucose and glutamine). At the end of the experiment, supplementation with chlortetracycline increased BW (21.5 vs 17.5 kg), BW gain (0.43 vs 0.32 kg/day) and ADFI (0.51 vs 0.37 kg/day), while no effect of treatment on G:F was observed, and on intestinal nutrient transport or SCFA concentration. Of the proteins identified across all tissues examined, 65 protein abundances were different among treatments. Among these proteins some were primarily involved with biological processes including metabolism and transport. Dietary chlortetracycline supplementation at 40 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Holman and Chénier (2013), a total of 12 male and 12 female piglets (from Landrace × Yorkshire sows, 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, and the basal diets (weaner, starter and fattener) were either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 5.5 mg/kg feed in weaner (for 21 days), starter (for 21 days) and fattener (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) faeces were sampled to enumerate total anaerobic bacteria. Dietary chlortetracycline supplementation (at 5.5 mg/kg diet) did not affect performance of pigs for fattening.

In the study of Hossain et al. (2012a), a total of 80 crossbred castrated male growing pigs (Landrace × Yorkshire; BW 51 kg) were allocated to four dietary treatments with four replicate pens per treatment of five animals each. Two were the relevant treatments and two basal diets (grower and finisher) were either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 30 mg/kg feed. The study lasted eight weeks. Animals' BW and FI were recorded biweekly and F:G ratio calculated. In addition, at the end of the trial, animals were slaughtered and the carcass weighed and graded and the backfat thickness determined as well as the loin sampled to determine the chemical composition (moisture, crude ash, crude fat (CF), CP, cholesterol, iron, calcium, and magnesium), shear force, cooking loss, juiciness, tenderness and flavour. The oxidative stability (malondialdehyde – MDA) of the loin in refrigeration after 3 weeks post-slaughter was also measured. In addition, blood was sampled and total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, glucose, cortisol and insulin concentrations measured as well as the spleen was sampled and the response to lipopolysaccharide (LPS) and concanavalin A (Con A) was determined in terms of splenocyte growth and IL-6 and TNF-α concentrations. At the end of the experiment, dietary chlortetracycline supplementation had no effect on growth performance of pigs. Pigs treated with chlortetracycline showed, compared to the control group, a reduced CP percentage in loin muscle (22.3% vs 23.6%) and improved juiciness (5.10 vs 4.22) and tenderness (4.85 vs 4.35). The serum insulin concentration was higher in the chlortetracycline treated animals than in the control ones, although it is worth to mention that the same difference was detected at the start of the trial. In addition, splenocyte growth was reduced with the chlortetracycline medicated diet, compared to the control diet, in response to Con A at 0.3 μg/mL, but increased in response to LPS at 10 μg/mL. Moreover, IL-6 concentrations response to Con A and TNF-α concentrations to LPS were lower in the chlortetracycline-treated animals than in the control ones. Dietary chlortetracycline supplementation (at 30 mg/kg diet) did not affect performance of pigs for fattening.

In the study of Hossain et al. (2012b), a total of 100 crossbred castrated male finishing pigs (Landrace × Large White; BW 77 kg) were allocated to five dietary treatments with five replicate pens per treatment and five animals each. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 30 mg/kg feed. The study lasted six weeks. Animals' BW and FI were recorded biweekly and F:G ratio calculated. In addition, at the end of the trial, animals were slaughtered and the carcass weighed and graded and the backfat thickness determined as well as the loin sampled to determine the chemical composition (moisture, crude ash, CF, CP), shear force, cooking loss, water holding capacity, pH, colour, juiciness, tenderness and flavour. The oxidative stability (malondialdehyde (MDA)) of the loin in refrigeration after four weeks post-slaughter was also measured. In addition, blood was sampled and total protein, cholesterol, albumin, globulin, blood urea nitrogen, white blood cells, red blood cells and haemoglobin concentrations measured as well as spleen was sampled and weighed and the T-helper and T-cytotoxic cells measured and the response of splenocyte growth and IL-6 and TNF-α to lipopolysaccharide (LPS) and concanavalin A (Con A) determined. At the end of the experiment, dietary chlortetracycline supplementation had no effect on growth performance of pigs. Pigs treated with chlortetracycline showed, compared to the control group, a reduced MDA concentration in loin (until week 3) and an increased stimulation of spleen cell growth to Con A (at 0.1, 0.3 and 1.0 μg/mL) and to LPS at 1.0 μg/mL. The response of IL-6 concentrations in spleen were also increased with the chlortetracycline diet compared to the control one. Dietary chlortetracycline supplementation (at 30 mg/kg diet) did not affect performance of pigs for fattening.

In the study of Jiang et al. (2019) a total of 96 nursery pigs (half Duroc × Landrace × Yorkshire, 35 days of age, and half Chinese native Licha-black, 42 days of age; BW of 11.2 kg for both breeds/genotypes) were allocated to four dietary treatments with 24 replicate cages (12 cages per breed) per treatment and one animal each. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted 42 days. Animals' BW was recorded weekly and FI was recorded daily and G:F ratio calculated. In addition, all animals were bled (day 42 of the study) and serum obtained to determine antioxidant enzymes (superoxide dismutase – T-SOD, glutathione peroxidase – (GSH-Px)) and malondialdehyde – MDA. Afterwards, animals were slaughtered and the liver sampled to measure the expression of the nuclear factor erythroid 2-related factor 2 (nrf2) and TNF-α genes and proteins as well as to quantify T-SOD, GSH-Px and MDA. Furthermore, positive cell optical density of nrf2 and TNF-α genes was determined in the liver. At the end of the trial, the pigs treated with chlortetracycline showed, compared to the control group, an improved BW gain (552 vs 487 g/day) and G:F ratio (0.45 vs 0.41). The antioxidant enzyme concentrations in serum were lower for GSH-Px, whilst hepatic MDA was greater in the chlortetracycline group than in the control group. In addition, compared to the control group, the livers from chlortetracycline treated animals showed greater TNF-α mRNA relative expressions, optic densities and protein expressions. Linked to this, the nrf2 to TNF-α gene expression ratio and protein expression ratio were lower in the chlortetracycline treated animals compared to the control ones as well as nrf2 protein expression was lower. Dietary chlortetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Ke et al. (2014), a total of 150 weaned piglets (Duroc × Landrace × Yorkshire, 21 days of age, BW 6.2 kg) were allocated to five dietary treatments and distributed in five replicate pens per treatment with six animals each. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 75 mg/kg feed. The study lasted 21 days. Animals' BW and FI were recorded at the end of the trial and the G:F ratio calculated. In addition, six animals per treatment were slaughtered at the end of the trial and blood, intestinal mucosa (distal jejunum), tissue and digesta (distal jejunum and proximal colon) were collected to determine diamine oxidase (DAO) activity in plasma and intestinal mucosa, perform the histomorphometry analysis and to enumerate *Escherichia coli* and *Streptococcus suis* by 16S rRNA-sequencing methodology, respectively. At the end of the trial, the pigs treated with chlortetracycline showed, compared to the control group, improved BW gain (290 vs 266 g/day) as well as *villi* height and *villus* to crypt ratios (758 vs 629 μm and 2.30 vs 1.80, respectively). In addition, chlortetracycline treated animals showed, compared to the control, lower counts of *E. coli* and *S. suis* in the jejunum (6.30 vs 7.20 gene copies/g and 5.19 vs 6.20 gene copies/g, respectively) and colon (7.62 vs 8.42 gene copies/g and 6.48 vs 7.67 gene copies/g, respectively). Moreover, DAO activity in plasma was lower in the chlortetracycline medicated animals than in the control ones, but greater in jejunal mucosa. Dietary chlortetracycline supplementation at 75 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Kijparkorn et al. (2009), a total of 20 crossbred mixed pigs (Hampshire × Landrace × Duroc; 12 barrows and 8 gilts, BW 52 kg) were allocated to four dietary treatments and distributed in five individual replicate pens per treatment (three barrows and two gilts per treatment). Two were the relevant treatments and a basal diet was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted eight weeks. Animals' BW and FI were recorded on weeks 4 and 8 and the G:F ratio calculated. All animals were also bled on weeks 4 and 8 to determine haematological parameters (red blood cell counts, haemoglobin, packed cell volume, total white blood cell counts and differential lymphocyte counts) and lipid peroxidation (thiobarbituric acid reactive substances). In addition, at the end of the trial faeces were collected to measure the apparent total tract digestibility of CP, ether extract, crude fibre, ash, calcium and phosphorus. At the end of the trial, the pig treated with chlortetracycline showed, compared to the control group, improved apparent total tract digestibility of total phosphorus (0.60 vs 0.50). Dietary chlortetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Ko and Yang (2008), a total of 90 crossbred finishing pigs (Landrace × Yorkshire; BW 70.5 kg) were allocated to five dietary treatments with three replicate pens per treatment and six animals each. Two were the relevant treatments and a basal diet (finisher) was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 30 mg/kg feed. The study duration was not defined. Animals' BW and FI were recorded biweekly and F:G ratio calculated. In addition, at the end of the trial, nine animals per treatment were slaughtered and the loin meat sampled to determine the chemical composition (moisture, crude ash, CF, CP), cholesterol and the oxidative status (thiobarbituric acid value, TBA) in refrigeration after three weeks post-slaughter. In addition, the spleen was sampled and the response to lipopolysaccharide (LPS) and concanavalin A (Con A) was determined in terms of splenocyte growth and IL-6 and TNF-α concentrations. At the end of the experiment, dietary chlortetracycline supplementation had no effect on growth performance of pigs. Pigs treated with chlortetracycline showed, compared to the control group, lower contents of crude ash and TBA in the loin meat. In addition, IL-6 and TNF-α concentrations in the spleen were higher in the chlortetracycline treated animals than in the control ones. Dietary chlortetracycline supplementation (at 30 mg/kg diet) did not affect performance of pigs for fattening.

In the study of Langlois et al. (1978), a total of five trials were carried out and overall outcomes were provided 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, BW 14 kg) were allocated to five dietary treatments with three replicate pens per treatment and four pigs each. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 44 mg/kg feed during 6, 11 and 16 weeks. All five trials lasted 16 weeks (until approx. BW of 98 kg). Animals' 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 chlortetracycline supplementation had no effect on growth performance of pigs. At week 11, the pig treated with chlortetracycline during 11 weeks showed, compared to the control group, lower faecal counts of coliforms (5.36 vs 6.28 $\log_{10}$ CFU/g faeces), but when treated with chlortetracycline during six weeks the chlortetracycline-resistant coliform counts were greater than in the control group

(6.80 vs 4.30 log_{10} CFU/g faeces) as well as this incidence increased in any of the treatment durations (90 vs 39%). Moreover, a greater number of faecal isolates resistant to neomycin and kanamycin were detected in the 11-week- chlortetracycline treated animals than in the control ones at week 11 (10 vs 5% and 13 vs 7%, respectively) and at week 16 (29 vs 11% for both antimicrobials). Dietary chlortetracycline supplementation (at 44 mg/kg diet) did not affect performance of pigs for fattening.

In the study of Liu et al. (2008) a total of 50 weaned barrows (Large White × Landrace; 16 days of age, initial BW 4.72 kg) were allocated to five dietary treatments with ten individual (replicate) metabolism cages per treatment. Two were the relevant treatments and a basal diet was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 80 mg/kg feed. The study lasted 21 days. Mortality, diarrhoea and health status were checked every day. Animals' BW and FI were recorded weekly and G:F ratio calculated. At days 0, 7, 14 and 21 faeces were collected to enumerate *E. coli* and *Lactobacillus*. In addition, at the end of the trial, faeces were also collected to measure the apparent digestibility of DM, gross energy, CF, CP, calcium and phosphorus contents and, afterwards, all animals were slaughtered and the duodenum, jejunum and ileum sampled to analyse the mucosal morphology. At the end of the trial, pigs treated with chlortetracycline showed, compared to the control group, improved final BW (11.3 vs 10.7 kg), BW gain (315 vs 285 g/day), ADFI (463 vs 436 g/day) and G:F ratio (0.68 vs 0.66), as well as reduced incidence of diarrhoea (5.7 vs 13.8%) and cumulative diarrhoea score (8 vs 19) and faecal counts of *E. coli* (3.38 vs 3.87 log_{10} CFU/g digesta). In addition, at the end of the trial, animals treated with chlortetracycline showed, compared to the control group, improved apparent digestibility of gross energy (85.4 vs 82.5%), DM (84.3 vs 81.4%), CF (74.2 vs 72.2%), CP (78.1 vs 74.7%), calcium (56.4 vs 52.0%) and phosphorus (46.5 vs 44.1%), as well as improved *villus* lengths (418 vs 406 μm in jejunum and 353 vs 331 μm in ileum) accompanied by improved *villus* to crypt ratios (1.86 vs 1.78 in the jejunum and 1.77 vs 1.62 in the ileum). Dietary chlortetracycline supplementation at 80 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Loh et al. (2013), a total of 40 mixed weaned piglets at (Large White × Landrace × Duroc; 26 days of age, initial BW 6.53 kg) were allocated to five dietary treatments with four replicate pens per treatment and two piglets per pen. Two were the relevant treatments and a basal diet was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 300 mg/kg feed. The study lasted five weeks. Mortality, diarrhoea and health status were checked every day. Animals' BW and FI were recorded weekly and the F:G ratio calculated. At the end of the trial, four male pigs per treatment were slaughtered and the ileal digesta and faeces sampled to measure pH, SCFA, protein and energy digestibility and lactobacilli and enterobacteria enumeration. At the end of the trial, pigs treated with chlortetracycline showed, compared to the control group, improved BW gain (293 vs 252 g/day) and reduced diarrhoea scores (0.09 vs 0.40). In addition, chlortetracycline treated animals showed lower counts of lactobacilli in faeces than control ones (6.12 vs 6.51 log_{10} CFU/g). Dietary chlortetracycline supplementation at 300 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Long et al. (2019) a total of 108 mixed weaned piglets (Duroc × (Landrace × Yorkshire), half barrows and half gilts, 28 days of age, with initial BW of 8.68 kg) were allocated to three dietary treatments with six replicate pens per treatment and six piglets (three barrows and three gilts) per pen. Two were the relevant treatments obtained from two basal diets which were either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 75 mg/kg feed. The study lasted 35 days. Mortality, diarrhoea and health status were checked every day. Animals' BW and FI were recorded at days 1, 14, 28 and 35 and the F:G ratio calculated. At days 14 and 28, faeces were collected to measure total apparent tract digestibility of DM, ether extract, CP, carbohydrate, gross energy and organic matter and nitrogen excretion, and to enumerate *E. coli*. In addition, at day 28, 6 pigs per treatment were bled and the serum triglyceride, blood urea nitrogen, IgG, IgM and IgA, antioxidant capacity (catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px)) and malondialdehyde (MDA) concentrations measured. The same pigs were afterwards slaughtered and the duodenal, jejunal, and ileal tissues sampled to analyse histomorphometry. At the end of the experiment, dietary chlortetracycline supplementation had no effect on growth performance of pigs. Pigs treated with chlortetracycline showed, compared to the control group, a reduced diarrhoea rate (0.71% vs 3.09%, between days 1 and 14). In addition, at day 28, chlortetracycline treated animals showed lower activities of serum antioxidant capacity, superoxide dismutase and catalase than control animals, but greater concentrations of malondialdehyde. Dietary chlortetracycline supplementation at 75 mg/kg feed did not have a growth-promoting effect in weaned piglets.

In the study of Ma et al. (2019) a total of 126 crossbred mixed piglets (Duroc × (Landrace × Large White)), half barrows and half gilts, 28 days of age; initial BW 7.33 kg) were allocated to three dietary treatments with seven replicate pens and six animals (three barrows and three gilts) per pen. Two were the relevant treatments and two basal diets (phase 1: day 1 to 14 and phase 2: day 15 to 28) were either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 75 mg/kg feed. The study lasted 28 days. Mortality, diarrhoea and health status were checked every day. Animals' BW and FI were recorded at days 1, 14 and 28 days and the G:F ratio calculated. In addition, in the same days, seven animals per treatment were bled and the serum IgA, IgG, IgM, malondialdehyde (MDA), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC) measured. At the end of the trial, the same pigs were slaughtered and the liver and mucosa from duodenum, jejunum and ileum and intestinal contents (from ileum and caecum) collected to measure hepatic Gpx1 and Gpx4 gene expression, mucosal IL-1β, TNF-α and IFN-γ as well as zonulin-1 (ZO-1), and occludin (OCLN), and to assess intestinal morphology, respectively. At the end of the trial, pigs treated with chlortetracycline showed, compared to the control group, improved BW gain (371 vs 354 g/day), G:F ratio (0.61 vs 0.57) and reduced diarrhoea rate (2.70 vs 5.70%). Regarding serum parameters, at day 14, chlortetracycline treated animals, compared to control ones, showed greater concentrations of IgM, T-SOD and GSH-Px and lower concentrations of MDA and, at day 28, greater concentrations of IgA and T-SOD. In addition, at the end of the trial, the pigs treated with chlortetracycline showed, compared to the control group, higher Gpx1 and Gpx4 gene expressions, lower concentrations of TNF-α in the duodenum and jejunum and greater concentrations in the jejunum of ZO-1 and OCLN. Dietary chlortetracycline supplementation at 75 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Mader and Brumm (1987), two experiments were carried out and the outcomes analysed independently. In Experiment 1, a total of 288 crossbred mixed growing pigs (unspecified breed; initial BW 19.1 kg) were allocated to four dietary treatments with three replicate pens per treatment and 24 animals per pen. Two were the relevant treatments obtained from three basal diets (receiver, grower and finisher) which were either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 55 mg/kg feed. Experiment 1 lasted 15 weeks. In Experiment 2, a total of 192 crossbred mixed growing pigs (breed not given, with initial BW of 19.3 kg) were allocated to the same four dietary treatments, as in Experiment 1, with three replicate pens per treatment and 16 animals per pen. Two were the relevant treatments and a basal diet (grower) was either not supplemented (control) or supplemented with chlortetracycline at a concentration of 55 mg/kg feed. Experiment 2 lasted eight weeks. Animals' BW and FI were recorded at the end of each phase (grower phase at 56 days and finisher phase at 17 weeks) and the F:G ratio calculated. At the end of Experiment 1, dietary chlortetracycline supplementation had no effect on growth performance of pigs. At the end of Experiment 2, the pigs treated with chlortetracycline showed, compared to the control group, improved BW gain (0.59 vs 0.58 kg/day) and ADFI (1.52 vs 1.48 kg/day). In one experiment, dietary chlortetracycline supplementation at 55 mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Maxwell et al. (1994), a total of 850 litters from nulliparous and multiparous sows were monitored over two reproductive cycles. The study was carried out in five research stations (average parities from 2.3 to 4.3) located at five different states in the US (number of litters contributed by each station ranged from 76 to 299), using sows of different genotypes (Yorkshire × Large White, Duroc × Yorkshire, Yorkshire, Yorkshire × Hampshire × Landrace, Dekalb 30), and different diets (maize–soybean meal in four locations and sorghum-soybean meal in the other). In each location, sows were randomly distributed into four experimental treatments, consisting in a non-medicated control diet, or the same diet supplemented with chlortetracycline (unspecified form) at 220 mg/kg feed fed for three weeks at the time of breeding, or at the end of gestation and during lactation, or at both times (breeding plus end gestation and lactation). Piglets were weaned from 21.0 to 41.1 days of age depending on location. Mortality and health status were checked every day. BW of sows was recorded at breeding, at days 90 and 110 of gestation, the day after farrowing, at day 21 of lactation and at weaning. Feed intake of sows was recorded from farrowing to day 21 of lactation. Litter size (total number of piglets and piglets alive) and piglet weights at farrowing and at weaning were recorded. In addition, the first-service and overall conception rates, the number of days to first-oestrus and the sow's rectal temperature were registered. Feeding chlortetracycline during the breeding season increased the sow weight at breeding (168 vs 163 kg) and the litter size at birth (10.8 vs 10.3), and decreased feed consumption (5.4 vs 5.5 kg/day) in the subsequent lactation period. Feeding chlortetracycline during lactation reduced lactation weight loss in sows (4.3 vs 6.1 kg) and improved subsequent conception rate at the first service (80 vs 73%) and overall conception rate (89 vs 84%). The results indicate that feeding chlortetracycline at 220 mg/kg feed during the breeding period or/and during lactation improved overall reproductive performance of sows.

The study by Messersmith et al. (1966) included two experiments involving 409 sows housed in eight farms. Experiment 1 included four breeding batches and a total of 179 sows (unspecified breed; nulliparous in two batches and multiparous in the other two) that were allocated (with their litters) to two dietary treatments: control non-medicated feed (87 sows) or the same diet supplemented with 220 mg chlortetracycline/kg (unspecified chemical form; Aureomycin chlortetracycline, Agricultural Division, American Cyanamid Co., Princeton, NJ, USA) feed (92 sows). Experiment 2 included five breeding batches and a total of 198 sows (breed not given, all multiparous) that were allocated (with their litters) to two dietary treatments: control non-medicated feed (97 sows) or the same diet supplemented with 110 mg chlortetracycline/kg feed (101 sows). Sows started to receive the medicated diets prior to breeding and were fed the same diets throughout a 21 to 24 day breeding period. The study lasted one reproductive cycle. Mortality and health status were monitored every day. The farrowing rate, the number of pigs born per litter and stillborn per litter, as well as the mortality of piglets 24 h and three weeks post-farrowing were measured. Farrowing rate was increased from 62% to 79% when sows were fed diets with 220 mg chlortetracycline/kg feed, and from 74% to 86% when the level of supplementation was 110 mg chlortetracycline/kg feed. Farrowing performance was improved with both chlortetracycline concentrations.

In the study of Myers and Speer (1973) a total of 249 sows (Yorkshire × Landrace) were housed in individual pens and allocated to four experimental treatments balanced by parity, weight and backfat thickness upon completion of a 3-week lactation period. The four treatments were arranged according to a 2 × 2-factorial design, with two levels of intake (basal vs flushing the first day post-mating) and two levels of chlortetracycline (unspecified form) in feed (either non-medicated feed or at a concentration of 1,000 mg/day from weaning to 15 days post-mating). The sows were fed 2.27 kg feed/day, this level being equivalent to 440 mg/kg feed. The study lasted one reproductive cycle. Reproduction performance parameters were measured (breeding, conception and farrowing rates, conception rate at first oestrus, interval to the first oestrus, matings per sow, weight at weaning, backfat thickness). Piglet data included total number born and born alive per litter, birth weight and the number of pigs weaned per litter and litter weight weaned. The addition of chlortetracycline at 440 mg/kg to breeding sows had no effects on reproductive or farrowing performance.

In the study of Nitikanchana et al. (2012), a total of 1,313 growing pigs (PIC 1,050 × 337, 22 kg initial BW) were distributed in 40 pens (31–33 pigs per pen, with similar number of barrows and gilts per pen) and allocated to four dietary treatments (arranged according to a 2 × 2-factorial design with ten replicates or pens per treatment). Pigs were fed a maize–soybean based diet that was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments obtained from basal diets which were either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 400 mg/kg feed during the first 15 days, then all pigs received the same control diet. The study lasted 35 days. BW and feed consumption (per pen) were determined at days 15 and 35 to calculate average daily gain (ADG) and G:F. ADG was increased in chlortetracycline pigs from day 1 to day 15 of the trial (685 vs 649 g/days in the control). Thus, chlortetracycline at 400 mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Papaioannou et al. (2002), a total of 240 sows/gilts (Large White × Landrace) were housed in individual pens and assigned to four dietary treatments. Two types of basal diets were used: a pregnancy feed and a lactation feed. The four experimental treatments were arranged according to a 2 × 2-factorial design, with two levels of a mycotoxin binder (zeolite) and two levels of dietary addition of chlortetracycline (either a non-supplemented control or supplemented with chlortetracycline (unspecified chemical form; AUROFAC® (Aureomycin), Roche) at a concentration of 800 mg/kg for two weeks post-service plus two weeks starting at five days pre-farrowing). The study lasted one reproductive cycle. Mortality and health status (inappetence, pyrexia, mastitis, vaginal discharge) were checked every day. BW and FI were recorded weekly and the F:G calculated. In addition, reproductive data (dates from the oestrus and service, pregnancy confirmation, farrowing, weaning and subsequent oestrus and service) were recorded and the litter performance parameters measured (number of piglets born alive and dead, malformed, weaned, mortality and piglet weight at birth and at weaning). Sows treated with chlortetracycline in diets with no zeolite showed, compared to the control group, reduced inappetence (25% vs 49%) and a shorter weaning-to-first oestrus interval after the second weaning on trial (8.8 vs 10.3 days). In the case of the farrowing and weaning performances, litters from chlortetracycline-treated sows, compared to the control, showed more pigs born alive (10.5 vs 9.1 pigs/litter), weaned (9.8 vs 8.1 pigs/litter) and greater weight of piglets at birth (1.4 vs 1.3 kg) and at weaning (6.4 vs 5.9 kg). Chlortetracycline at 800 mg/kg feed improved reproductive and litter performance in breeding sows.

In the study of Ribeiro de Lima et al. (1981), a total of 252 crossbred pigs (Yorkshire-Hampshire; initial BW 20 kg, 65–76 days of age) were distributed in 63 pens (two barrows and two gilts pigs per pen) and allocated to several dietary treatments (three replicates or pens per treatment) in three separate trials (six treatments (72 pigs), in two trials and nine treatments (108 pigs) in the other trial). In the three trials, there were two relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; Aureomycin-50, American Cyanamid Co., Inc, Princeton, NJ, USA) at a concentration of 55 mg/kg feed. Trial 1 lasted 105 days (from 17.4 to 95 kg BW), trial 2 102 days (from 19.9 to 90 kg BW) and trial 3 92 days (from 23.3 to 93 kg BW). Individual pig weights and FI for each pen were measured every two weeks and feed conversion ratio calculated. Compared to the control, in the first trial pigs fed the chlortetracycline-supplemented diet showed higher average daily weight gain (778 vs 682 g/day) and improved F:G (2.83 vs 2.94), but there were no differences between both treatments in the other two trials. In one trial, dietary chlortetracycline supplementation at 55 mg/kg feed had growth-promoting effects in pigs for fattening.

In the study of Sarker et al. (2010d), a total of 90 finishing pigs (Landrace × Yorkshire, both sexes, 70.8 kg initial BW) were distributed in 15 pens (six pigs per pen) and allocated to five dietary treatments (three replicates or pens per treatment). Pigs were fed a maize–wheat-soybean based finishing diet that was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments with basal diets either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at a concentration of 30 mg/kg feed. The study lasted 42 days. BW and FI were measured every two weeks and feed conversion ratio calculated. At the end of the trial, pigs were slaughtered and carcass weight, backfat thickness and carcass grade were determined. Carcass composition and thiobarbituric acid (TBA) value were also measured. Meat quality (shear force, cooking loss, meat colour), including sensory evaluation (juiciness, tenderness and flavour), was assessed. Pigs fed chlortetracycline-supplemented diets showed, compared to the control group, greater total weight gain after 42 days (42.3 vs 39.4 kg) and higher ash content in meat (2.58% vs 2.09%). Thus, chlortetracycline at 30 mg/kg feed had growth-promoting effect in pigs for fattening.

In the study of Sbiraki et al. (2003), a total of 400 gilts/sows (Landrace × Large White) were monitored for two consecutive breeding cycles. The females were distributed in two experimental groups, a control and a treated group receiving chlortetracycline (unspecified chemical form; Aurofac, a granular premix containing 10% of chlortetracycline; Hoffmann-La-Roche, now Alpharma, Oslo, Norway) in feed (10 g/day for each animal) during the lactation of the first cycle, from five days before farrowing to the first service after weaning. During the second breeding cycle, all animals were fed the non-medicated diets. Sows were fed cereal-soybean based gestation and lactation diets. Weaning was at 22–28 days post-farrowing (mean duration of lactation was 23.1 days in cycle 1 and 23.7 days in cycle 2). Phase 2 began immediately after Phase 1 and ended when the sows were first mated after their litters had been weaned. Feed intake of sows was adjusted to litter size and to gestation period. Health status of sows and piglets was assessed daily, and signs of disease of sows recorded (poor appetite, fever, clinical mastitis and vaginal discharge). Pregnancy was confirmed by ultrasound. During each phase of the study (Phase 1 and Phase 2), each female was weighed at farrowing, at weaning and at the first subsequent oestrus. Feed intake was recorded in both phases during lactation and during weaning-to-oestrus interval and G:F calculated. The duration of the interval from weaning to first oestrus and farrowing interval (number of days between the first and second farrowing) were also recorded. For litters, the following data were recorded: numbers of live-born and stillborn piglets, number of piglets that died during lactation, number of weaned piglets, individual BW of piglets at birth and weaning. Piglets were monitored daily for diarrhoea. Considering the FI during lactation, the concentrations of chlortetracycline in the treated group would be approximately 2,000 mg/kg feed during lactation. Compared to the control, chlortetracycline-treated sows ate less feed from weaning to first oestrus (22.9 vs 25.5 kg), lost less weight during lactation (−8.5 vs −10.3 kg) and from weaning to first oestrus (−2.1 vs −2.5 kg) and less females had to be excluded because of anoestrus (6 vs 12%). The duration of weaning to first oestrus (8.1 vs 9.1 days) and of farrowing interval (146 vs 147 days) were shorter in chlortetracycline-treated than in control females. The health status of chlortetracycline-treated sows was improved in comparison with the control group with a reduced percentage of animals showing poor appetite (38% vs 49%), clinical mastitis (16% vs 28%) and vaginal discharge (7% vs 14%). Litters from chlortetracycline-treated sows showed, when compared to control animals, more weaned piglets (9.2 vs 8.8) with a higher BW at weaning (6.1 vs 5.9 kg) and a reduced diarrhoea score (0.59 vs 0.93). During the second cycle, the health status of chlortetracycline-treated sows was improved in comparison with the control group with less animals showing poor appetite (36% vs 50%) and vaginal discharge (5% vs 13%). In the second cycle, litters from chlortetracycline-treated sows showed, compared to control, more total born (10.4 vs 10.1), live-born (9.8 vs 9.5) and weaned (9.1 vs 8.6) piglets, and reduced diarrhoea score of piglets (0.70 vs 0.86). Chlortetracycline-treated sows showed, when compared to control, a reduced proportion of females with return to oestrus (5% vs 12%) in the second cycle. Dietary supplementation during lactation with chlortetracycline at 10 g/day per sow (~ 2,000 mg/kg feed) improved reproductive and litter performance in breeding sows.

Two experiments were reported in the study of Shen et al. (2009). In Experiment 1, 192 piglets (Landrace × Large White; initial BW 7.5 kg) weaned at 28 days of age, were distributed in 48 pens (four piglets per pen) and allocated to one of six dietary treatments (eight pens or replicates per treatment). Two were the relevant treatments obtained from a basal diet (maize–soybean–spray-dried plasma-fish meal) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; Jinhe Biotechnology Co. Ltd., Tuoketuo, China) at 80 mg/kg feed. The study lasted 21 days. Feed and water were available ad libitum, FI was recorded and piglets were weighed at the beginning and at the end of the experiment to calculate ADG and G:F. Compared to the control, piglets fed the chlortetracycline-supplemented diet showed higher ADG (412 vs 362 g/day) and FI (749 vs 655 g/day) with no differences in G:F. In Experiment 2, 24 nursery piglets (Landrace × Large White, initial BW 5.8 kg, weaned at 21 days of age) were housed in individual pens and allocated to one of three dietary treatments (eight piglets or replicates per treatment). Two were the relevant treatments obtained from a basal diet (maize–soybean–sprayed plasma and fish meal) which was either not supplemented (control) or supplemented with chlortetracycline at 80 mg/kg feed. The study lasted 21 days. FI and BW were measured weekly and G:F was calculated. During the last three days of the experiment, faeces were collected to estimate the apparent digestibility of DM, gross energy and protein (chromium oxide used as indigestible marker). On days 0, 7, 14 and 21 blood samples were collected to determine blood CD4+ and CD8+ lymphocyte subset concentrations. At the end of the experiment, piglets were euthanised and samples of duodenum, jejunum and ileum were collected for assessment of microscopic morphology, gut microbiota and immune function. Samples of jejunum were processed to determine cytokines. The digesta from caecum and colon were used for the analysis of VFA. Compared to the control, piglets fed the chlortetracycline-supplemented diet showed higher ADG (338 vs 275 g/day), increased digestibility of gross energy (82 vs 75%), DM (81 vs 73%) and protein (79 vs 71%), reduced *E. coli* counts in caecum (4.7 vs 5.3 $\log_{10}$ CFU/g digesta). In both experiments, chlortetracycline at 80 mg/kg feed showed growth-promoting effects in weaned piglets.

In the study published by Song et al. (2013) a total of 96 piglets (Duroc × (Landrace × Large White), initial BW 5.6 kg, weaned at 21 days of age) were distributed in 24 pens (four piglets per pen) and allocated to one of four dietary treatments (6 pens or replicates per treatment). Two were the relevant treatments obtained from a basal diet (maize–soybean–sprayed plasma and fish meal) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at 75 mg/kg feed. The study lasted 2 weeks. Feed and water were available ad libitum, FI was recorded and piglets were weighed at the beginning and at the end of the experiment to calculate ADG and G:F. Faecal score was assessed using a 1-to-4 scale (1 = normal; 4 = severe scours). Blood samples were taken for the analysis of D-lactate and diamine oxidase (DAO). At the end of the trial, one pig per replicate was euthanised and the intestinal contents from ileum and proximal colon were collected for microbial population enumeration. Ileal mucosa was analysed for pro-inflammatory cytokines (IL-6; TNF-α and IFN-Υ). Piglets fed the chlortetracycline-supplemented diet showed, compared to the control group, greater ADG (257 vs 224 g/day) and G:F (0.826 vs 0.746). Faecal score was lower in chlortetracycline-treated piglets (1.95 vs 3.94). Chlortetracycline-treated piglets showed less *Clostridium* (5.6 vs 6.5 $\log_{10}$ CFU/g in ileum and 6.8 vs 7.8 $\log_{10}$ CFU/g in colon) and *E. coli* (6.7 vs 7.6 $\log_{10}$ CFU/g in ileum and 7.7 vs 8.7 $\log_{10}$ CFU/g in colon). Chlortetracycline-treated piglets had lower blood levels of D-lactate and of DAO, and lower levels of mucosal IL6 and TNF-α. The antimicrobial chlortetracycline at 75 mg/kg feed was effective for alleviating diarrhoea and inflammation and improving intestinal microbiota and mucosal barrier integrity, showing growth promotion effects in weaned piglets.

In the study of Stahly et al. (1980), a total of 183 piglets (Hampshire × Yorkshire; initial BW 6.75 kg, weaned at 28 days of age) were distributed in 32 pens (4–8 piglets per pen depending on litter size) and allocated to one of four dietary treatments (eight pens or replicates per treatment from two separate trials with four replicates per trial). Two were the relevant treatments obtained from a basal diet (maize–soybean) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; Aureomycin 50, American Cyanamid, Co., Princeton, NJ, USA) at 55 mg/kg feed. The study lasted 28 days. Feed and water were available ad libitum, FI and piglet weight were determined at 14-day intervals to calculate ADG and F:G. Piglets fed the chlortetracycline-supplemented diet showed, compared to the control group, greater ADG (230 vs 188 g/day) and daily FI (419 vs 370 g/day), and improved F:G (1.83 vs 2.01). The antimicrobial chlortetracycline at 55 mg/kg feed had growth-promotion effects in weaned piglets.

The study of Teague et al. (1966) included two pooled data analyses using information from 20 trials carried out between 1950 and 1963 with pigs for fattening (Duroc), with chlortetracycline (unspecified chemical form; from 1951 to 1954 Aurofac 2A feed supplement containing 7.94 g chlortetracycline/kg, from 1957 to 1963 Aurofac 10 feed supplement containing 22.05 g chlortetracycline/kg; American Cyanamid Co) ranging from 11 to 88 mg/kg feed. Each trial consisted of a direct comparison in growth performance between pigs fed basal diets either not supplemented (control) or supplemented with chlortetracycline. At the start of the trials, pigs were 7–9 weeks old. Average daily weight gain (ADG) and F:G were calculated from the start of the experiment to 54 kg BW, and from 54 kg to market weight. In the first data analysis, results from ten pairwise comparative trials were pooled. In five of these trials, there were two lots (pens) and between 10 and 17 pigs per treatment in each trial. Treatments were control non-supplemented diet and the same diet supplemented with 10 mg chlortetracycline/kg feed. In the other five trials, there were two or three lots (pens) and between 16 and 27 pigs per treatment in each trial. Treatments were control non-supplemented diet and the same diet supplemented with 11 mg chlortetracycline/kg feed. By pooling data analysis from the ten trials adjusted for year and initial weight, it was observed that pigs fed diets with 10–11 mg chlortetracycline/kg showed greater ADG than control (0.65 vs 0.62 kg/day) up to 54 kg BW, but not at higher BW, with no effect on feed efficiency. In the second data analysis, results from ten pairwise comparative trials were pooled. In each of these trials, there were two or three lots (pens) and between 14 and 27 pigs per treatment. Treatments were control non-supplemented diet and the same diet supplemented with 22 mg chlortetracycline/kg feed. By pooling data analysis adjusted for year and initial weight, it was observed that pigs fed diets with 22 mg chlortetracycline/kg showed greater ADG than control (0.67 vs 0.62 kg/day) up to 54 kg BW, but not at heavier BW, with no effect on feed efficiency. Other trials reported in the publication by Teague et al. (1966) showed effects of supplementing diets for pigs for fattening with chlortetracycline at 11, 22, 44 or 88 mg/kg increasing ADG and enhancing F:G. Thus, chlortetracycline at 11 or 22 mg/kg feed showed growth-promoting effect in pigs for fattening. These growth-promoting effects were also observed in animals fed diets containing 44 or 88 mg chlortetracycline/kg feed.

In the study of Thu et al. (2011) a total of 120 piglets (Duroc × (Landrace × Large White), initial BW 6.3 kg, weaned at 26 days of age) were distributed in 30 pens (four piglets per pen) and allocated to one of five dietary treatments (six pens or replicates per treatment). Two were the relevant treatments obtained from a basal diet (maize–soybean) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at 300 mg/kg feed. The study lasted five weeks. Feed and water were available ad libitum, FI and piglet weights were recorded weekly and ADG and F:G were calculated. Faecal score was assessed using a 1-to-3 scale (1 = normal; 3 = watery) on days 3, 5, 10, 12, 17 and 24. At the end of the trial, three piglets per treatment were euthanised and intestinal tissues (duodenum, jejunum and ileum) were collected for *villi* height and crypts depth measurements. Faecal samples were collected for microbial counts and determination of SCFAs. At the end of the trial, piglets receiving chlortetracycline showed, compared to the control group, greater final BW (14.1 vs 12.8 kg) and ADG (213 vs 186.7 g/day) and better F:G (1.89 vs 2.17). Piglets fed chlortetracycline had lower diarrhoea score (0.24 vs 0.56) and larger duodenal *villi* height (499 vs 403 μm). The antimicrobial chlortetracycline at 300 mg/kg feed had growth promotion effects in weaned piglets.

In the study of Wang et al. (2012) a total of 90 piglets (Duroc × (Landrace × Large White); initial BW 7.2 kg, weaned at 21 days of age) were distributed in nine pens (ten piglets per pen) and allocated to one of three dietary treatments (three pens or replicates per treatment). In the two relevant treatments, the basal diet (maize–soybean–fish meal) was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at 100 mg/kg feed. The study lasted 28 days. Feed intake and piglet weights were recorded and ADG and F:G were calculated. Faecal score was assessed. At the end of the trial, two piglets per pen were euthanised and, serum and intestinal digesta and tissue (duodenum, jejunum and ileum) were collected for histomorphology and microbial counts. At the end of the trial, piglets receiving chlortetracycline showed, compared to the control group, greater final weight (17.5 vs 15.1 kg), ADG (360 vs 287 g/day) and FI (685 vs 597 g/day) and better F:G (1.90 vs 2.08). Piglets fed chlortetracycline-supplemented diet showed less *E. coli* in duodenum (6.7 vs 7.9 $\log_{10}$ CFU/g), jejunum (7.1 vs 9.2 $\log_{10}$ CFU/g) and caecum (9.1 vs 9.7 $\log_{10}$ CFU/g) and more *Lactobacilli* in duodenum (5.8 vs 5.2 $\log_{10}$ CFU/g) and jejunum (5.9 vs 5.3 $\log_{10}$ CFU/g). Furthermore, chlortetracycline-treated piglets showed shorter crypt depth in duodenum (150 vs 169 μm), jejunum (149 vs 154 μm) and ileum (133 vs 138 μm) and higher *villus* height to crypt depth ratio in the duodenum (2.54 vs 2.28). The antimicrobial chlortetracycline at 100 mg/kg feed had growth promotion effects in weaned piglets.

In the study of Wang et al. (2019) a total of 108 piglets (Duroc × (Landrace × Large White); initial BW 7.1 kg, weaned at 28 days of age) were distributed in 18 pens (three female and three male piglets per pen) and allocated to one of three dietary treatments (six pens or replicates per treatment). Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; commercially available chlortetracycline with a purity of 15%) at 75 mg/kg feed. The study lasted 28 days. Feed and water were offered ad libitum, and FI and piglet weights were recorded every 2 weeks, and ADG and F:G were calculated. Faecal score was assessed. Diarrhoea prevalence was recorded. At the end of the trial six piglets per treatment were selected to collect blood samples (determination of immunoglobulins and inflammatory cytokines) and three piglets per treatment were euthanised and samples of digesta were collected to determine SCFAs and microbiota composition. At the end of the trial, piglets receiving chlortetracycline showed, compared to the control group, lower concentrations of lactic and propionic acids in colonic digesta and lower levels in blood serum of IL-10, IL-1β, IL-6 and IFN-ϒ. At a phylum level, in the colon, Firmicutes were higher in chlortetracycline group while Bacteroidetes were lower; in caecum Spirochaetae were less abundant. No differences were observed for growth parameters. The antimicrobial chlortetracycline at 75 mg/kg feed did not show growth promotion effects in weaned piglets.

In the study of Williams et al. (2018) a total of 300 piglets (DNA 200 × 400 Columbus NE, 5.9 kg initial BW) weaned at 21 days of age, were distributed in 60 pens (five piglets per pen) and allocated to one of six dietary treatments (ten pens or replicates per treatment). Two were the relevant treatments obtained from two basal diets —phase 1 days 0–14 (maize–soybean–whey) and phase 2 days 14–42 (maize–soybean)— which were either not supplemented (control) or supplemented with chlortetracycline at 400 mg/kg feed (unspecified chemical form; Zoetis Services, LLC, Florham Park, NJ, USA). The study lasted 42 days. Feed and water were offered ad libitum. Feed intake and piglet weights were recorded weekly at pen level, and ADG and G:F were calculated. On days 0, 21 and 42 faecal samples were collected from three randomly selected piglets per pen for bacterial isolation and antimicrobial resistance test of faecal *E. coli* isolates. At the end of the trial, piglets receiving chlortetracycline-supplemented diets showed, compared to the control group, greater final weight (25.6 vs 24.2 kg), ADG (469 vs 424 g/day) and FI (726 vs 644 g/day), with no differences in G:F. The antimicrobial chlortetracycline at 400 mg/kg feed showed growth promotion effects in weaned piglets.

In the study of Zhao et al. (2015) a total of 150 piglets (Duroc × (Landrace × Large White); initial BW 9.2 kg) weaned at 28 days of age, were distributed in 30 pens (five piglets per pen) and allocated to one of five dietary treatments (six pens or replicates per treatment). Two were the relevant treatments obtained from a basal diet (maize–soybean–fish meal) which was either not supplemented (control) or supplemented with chlortetracycline (unspecified form) at 150 mg/kg feed. The study lasted 21 days. Feed and water were offered ad libitum, FI per pen was recorded and piglets were weighed on days 1, 10 and 21, and ADG and F:G were calculated. Diarrhoea incidence was monitored. At the end of the trial six pigs for treatment were euthanised and blood samples were taken for determination of serum glucose, BUN, T3, T4, GH and IGF-1. In addition, samples of digesta from caecum, colon and rectum were taken for microbiota enumeration (*E. coli*, *Bifidobacterium* and *Lactobacillus*). Piglets receiving chlortetracycline-supplemented diet showed, compared to the control group, lower diarrhoea incidence (3.67% vs 5.61%) and higher serum levels of T3, T4 and IGF-1. No differences were observed for growth parameters, except that initial weight was greater in control than in chlortetracycline piglets. The antimicrobial chlortetracycline at 150 mg/kg feed did not show growth promotion effects in weaned piglets.

3.3.2.3.3. *Studies in poultry*

In the study of Aguirre et al. (2015) a total of 300 one-day-old Cobb chickens for fattening were allocated to five dietary treatments and distributed in six pens per treatment, in groups of ten chickens per each pen. Three basal diets based on maize and soybean meal (booster, starter and finisher) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of 150 mg chlortetracycline/kg feed (unspecified form; 1 g chlortetracycline 15%/kg feed). Viability was 100%. Chicken weights were recorded on days 7, 28 and 42, cumulative FI was recorded on days 8, 28 and 42 and cumulative BW gain and F:G were calculated. A digestibility trial was conducted for 6 days starting on day 23. Three chickens per treatment randomly selected (replicates not indicated) were transferred to individual cages for faeces collection. Crude protein, gross energy and apparent metabolisable energy digestibility were assessed and at the end of the experiment (42 days), these chickens were slaughtered. Intestines of each slaughtered bird were collected for histological analysis. At the end of the trial, the birds treated with chlortetracycline at 150 mg/kg feed, compared to the control group, showed higher final BW (2,078 vs 1,862 g), higher cumulative BW gain (1,921 vs 1,701 g) and improved F:G (1.90 vs 2.19). Crude protein and gross energy digestibility of animals treated with chlortetracycline was improved, (75.5 vs 70.1% and 83.1 vs 78.6%, respectively), compared to the control group. In addition, in birds treated with chlortetracycline, compared to the control group, an increase of duodenal *villi* height (820 vs 673 μm) and a decrease of crypt depth (334 vs 412 μm) were observed. Dietary chlortetracycline supplementation at 150 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Alvares et al. (1964), a total of 1,440 1-day-old male Vantress-Arbor Acres chickens for fattening were used in three experiments (one preliminary study and two experiments). In each experiment, birds were allocated to 12 dietary treatments and distributed in four lots per treatment, in groups of 15 birds per each lot. Two successive experiments with 24 lots each were carried out, with means for the parameters established from both experiments. A basal diet was either not supplemented (control) or supplemented with different treatments. Six were the relevant treatments: three control diets (basal diet containing sucrose, dextrose or starch) and three treatments consisting of chlortetracycline supplementation (unspecified form) at a concentration of 100 mg/kg feed, for the diet containing sucrose, dextrose or starch. Mortality and health status were not indicated. Chicken BW was recorded on days 0, 14 and 28 and cumulative weight gain and feed conversion ratio were calculated. At the end of the trial, the birds treated with chlortetracycline at 100 mg/kg feed, compared to the control group, showed higher BW gain (319 vs 265 g) and improved feed utilisation efficiency (0.55 vs 0.52). No effect of the supplementation with chlortetracycline was observed with dextrose and starch diets. Dietary chlortetracycline supplementation at 100 mg/kg feed had a growth-promoting effect in chickens for fattening, only with sucrose diet.

In the study of Bagal et al. (2016), a total of 400 1-day-old Cobb chickens for fattening were allocated to 10 dietary treatments and distributed in four pens per treatment, in groups of 10 birds per pen. Two basal diets, based on maize and soybean meal (starter and grower), were either not supplemented (control) or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of chlortetracycline supplementation (unspecified form) at a concentration of 335 mg/kg feed. Mortality and health status were not indicated. Chicken weight and FI were recorded on days 0, 15, 30 and 45 and cumulative weight gain and feed conversion ratio were calculated. At the end of the trial (45 days), the birds treated with chlortetracycline at 335 mg/kg feed, compared to the control group, showed higher cumulative weight gain (2,404 vs 2,236 g), higher cumulative FI (4,702 vs 4,222 g) and improved F:G (1.95 vs 1.97). Dietary chlortetracycline supplementation at 335 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Bai et al. (2013), a total of 696 1-day-old male Cobb chickens for fattening were allocated to four dietary treatments and distributed in six pens per treatment, in groups of 29 birds per each pen. Two basal diets based on maize and soybean meal (starter and grower) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline supplementation (unspecified form) at a concentration of 100 mg/kg feed. Mortality and health status were not indicated. Animal weight and daily FI were recorded on days 1, 21 and 42 and daily weight gain and F:G were calculated. On days 21 and 42, two birds from each replicate were selected according to the average BW of the pen, and slaughtered. At the end of the trial (42 days), the birds treated with chlortetracycline at 100 mg/kg feed, compared to the control group, showed no effect on growth performance. Dietary chlortetracycline supplementation at 100 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

The study of Begin (1971) reported three different experiments with different concentrations of chlortetracycline. In Experiment 1, a total of 120 one-day-old male Inbred-Hybrid (light) chickens for fattening were allocated to four dietary treatments and distributed in three pens per treatment, in groups of ten birds per each pen. In Experiment 2, a total of 90 one-day-old male New Hampshire × Columbian cross (heavy) chickens for fattening were allocated to three dietary treatments and distributed in three pens per treatment, in groups of ten birds per each pen. In Experiment 3, a total of 120 one-day-old male New Hampshire × Columbian cross heavy chickens for fattening were allocated to four dietary treatments and distributed in three pens per treatment, in groups of ten birds per each pen. A basal diet based on maize and soybean meal was either not supplemented (control) or supplemented with different treatments. The relevant treatments were: in Experiments 1 and 3, a control diet and three treatments consisting of chlortetracycline supplementation (unspecified chemical form; Aurofac 50 from American Cyanamid Co., Inc.) at concentrations of 50, 100 and 200 mg/kg feed; in Experiment 2, a control diet and two treatments consisted of chlortetracycline supplementation at a concentration of 100 and 200 mg/kg feed. In Experiment 3, the determination of carcass energy gain was accomplished by slaughtering 10-day-old chicks (neither number nor origin was indicated) to obtain the initial gross energy content of the birds and at the end of the experiment (day 28), five chickens/each experimental group were fasted for 16 h and selected for final energy determinations, after slaughter. Mortality and health status were not indicated. In the three experiments, chicken BW and cumulative FI were recorded at the start (day 1) and at the end (day 28) of the trials, and cumulative weight gain and feed conversion ratio were calculated. Excreta samples were collected from each pen on two consecutive days during the fourth week of each experiment, in order to calculate the metabolisable energy of the experimental diets, corrected for nitrogen equilibrium. Energy utilisation values were calculated in the three experiments. At the end of the trial, in Experiment 1, the birds treated with chlortetracycline at 50, 100 or 200 mg/kg feed compared to the control group, showed higher BW gain (272, 287 and 277 g, respectively, vs 265 g), improved feed utilisation efficiency (0.52, 0.52 and 0.50, respectively, vs 0.47) and improved metabolisable energy utilisation efficiency (gain to metabolisable energy ratio) (0.18, 0.18 and 0.17, respectively, vs 0.16); in Experiment 2, the birds treated with chlortetracycline at the concentration of 100 or 200 mg/kg feed compared to the control group, showed higher BW gain (311 and 337 g, respectively, vs 300 g), improved feed utilisation efficiency (0.55 and 0.53, respectively, vs 0.49) and improved metabolisable energy utilisation efficiency (gain to metabolisable energy ratio) (0.19 and 0.18, respectively, vs 0.17); in Experiment 3, the birds treated with chlortetracycline at 50, 100 or 200 mg/kg feed compared to the control group, showed higher BW gain (327, 327 and 323 g, respectively, vs 298 g), improved feed utilisation efficiency (0.55, 0.57 and 0.55, respectively, vs 0.53), higher carcass energy gain (591, 561 and 587 kcal, respectively, vs 521 kcal) and improved metabolisable energy efficiency (33.6, 34 and 34%, respectively, vs 31%). There was the same effect of chlortetracycline on the different performance parameters, whatever its concentrations. Dietary chlortetracycline supplementation at 50, 100 and 200 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Bostami et al. (2016), a total of 300 1-day-old mixed-sex Ross (exact strain not specified) chickens for fattening were allocated to three dietary treatments and distributed in ten pens per treatment, in groups of ten chickens per pen. Two basal diets based on maize and soybean meal (starter and finisher) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of 1,000 mg chlortetracycline hydrochloride/kg feed (corresponding to 930 mg chlortetracycline/kg feed). Mortality was recorded daily. Chicken weights and FI were recorded on days 0, 21, 28 and 35 and daily weight gain and F:G were calculated. At the end of the trial (42 days), three birds/each pen were randomly selected to perform microbial analysis. At the end of the trial, the birds treated with chlortetracycline hydrochloride at 1,000 mg/kg feed, compared to the control group, showed higher daily weight gain (63.2 vs 57.6 g), improved F:G (1.49 vs 1.60) and reduced mortality (4 vs 8%). The caecal digesta of the treated animals showed a reduction in *E. coli* (6.55 vs 7.47 $\log_{10}$ CFU/g) and *Salmonella* (6.21 vs 6.59 $\log_{10}$ CFU/g). Dietary chlortetracycline hydrochloride supplementation at 1,000 mg/kg feed (corresponding to 930 mg chlortetracycline/kg feed) had a growth-promoting effect in chickens for fattening.

In the study of Chen et al. (2018) a total of 144 1-day-old male Arbor Acres Plus chickens for fattening were allocated to three dietary treatments and distributed in six cages per treatment, in groups of eight birds per each cage. Two basal diets based on maize and soybean meal (starter and grower) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 40 mg/kg feed. Mortality and health status were not indicated. Chicken weight and daily FI were recorded on days 21 and 42 (after 12 h fasting) and daily weight gain and feed conversion ratio were calculated. On days 21 and 42, 6 birds per treatment were slaughtered and thymus, bursa and spleen were weighed, content of caeca was collected and cultured to determine colonies of *E. coli* and *Lactobacillus*; the jejunum and ileum were also excised and their mucosa was collected for immune and oxidative markers. At the end of the trial (42 days), the birds treated with chlortetracycline at 40 mg/kg feed compared to the control group, showed higher daily weight gain (60.7 vs 55.6 g) and improved F:G (1.69 vs 1.85). At day 42, relative thymus weight was also higher than in controls (5.98 vs 4.12 g/kg), the jejunum and ileum of treated chickens exhibited higher concentration of IgA in jejunum and higher concentration of IgG in ileum. Dietary chlortetracycline supplementation at 40 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Dong et al. (2011) a total of 360 one-day-old male Arbor Acres chickens for fattening were allocated to five dietary treatments and distributed in six cages per treatment, in groups of 12 birds per each cage. Two basal diets based on maize and soybean meal (starter and grower) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 150 mg/kg feed. Mortality and health status were not indicated. Animal weight and daily FI were recorded on days 0, 21, 28 and 42 (after 12 h fasting) and feed conversion ratio was calculated. On day 42, one bird per replicate was slaughtered after 12 h fasting, blood and liver samples were collected and a sample of the left breast muscle and the right breast muscle were collected. At the end of the trial (42 days), the birds treated with chlortetracycline at 150 mg/kg feed, compared to the control group, showed higher BW (2,607 vs 2,488 g) and higher daily FI (112.2 vs 107.5 g/day). Dietary chlortetracycline supplementation at 150 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Guo et al. (2020), a total of 240 one-day-old mixed-sex Arbor Acres chickens for fattening were allocated to six dietary treatments and distributed in five cages per treatment, in groups of eight birds per cage. Two basal diets based on maize and soybean meal (starter and grower) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form, purity 100%) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were not indicated. Chicken weight and daily FI were recorded on days 21 and 42, and daily weight gain and feed conversion ratio were calculated. At the end of the trial (42 days), the supplementation of chlortetracycline at 50 mg/kg feed had no effect on growth performance. Dietary chlortetracycline supplementation at 50 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Han et al. (2012) a total of 252 one-day-old male Arbor Acres chickens for fattening were allocated to three dietary treatments and distributed in 14 cages per treatment, in groups of six birds per cage. Two basal diets based on maize and soybean meal (starter, 1–21 days and grower, 22–42 days) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 80 mg/kg feed in the starter diet and 50 mg/kg feed in the grower diet. Mortality and health status were not indicated. Chicken weight and daily FI were recorded on days 21 and 42 (after 12 h fasting) and daily weight gain and feed conversion ratio were calculated. The excreta were collected from each cage from day 19 to day 21, and from day 22 to day 42 to measure digestibility of nutrients and energy. At 42 days of life, one bird per cage was slaughtered and the content of caeca was collected and cultured to determine colonies of *E. coli* and *Lactobacillus* and the duodenum, jejunum and ileum were also excised for the analysis of their morphology. At the end of the trial (42 days), the birds treated with chlortetracycline at 80 mg/kg feed in the starter diet and at 50 mg/kg feed in the grower diet, compared to the control group, showed higher daily weight gain (54 vs 50 g/day), higher daily FI (92 vs 85 g/day) and lower count of *E. coli* (5.31 vs 6.19 $\log_{10}$ CFU/g wet digesta). The dietary supplementation of chlortetracycline had no effect on nutrient and energy digestibility and on intestinal morphology. Dietary chlortetracycline supplementation at 80 mg/kg feed from 1 to 21 days of age and at 50 mg/kg feed from 22 to 42 days of life had a growth-promoting effect in chickens for fattening.

In the study of He et al. (2019) a total of 168 one-day-old mixed-sex Arbor Acres chickens for fattening were allocated to three dietary treatments and distributed in seven cages per treatment, in groups of eight birds per cage. Two basal diets based on maize and soybean meal (starter and grower) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 75 mg/kg feed. Mortality and health status were not indicated. Chicken weight and daily FI were recorded on days 0, 21 and 42 and daily weight gain and feed conversion ratio were calculated. On days 39 and 42, excreta samples were collected from each replicate to determine total tract digestibility. At 42 days of age, birds were slaughtered. At the end of the trial (42 days), the supplementation with chlortetracycline at 75 mg/kg feed, compared to the control group, had no effect on growth performance, but increased the digestibility of DM and organic matter, dietary chlortetracycline supplementation at 75 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Hong et al. (2019) a total of 180 one-day-old male Cobb 500 chickens for fattening were allocated to three dietary treatments and distributed in four pens per treatment, in groups of 15 birds per cage. Two basal diets based on maize and soybean meal (starter and grower) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified chemical form; Hubei Huada Real Technology Co., Wuhan City, Hubei Province, China) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were not indicated. Chickens weight and daily FI were recorded on days 7, 14, 21 and 35 and daily weight gain and feed conversion ratio were calculated at the end of the trial (day 42). On days 7, 14, 21 and 35, 8 birds per treatment were slaughtered to sample the caecal content. At the end of the trial (42 days), the birds treated with chlortetracycline at 50 mg/kg feed, compared to the control group, showed higher final BW (2,206 vs 1,901 g), higher daily weight gain between 14 and 21 days (70 vs 51.3 g/day) and between 21 and 35 days (92.8 vs 80.2 g/day) and improved F:G (1.63 vs 2.02). Furthermore, compared to the control group, the chickens treated with chlortetracycline showed greater proportion of caecal Firmicutes bacteria (0.7% vs 0.6%) while caecal Bacteroidetes were in lower proportion (0.15% vs 0.25%). Dietary chlortetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Hossain et al. (2012c), a total of 140 1-day-old Ross chickens for fattening were allocated to four dietary treatments and distributed in five cages per treatment, in groups of seven birds per cage. Two basal diets based on maize and soybean meal (starter and finisher) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were not indicated. Chicken weight and daily FI were recorded on days 1, 21, 28 and 35, daily weight gain was recorded daily and feed conversion ratio was calculated at the end of the trial (day 35). At the end of the experiment, 15 chickens per treatment were selected and slaughtered to determine the weight of organs and of thighs and breast meat. At the end of the trial (35 days), the supplementation of chlortetracycline at 50 mg/kg feed, compared to the control group, had no effect on growth performance but showed greater breast relative weight (14.5% vs 12.5%), lower thigh relative weight (12% vs 13%) and lower large intestine relative weight (0.12% vs 0.19%). Dietary chlortetracycline supplementation at 50 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Hosseini and Meimandipour (2018), a total of 250 one-day-old male Ross 308 chickens for fattening were allocated to five dietary treatments and distributed in five pens per treatment, in groups of ten birds per each pen. Two basal diets based on maize and soybean meal (starter 1–21 days and grower 22–42 days) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 80 mg/kg feed in the starter diet and 50 mg/kg feed in the grower diet. Mortality and health status were checked every day. Chicken weight and daily FI were recorded on days 1, 21, 28 and 42 and daily weight gain and feed conversion ratio were calculated. On day 42, all the birds were slaughtered. At the end of the trial (42 days), the birds treated with chlortetracycline at 80 mg/kg feed in the starter diet and at 50 mg/kg feed in the grower diet, compared to the control group, showed higher cumulative BW gain (2,025 vs 1,925 g). Dietary chlortetracycline supplementation at 80 mg/kg feed from 1 to 21 days of age and then at 50 mg/kg feed from 22 to 42 days of life had a growth-promoting effect in chickens for fattening.

The study of Huang et al. (2018) reported two experiments. In Experiment 2, a total of 600 one-day-old Arbor Acres chickens for fattening were allocated to five dietary treatments and distributed in ten cages per treatment, in groups of 12 birds per cage for 42 days. In Experiment 3, a total of 600 one-day-old local Yellow-Feather chickens for fattening were allocated to five dietary treatments and distributed in ten cages per treatment, in groups of 12 birds per each cage for 56 days. A basal diet was either not supplemented (control) or supplemented with different treatments. In the 2 experiments two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified chemical form; Citifac®, chlortetracycline 20% w/w premix) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were checked daily. In both experiments, chicken BW and cumulative FI were recorded for each replicate at the start (day 1) and at the end of the trial (day 42 for Experiment 2 and day 56 for Experiment 3), and cumulative weight gain and feed conversion ratio were calculated. In Experiment 2, ten birds per treatment were selected on days 21 and 42, slaughtered and ileal tissue was sampled. Moreover, five additional birds were slaughtered at the end of each study and the digesta from the duodenum, jejunum, ileum and caecum and colorectum was collected to sequence the microbiota. At the end of the trial, (1) in Experiment 2, the chlortetracycline supplementation had no effect on growth performance; (2) in Experiment 3, the birds treated with chlortetracycline at the concentration of 50 mg/kg feed compared to the control group, showed higher cumulative FI (3,548 and 3,443 g). In both experiments the birds treated with chlortetracycline at 50 mg/kg feed, compared to the control group, showed lower abundances of *Corynebacterium*, *Brachybacterium* and *Dietzia* and higher abundances of *Kitasatospora* and *Streptomyces*. Dietary chlortetracycline supplementation at 50 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Kim and Choi (2014) a total of 150 one-day-old male Arbor Acres chickens for fattening were allocated to five dietary treatments and distributed in three pens per treatment, in groups of ten birds per each pen. Two basal diets based on maize, soybean meal and wheat bran (starter, 1–21 days and finisher, 22–35 days) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified chemical form; CTC Bio Inc., Seoul, South Korea) supplementation at a concentration of 500 mg/kg feed. Mortality and health status were not specified. Chicken weights were recorded daily from the start until the end of the experiment (35 days), FI was recorded weekly per pen and cumulative daily weight gain and feed conversion ratio were calculated. At the end of the trial (35 days), the birds treated with chlortetracycline at 500 mg/kg feed, compared to the control group, showed no effect on growth parameters. Dietary chlortetracycline supplementation at 500 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Li et al. (2007) a total of 196 one-day-old male Arbor Acres chickens for fattening were allocated to four dietary treatments and distributed in seven cages per treatment, in groups of seven birds per cage. Two basal diets (starter, 1–21 days and grower, 22–42 days) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 80 mg/kg feed in the starter diet and 50 mg/kg feed in the grower diet. Mortality and health status were not specified. Chicken weight and cumulative FI were recorded on days 21 and 42, and daily FI daily weight gain and feed conversion ratio were calculated. At the end of each phase (days 21 and 42) excreta from each cage were collected to measure digestibility. At 42 days of age, seven birds per treatment (one bird per each cage) were bled, slaughtered and caeca content was sampled to enumerate *Lactobacillus* and *E. coli*. At the end of the trial (42 days), the birds treated with chlortetracycline at 80 mg/kg in the starter diet and at 50 mg/kg feed in the grower diet, compared to the control group, showed higher daily weight gain (49.5 vs 47.3 g/day) and lower counts of *E. coli* (5.32 vs 6.20 $\log_{10}$ CFU/g digesta), but no effect on digestibility. Dietary chlortetracycline supplementation at 80 mg/kg in the starter diet (1–21 days) and at 50 mg/kg feed in the grower diet (22–42 days) had a growth-promoting effect in chickens for fattening.

In the study of Li et al. (2020) a total of 144 one-day-old male Arbor Acres chickens for fattening were allocated to three dietary treatments and distributed in six cages per treatment, groups of eight 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 chlortetracycline (unspecified form) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were not specified. Chicken weight and FI were recorded at the start and at the end (21 days) of the study, and daily FI daily weight gain and feed conversion ratio were calculated. At 21 days of age, six birds/ treatment (one bird per cage) were selected, slaughtered (after a 12-h fast), the immune organs were weighed and duodenal, jejunal and ileal mucosa were sampled. At the end of the trial (21 days), the birds treated with chlortetracycline at 50 mg/kg feed, compared to the control group, showed improved F:G (1.53 vs 1.60), higher duodenal *villi* length (1,729 vs 1,523 μm) and higher *villus* height to crypt depth ratios in the duodenum (9.80 vs 7.24) and the jejunum (5.75 vs 4.51), and shorter crypt depth in the jejunum (209 vs 289 μm) and the ileum (125 vs 188 μm). Dietary chlortetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Li et al. (2020) a total of 120 one-day-old mixed sex Arbor Acres chickens for fattening were allocated to three dietary treatments and distributed in 40 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 of chlortetracycline (unspecified chemical form; chlortetracycline (20%), 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 feed conversion ratio were calculated. At 42 days of age, all the birds were bled and slaughtered. At the end of the trial (42 days), the birds treated with chlortetracycline at 50 mg/kg feed, compared to the control group, showed higher BW (2,389 vs 2,107 g), higher daily FI (96 vs 85 g/day), higher carcass weight (2,312 vs 2,033 g) and higher blood triglycerides concentration (3 vs 2 mg/mL). Dietary chlortetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Liao et al. (2015) a total of 320 one-day-old male Arbor Acres chickens for fattening were allocated to five dietary treatments and distributed in eight cages per treatment, in groups of eight birds per cage. Two basal diets based on maize and soybean meal (starter 1−21 days and grower 22−42 days) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form; Aureomycin) supplementation at a concentration of 150 mg/kg feed. Mortality and health status were checked daily. Chicken weight and FI were recorded on days 21 and 42, and daily FI daily weight gain and feed conversion ratio were calculated. The birds treated with chlortetracycline at 150 mg/kg feed, compared to the control group, showed higher daily gain from day 22 to day 42 (82.7 vs 76.0 g/day). Dietary chlortetracycline supplementation at 150 mg/kg feed had a growth-promoting effect on the performance of chickens for fattening.

In the study of Mahfuz et al. (2019), a total of 252 1-day-old male Arbor Acres chickens for fattening were allocated to four dietary treatments and distributed in seven cages per treatment, in groups of nine birds per cage. Two basal diets based on maize and soybean meal (starter and finisher) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 80 mg/kg feed. Mortality and health status were not specified. Animal weight and FI were recorded weekly, and daily FI daily weight gain and feed conversion ratio were calculated. Blood from seven birds per treatment (one bird per cage) was collected on days 14, 21, 28 and 42. At the end of the trial (42 days), the birds treated with chlortetracycline at 80 mg/kg feed, compared to the control group, showed no effect on growth parameters, they only showed lower plasma total cholesterol concentration. Dietary chlortetracycline supplementation at 50 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Proudfoot et al. (1988), a total of four experiments were carried out and the outcomes analysed separately by pooling results from trials 1 and 2 apart from trials 3 and 4. In Experiment 1/2, a total of 800 1-day-old male Arbor Acres chickens for fattening were allocated to four dietary treatments and distributed in four pens per treatment, in groups of 50 birds per pen. Two basal diets based on maize, soybean meal and wheat (starter and finisher) were either not supplemented (control) or supplemented with different treatments. All the four treatments were relevant: a control (a) and three treatments consisting of chlortetracycline (unspecified form) supplementation at a concentration of 5.5 mg/kg feed (treatment b), or a quantity in drinking water to give equivalence of 5.5 mg/kg feed (treatment c), or a quantity in drinking water with a concentration of half of the concentration used in treatment c (treatment d). In Experiment 3/4, a total of 2,400 one-day-old male Arbor Acres chickens for fattening were allocated to six dietary treatments and distributed in four pens per treatment, in groups of 100 birds per pen. Two basal diets based on maize, soybean meal and wheat (starter and finisher) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline supplementation at a concentration of 5.5 mg/kg feed. Mortality and health status were checked daily in all the experiments. Chicken weight and cumulative FI were recorded on days 21 and 42, and feed conversion ratio was calculated. As in the paper the water intake is not reported and the chlortetracycline concentration in the drinking water is not provided, only the treatment given in feed will be considered. At the end of the trial (42 days), the birds treated with chlortetracycline at 5.5 mg/kg feed, compared to the control group, showed no difference in any of the performance parameters. Dietary chlortetracycline supplementation at 5.5 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Qu et al. (2019), a total of 144 1-day-old male Arbor Acres chickens for fattening were allocated to three dietary treatments and distributed in six cages per treatment, in groups of eight birds per each cage. Two basal diets (starter, 1–21 days and grower, 22–42 days) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified chemical form; Jinhe Biotechnology Co. Ltd. Hohhot, China) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were checked daily. Chicken weight and cumulative FI were recorded on days 21 and 42 (after a 12-h fast), and daily FI daily weight gain, and feed conversion ratio were calculated. At the end of the trial (42 days), the birds treated with tetracycline at 50 mg/kg feed compared to the control group, showed higher daily weight gain (52.6 vs 49.7 g/day). Dietary chlortetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Shi et al. (2005) two experiments were reported. In Experiment 1, a total of 294 one-day-old male Arbor Acres chickens for fattening were allocated to seven dietary treatments and distributed in six cages per treatment, in groups of seven birds per cage. In Experiment 2 a total of 42 one-day-old male Arbor Acres chickens for fattening were allocated to seven dietary treatments and distributed in six individual cages per treatment. In both experiments, two basal diets based on maize and soybean meal (starter days 1 to 21 and finisher days 22–42) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 50 mg/kg feed. Mortality and health status were checked daily. In Experiment 1, chicken weight and cumulative FI were recorded on days 0, 21 and 42, and daily FI daily weight gain and feed conversion ratio were calculated. In Experiment 2, FI was recorded on days 19–21 and 40–42, and excreta were collected to determine gross energy and nitrogen contents. Apparent metabolisable energy, N retained per day and the efficiency of utilisation of nitrogen were calculated at the end of the trial (42 days). The birds treated with chlortetracycline at 50 mg/kg feed, compared to the control group, showed no differences in any of the measured parameters, in both experiments. Dietary chlortetracycline supplementation at 50 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Stutz and Lawton (1984), in Experiment 2, a total of 168 two-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 chlortetracycline (unspecified form) supplementation at a concentration of 55 mg/kg feed. The experiment lasted eight 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 (chlortetracycline treatment) were slaughtered for relative ileal weight determination, whereas ileal digesta from 12 (control) or six (chlortetracycline treatment) chickens were used for enumeration of *C. perfringens*. At the end of the experiment, the birds treated with chlortetracycline at 55 mg/kg feed, compared to the control group, showed higher daily weight gain (127 vs 111 g/day) and an improved F:G (1.21 vs 1.26), and had decreased relative ileum weight (1.10% vs 1.62% BW) and lower *C. perfringens* count (2.4 vs 3.8 log10/g digesta). Dietary chlortetracycline supplementation at 55 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Tang et al. (2014) a total of 300 one-day-old Arbor Acres chickens for fattening were allocated to five dietary treatments and distributed in six pens per treatment, in groups of ten birds per pen. Two basal diets based on maize and soybean meal (starter days 1–21 and grower days 22–42) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 40 mg/kg feed. Mortality was recorded. Chicken weight and FI were recorded on days 21 and 42 and F:G was calculated. On days 21 and 42, 6 chickens per treatment were slaughtered and jejunum and ileum were sampled and caeca content was collected for enumeration of *E. coli* and *Lactobacillus*. At the end of the trial (42 days), the birds treated with chlortetracycline at 40 mg/kg feed, compared to the control group, showed lower *E. coli* count (6.86 vs 7.32 $\log_{10}$ CFU/g digesta) but showed no effect on growth performance. Dietary chlortetracycline supplementation at 40 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Ürüşan and Bölükbaşı (2017), a total of 350 (175 males and 175 females) 1-day-old Ross PM 308 chickens for fattening were allocated to seven dietary treatments and distributed in five pens per treatment, in groups of 10 birds per each pen. Two basal diets based on maize, soybean meal and full-fat soybean (starter, days 1–21 and finisher, days 22–42) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 10 mg/kg feed. Mortality and health status were not specified. Chicken weight and FI were recorded and F:G was calculated. At 42 days of age, five chickens per treatment were slaughtered, carcass yield was calculated and intestinal content from jejunum was collected for enumeration of microbial population. At the end of the trial (42 days), the birds treated with chlortetracycline at 10 mg/kg feed, compared to the control group, showed higher slaughtering weight (2,782 vs 2,392 g), higher hot and cold carcass weights (2,092 vs 1,813 g and 2,050 vs 1,773 g, respectively), higher content of aerobe mesophilic bacteria (8.49 vs 7.92 CFU/g) and lactic bacteria (7.74 vs 7.28 CFU/g). Dietary chlortetracycline supplementation at 10 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Uuganbayar et al. (2005), a total of 180 40-week-old Tetra Brown laying hens were allocated to six dietary treatments with five replicates per treatment, in groups of six birds per treatment (each group consisted of three adherent cages of two birds). One basal diet based on maize grain and soybean was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified form) supplementation at a concentration of 500 mg/kg feed. Mortality and health status were not specified. Feed intake was recorded weekly. Egg production rate, egg weight, egg mass and feed conversion ratio were calculated. Fifteen eggs per treatment were selected for eggshell thickness measurements. At the end of the trial (56 days), the dietary supplementation with chlortetracycline at 500 mg/kg, compared to the control group, had no effect on laying performance. Dietary chlortetracycline supplementation at 500 mg/kg feed did not have a growth-promoting effect in laying hens.

In the study of Zhang et al. (2015) a total of 700 one-day-old Arbor Acres chickens for fattening were allocated to seven dietary treatments and distributed in five pens per treatment, in groups of 20 birds per each pen. Two basal diets based on maize and soybean meal (starter days 1–21 and grower days 22–42) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of chlortetracycline (unspecified chemical form; Aureomycin 0.1%, Agrichina company, Beijing, China) supplementation at a concentration of 1,000 mg/kg feed. Mortality and health status were not recorded. Chicken weight and FI were recorded weekly and F:G was calculated. At the end of the trial (42 days) 42 samples of caecal content were analysed to determine caecal microbiota. At the end of the trial (42 days), the dietary supplementation with chlortetracycline at 1,000 mg/kg feed, compared to the control group, had no effect on growth performance and caecal microbiota. Dietary chlortetracycline supplementation at 1,000 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

3.3.2.3.4. Studies in fish

In the study of Kim et al. (2009), a total of 225 native wild crucian carp (*Carassius auratus*), 20 g BW, were distributed in 15 tanks in groups of 15 animals and allocated to five dietary treatments (three replicates/treatment). Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with chlortetracycline (unspecified chemical form; chlortetracycline purity 98.8%; Wuhan Hezhongs) at a concentration of 50 mg/kg feed. The fish were fed at a rate of 4% (wet weight basis) of their total biomass per day. The daily ration was divided into two equal portions. The study lasted 60 days. Animals' weight and cumulative FI were recorded at the end of the trial and the G:F calculated. In addition, at the end of the trial, nine animals per treatment were slaughtered and skin, gill and intestine sampled to enumerate total aerobes, *E. coli* and lactobacilli as well as to examine intestinal morphology. At the end of the trial, the carp treated with chlortetracycline showed, compared to the control group, lower counts in the intestine of total aerobes (ca. 4.75 vs 5.5 $\log_{10}$ CFU/cm^2), *Vibrio* (ca. 3.75 vs 4.5 $\log_{10}$ CFU/cm^2) and *E. coli* (1.9 vs 2.25 $\log_{10}$ CFU/cm^2), and improved structure of the intestine mucosae by increased *villus* height of both intestinal parts – in the mid (65.7 vs 51.5 μm) and distal (50.7 vs 42.3 μm) intestine. Dietary supplementation with chlortetracycline at a concentration of 50 mg/kg feed showed growth-promoting effects in crucian carp (*Carassius auratus*).

3.3.2.4. Discussion

From the studies examined, the test item has been described as (i) 'chlortetracycline hydrochloride' (2 studies), (ii) a chlortetracycline commercial preparation (unspecified chemical form; 30 studies) or (iii) 'chlortetracycline' (unspecified form; 58 studies). Therefore, for the cases (ii) and (iii), an uncertainty on the exact product used/concentration applied has been identified.

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

3.3.2.4.1. Ruminants

The 15 studies (16 publications) considered as suitable for the assessment included four studies in calves, eight in cattle for fattening and three in lambs. Except for one study (Stanford et al., 2015), treatments contained groups of animals treated with only one chlortetracycline concentration and did not allow to assess any dose-related effects.

In the four studies in calves, three studies including a dietary chlortetracycline supplementation at 45 mg chlortetracycline/kg DM (Bush et al., 1959; Brown et al., 1960) and 80 mg chlortetracycline/kg DM (Murdock et al., 1961) showed growth-promoting effects; no effects were observed at 70 mg chlortetracycline/kg DM (Hibbs and Conrad, 1958).

In eight studies in fattening cattle, only two studies including a dietary chlortetracycline supplementation at 35 mg chlortetracycline/kg DM (Beacom et al., 1988; Baldwin et al., 2000) showed growth-promoting effects. On the contrary, in other four studies, a dietary chlortetracycline supplementation at the same range of concentration in feed did not affect animal performance: 11 and 36–39 mg chlortetracycline/kg DM (Stanford et al., 2015); 38 mg chlortetracycline/kg DM (Kitts et al., 2007); 40 mg chlortetracycline/kg DM (Kitts et al., 2006; Reid et al., 2014). Equally, no effects on growth performance were observed when chlortetracycline was supplemented at 9 mg chlortetracycline/kg DM (Brown et al., 1975) or 589 mg chlortetracycline/kg DM (Cabral et al., 2013).

The three studies in lambs for fattening showed rather inconsistent results: dietary chlortetracycline supplementation had growth-promoting effects at 25 mg chlortetracycline/kg DM (Rumsey et al., 1982; only in one out of three trials); no effects at 63 mg chlortetracycline/kg DM (Ternus et al., 1971) or negative effects on performance at 11 mg chlortetracycline/kg DM (Mir, 1989).

3.3.2.4.2. Pigs

The 44 studies considered as suitable for the assessment covered three animal categories within pigs: weaned piglets (22), pigs for fattening (17) and sows (5). In most studies, treatments included groups of animals treated with only one chlortetracycline concentration and did not allow to assess any dose-related effects.

In 19 studies in weaned piglets, dietary chlortetracycline supplementation at 40 to 500 mg/kg feed had growth-promoting/increase yield effects (Helm et al. (2019), 40 mg chlortetracycline/kg feed; Jiang et al. (2019), 50 mg chlortetracycline/kg feed; Stahly et al. (1980), 55 mg chlortetracycline/kg feed; Feldpausch et al. (2018), 55 and 441 mg chlortetracycline/kg feed; Song et al. (2013), Ke et al. (2014), Han et al. (2018) and Ma et al. (2019), 75 mg chlortetracycline/kg feed; Liu et al. (2008) and Shen et al. (2009), 80 mg chlortetracycline/kg feed; Choi et al. (2011a,b) and Wang et al. (2012), 100 mg chlortetracycline/kg feed; Thu et al. (2011), Cha et al. (2013) and Loh et al. (2013), 300 mg chlortetracycline/kg feed; Williams et al. (2018), 400 mg chlortetracycline/kg feed; Capps et al. (2020) 440 mg chlortetracycline/kg feed; Amachawadi et al. (2011), 500 mg chlortetracycline/kg feed). Other three studies in weaned piglets showed that dietary chlortetracycline supplementation did not have a growth-promoting effect in piglets at 75 mg chlortetracycline/kg feed (Long et al. (2019) and Wang et al. (2019)) or 150 mg chlortetracycline/kg feed (Zhao et al. (2015)).

In nine studies in pigs for fattening, dietary chlortetracycline supplementation at 11 to 1,000 mg/kg feed had growth-promoting/increase yield effects in pigs. Specifically, these positive effects were shown in seven studies (Teague et al. (1966) from 11 to 88 mg chlortetracycline/kg feed; Brown et al. (1952), 22 mg chlortetracycline hydrochloride/kg feed, corresponding to 20.5 mg chlortetracycline/kg feed; Ahmed et al. (2018), 30 mg chlortetracycline/kg feed; Kijparkorn et al. (2009), 50 mg chlortetracycline/kg feed; Brumm and Peo (1985), 110 mg chlortetracycline/kg feed; Nitikanchana et al. (2012), 400 mg chlortetracycline/kg feed; Chen et al. (2005), 1,000 mg chlortetracycline/kg feed). In two studies in pigs for fattening, dietary chlortetracycline supplementation at 55 mg/kg feed had growth-promoting/increase yield effects in pigs in two experiments (Ribeiro de Lima et al. (1981) and Mader and Brumm (1987), one experiment), 55 mg chlortetracycline/kg feed), but also did not show any growth-promoting effect in other three experiments (Ribeiro de Lima et al. (1981) and Mader and Brumm (1987), 55 mg chlortetracycline/kg feed). Other seven studies in pigs for fattening showed that dietary chlortetracycline supplementation did not have a growth-promoting effect (Holman and Chénier (2013), 5.5 mg chlortetracycline/kg feed; Hossain et al. (2012a,b), Ko and Yang (2008) and Sarker et al. (2010d), 30 mg chlortetracycline/kg feed; Langlois et al. (1978), 44 mg chlortetracycline/kg feed; Chen et al., 2006, 1,000 mg chlortetracycline/kg feed). In contrast, one study in pigs for fattening showed that dietary chlortetracycline supplementation adversely affected growth performance and feed utilisation of pigs (Cheng et al., 2018, 150 mg chlortetracycline/kg feed).

In four studies in sows, dietary chlortetracycline supplementation at 110 to 2,000 mg/kg feed improved reproductive performance in sows (Messersmith et al. (1966), 110 and 220 mg chlortetracycline/kg feed; Maxwell et al. (1994), 220 mg chlortetracycline/kg feed; Papaioannou et al. (2002), 800 mg chlortetracycline/kg feed; Sbiraki et al. (2003), 2,000 mg chlortetracycline/kg feed). Another study in sows for reproduction showed that dietary chlortetracycline supplementation did not affect performance (Myers and Speer (1973), 440 mg chlortetracycline/kg feed).

3.3.2.4.3. Poultry

The 29 studies considered as suitable for the assessment covered two animal categories within poultry: chickens for fattening (27) and laying hens (2). In most studies, treatments included groups of birds treated with only one chlortetracycline concentration and did not allow to assess any dose-related effects.

In 17 studies in chickens for fattening, dietary chlortetracycline supplementation at concentrations ranging from 10 to 930 mg/kg feed improved growth performance of chickens for fattening (Ürüşan and Bölükbaşı (2017), 10 mg chlortetracycline/kg feed; Chen et al. (2018), 40 mg chlortetracycline/kg feed; Qu et al. (2018), Hong et al. (2019), Li et al. (2019a, 2020), 50 mg chlortetracycline/kg feed; Begin (1971), 50, 100 and 200 mg chlortetracycline/kg feed; Stutz and Lawton (1984), 55 mg chlortetracycline/kg feed; Han et al. (2012), Hosseini and Meimandipour (2018) and Li et al. (2007), 80 mg chlortetracycline/kg feed from day 1 to day 21 of age and then 50 mg chlortetracycline/kg feed from day 22 to day 42 of age; Alvares et al. (1964), 100 mg chlortetracycline/kg feed; Dong et al. (2011), Aguirre et al. (2015) and Liao et al. (2015), 150 mg chlortetracycline/kg feed; Bagal et al. (2016), 335 mg chlortetracycline/kg feed; Bostami et al. (2016), 1,000 mg chlortetracycline hydrochloride/kg feed, corresponding to 930 mg chlortetracycline/kg feed). Moreover, two studies out of 17 presented some limitations: in the study of Alvares et al. (1964), the positive effect of dietary chlortetracycline supplementation was observed in feed containing sucrose but not in feed containing starch or dextrose; in the study of Liao et al. (2015), the positive effect of dietary chlortetracycline supplementation was observed only on weight gain from 22 to 42 days of age.

Other ten studies in chickens for fattening showed that dietary chlortetracycline supplementation in the range of 5.5 to 500 mg/kg feed did not affect performance of chickens for fattening (Proudfoot et al. (1988), 5.5 mg chlortetracycline/kg feed; Tang et al. (2014), 40 mg chlortetracycline/kg feed; Shi et al. (2005), Hossain et al. (2012c), Huang et al. (2018), Mahfuz et al. (2019) and Guo et al. (2020), 50 mg chlortetracycline/kg feed; He et al. (2019), 75 mg chlortetracycline/kg feed; Bai et al. (2013), 100 mg chlortetracycline/kg feed; Kim and Choi (2014), 500 mg chlortetracycline/kg feed).

The two studies in laying hens showed that dietary chlortetracycline supplementation did not affect laying performance of hens (Uuganbatar et al., 2005, 500 mg chlortetracycline/kg feed; Zhang et al., 2015, 1,000 mg chlortetracycline/kg feed).

3.3.2.4.4. Fish

Only one study in fish (native wild crucian carp, *Carassius auratus*) was identified as a relevant (Kim et al., 2009). Dietary chlortetracycline supplementation at 50 mg/kg had growth-promoting effects in crucian carp.

3.3.2.5. Concluding remarks

It is judged 66−90% certain ('likely') that chlortetracycline has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 40 to 500 mg/kg complete feed (19 studies).

It is judged 50−66% certain that chlortetracycline has growth-promoting/increase yield effects in pigs for fattening at concentrations ranging from 11 to 1,000 mg/kg complete feed (nine studies) and in chickens for fattening at concentrations ranging from 10 to 929.3 mg/kg complete feed (17 studies).

It is judged 33–66% certain ('about as likely as not') that chlortetracycline has growth-promoting/ increase yield effects: in calves at concentrations ranging from 45 to 80 mg/kg DM (three studies), in cattle for fattening at concentrations ranging from 35 to 40 mg/kg DM (two studies), in lambs for fattening at the concentration of 25 mg/kg DM (one study), in sows at concentrations ranging from 110 to 2,000 mg/kg complete feed (four studies) and in fish at a concentration of 50 mg/kg complete feed (one study).

It is judged 33–66% certain ('about as likely as not') that chlortetracycline has negative effects on performance of lambs for fattening at a concentration of 11 mg/kg DM (one study) and on performance and feed utilisation of pigs for fattening at a concentration of 150 mg/kg complete feed (one study).

No data are available in the scientific literature showing effects of chlortetracycline on growth promotion/increased yield when added (i) to calves feed at concentrations below 45 mg/kg DM, (ii) to cattle for fattening feed at concentrations below 35 mg/kg DM, (iii) to lambs for fattening feed at concentrations below 25 mg/kg DM, (iv) to weaned piglets feed at concentrations below 40 mg/kg, (v) to pigs for fattening feed at concentrations below 11 mg/kg, (vi) to sows feed at concentrations below 110 mg/kg, (vii) to chickens for fattening feed at concentrations below 10 mg/kg, (viii) to fish feed at concentrations below 50 mg/kg, or (ix) to feed of any other food-producing animal species or categories.

3.3.3. Oxytetracycline

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 (see also the Virtual Issue), resulted in 1,910 papers mentioning oxytetracycline 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 oxytetracycline.[4] After removing the reports not matching the eligibility criteria, 168 publications were identified.

3.3.3.2. Evaluation of the studies

The 168 publications identified in the literature search were appraised for suitability for the assessment of the effects of oxytetracycline 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; see also the Virtual Issue).[5] A total of 119 publications were not considered suitable for the assessment 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 B.3 (Table B.3).

The publications considered suitable for the assessment are described and assessed in Section 3.3.3.3.

3.3.3.3. Assessment of the effects of oxytetracycline on growth performance and yield

Forty-nine publications were considered suitable for the assessment of the effects of oxytetracycline on growth and yield performance in food-producing animals. The effects of the administration of the antimicrobial on the endpoints described in Section 2.2.2.2.1 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 oxytetracycline used —either as the base or as any specific form/commercial preparation—, and the concentration(s) applied as reported in each study.

3.3.3.3.1. Studies in ruminants

In the study of Yuangklang et al. (2005), a total of 38 Dutch Friesian-Holstein calves (one week of age, 41 kg BW, sex not specified) were housed individually and allocated to different treatments (19 replicates/ treatment). The study lasted 25 weeks. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline at 80 mg/kg milk replacer. The following parameters were measured: daily FI final BW, BW gain, digestibility coefficients of DM, CP, total fat, ash, calcium, phosphorus, magnesium, faecal bile acid excretion. During the first six weeks of life, the calves received a commercial starter diet and milk replacer without any supplementary treatment. From experimental weeks 19 to 23 the calves' diet included a finisher diet and a milk replacer which either contained or not oxytetracycline (unspecified form) at a level of 80 mg/kg. Animals treated with oxytetracycline, compared with the control group, showed an increased coefficient of digestibility of magnesium (0.41 vs 0.32). Dietary supplementation at 80 mg/kg milk replacer had not a growth-promoting effect in veal calves.

3.3.3.3.2. Studies in pigs

In the study of Akinfala and Tewe (2004), a total of twenty pigs (Large White × Hampshire, sex not specified), with a mean BW of 13.3 kg, were allocated individually in pens. Each pen had feeders and nipples to provide ad libitum access to feed and water. The basal diets were whole cassava plant meal-based. Feed was given to the pigs during the experiment in an amount corresponding to 3.5% of BW. All pigs were allocated to four different treatments via feed. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 450 mg/kg feed. The study lasted a total of nine weeks. Endpoints included: FI daily gain, feed conversion ratio, final weight, haematological parameters (PCV, red and white blood cells, Hb); apparent digestibility coefficients of DM, N × 6.25, CF, NDF, ADF, lignin, cellulose and haemicellulose. At the end of the experiment the final BW and weight gain were higher in the group containing oxytetracycline than in the control group (43.9 vs 34.0 kg for final BW and 0.47 vs 0.38 kg/day for weight gain). Oxytetracycline supplementation compared to the control group increased content of RBC and digestibility of haemicellulose. Oxytetracycline supplementation at 450 mg/kg feed showed growth-promoting effect in pigs for fattening.

In the study of Han et al. (2014) a total of 60 crossbred piglets weaned at 28 days old ((Landrance × Yorkshire) × Duroc; sex unspecified, BW 8.1 kg), were randomly assigned to five different dietary treatments with four replications per treatment and three pigs per pen in a completely randomised design. Each pen contained feeders and nipples to provide ad libitum access to feed and water. The basal diets were based on maize and soybean meal, without or with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 500 mg/kg feed. The study lasted 28 days. Endpoints included growth performance parameters (BW, ADG, ADFI and G:F), blood parameters, nutrient digestibility (DM, N, DE) and faecal noxious gas (ammonia, hydrogen sulfide, amine, R-SH) emission. At the end of the experiment the animals receiving oxytetracycline had higher ADG and ADFI compared to control: 474 vs 412 g and 717 vs 688 g, respectively. The G:F was positively changed (0.637 vs 0.614 in control group). Dietary oxytetracycline supplementation at 500 mg/kg feed showed growth-promoting effects in weaned piglets.

3.3.3.3.3. Studies in poultry

In the study of Aalaei et al. (2018, 2019), a total of 300 breeder hens, 51-week-old (Ross 308), were allocated to five dietary treatments (60 birds/treatment), each including six pens with 10 birds. The diets were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 500 mg/kg feed. The study lasted ten weeks. Egg production (hen-day and hen-house egg, egg weight, egg mass), BW gain and F:G were recorded. Hens were artificially inseminated to evaluate hatchability and fertility. Two hens from each pen (12 per treatment) were killed for evaluation of reproductive organs and intestinal morphology. Samples of foregut were collected to evaluate TLR (Toll-like receptor), mRNA expression of TLR2 and TLR4. Five hens from each pen (30 hens per treatment) were selected to measure feather (on a 1-to-5 scale from normal to naked with injuries) and faecal (on a 0-to-4 scale from normal to diarrhoea) scores. The toe web swelling reaction (lymphoproliferative response to phytohaemagglutinin) was measured in 12 animals per treatment and blood samples were collected to determine monocytes, lymphocytes and heterophils. Furthermore, at the end of trial, blood samples were collected from 12 animals per treatment to determine serum malondialdehyde, serum glutathione peroxidase and lipid peroxidation (thiobarbituric acid-reactive substances (TBARS)). At the end of the trial, the hens treated with oxytetracycline compared to the control group, showed higher faecal score (2.46 vs 1.50), lower *E. coli* counts (5.78 vs 8.93 $\log_{10}$ CFU/g) and increased expression levels were observed for the mRNA of TLR-2 and TLR-4. Dietary oxytetracycline supplementation at 500 mg/kg feed had no promoting effect on the performance of laying hens.

In the study of Ahmed et al. (2014) a total of 140 one-day-old chickens for fattening (Ross, unspecified strain) were allocated to four dietary treatments (35 birds/treatment) each including five replicates with seven birds. Two diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted 35 days. BW and FI were recorded weekly and F:G calculated. Excreta samples were collected weekly until the fourth week for gas measurements (ammonia and hydrogen sulfide). At the end of the trial three birds per replicate (15 broilers per treatment) were slaughtered and samples of muscles (from breast and thigh) were collected to calculate proximate composition and oxidative stability. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher ammonia emission during the fourth week of trial and lower lipid peroxidation (measured as malondialdehyde contents in muscle) after the first and the second week of meat storage. Dietary oxytetracycline supplementation at 50 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Alonge et al. (2017a), a total of 180 one-day-old chickens for fattening (Arbor Acres) were allocated to five dietary treatments (36 birds/treatment), each including three replicates with 12 birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 600 mg/kg feed. The study lasted eight weeks. BW and FI were recorded weekly and F:G calculated. At the end of weeks 4 and 8, four birds per replicate (12 per treatment) were selected for evaluation of digestibility of DM, CP, crude fibre, nitrogen-free extract, ether extract and ash. Birds receiving oxytetracycline compared to the control group, showed lower mortality during weeks 4–8 (2.78% vs 8.33%); only on week 4 higher digestibility of DM (84.2% vs 80.5%), CP (78.2% vs 74.5%) and nitrogen free extract (80.1% vs 73.1%) was observed, but not on week 8. Dietary oxytetracycline supplementation at 600 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Attia et al. (2017), a total of 245 1-day-old chickens for fattening Cobb 500 (unsexed) were allocated to seven dietary treatments (35 birds/treatment), each including five pens with seven birds. The diets (starter 0–21, grower 22–35, finisher 36–42) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form; Terramycin® (oxytetracycline, 40% purity) purchased from Delta Vet Center Company, Egypt) at a concentration of 200 mg/kg feed. The study lasted 42 days. BW and FI were evaluated at the end of each feeding phase to calculate average daily gain, daily FI and F:G. Dead birds were recorded on a daily basis. At the end of the study five birds per treatment were slaughtered for examination of intestinal morphology (*villus* height, crypt depth, crypt/*villus* in duodenum, jejunum and ileum) and caecal microbiota count. At 42 days of age, ten birds per treatment were kept in their pens (2 bird/pen) for the evaluation of total tract nutrient digestibility (DM, nitrogen, ether extract). Birds receiving oxytetracycline compared to the control group, showed higher final BW (2,526 vs 2,322 g), higher average daily weight gain (59.1 vs 54.3 g), better feed conversion ratio (1.66 vs 1.72) and lower mortality (0% vs 11.8%). The intestinal digesta of the treated birds showed a reduction in lactobacilli (4.08 vs 4.98 $\log_{10}$ CFU/g) and coliform bacteria (1.65 vs 2.55 $\log_{10}$ CFU/g). The results of digestibility study showed higher digestibility of DM (82.6% vs 78.7%) and CP (75.2% vs 71.7%). Dietary oxytetracycline supplementation at 200 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Bostami et al. (2017) a total of 240 one-day-old chickens for fattening (Ross 308, mixed sex) were allocated to five dietary treatments (48 birds/treatment), each including six replicates with eight birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 500 mg/kg feed. The study lasted 35 days. BW and FI were recorded weekly and feed conversion ratio calculated. As health-relevant parameters, besides mortality, levels of IgG, IgM and IgA were measured at the end of the study in three randomly selected birds/pen. Relative organ development was measured based on the final BW before slaughter of the bird. At the end of the trial, two chickens from each replication were slaughtered and sampled for the determination of chemical meat composition (moisture, CP, CF, crude ash, calcium, magnesium, iron, sodium) and a full fatty acid (FA) profiling including lipid oxidation (by TBARS test). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed lower mortality (2.9% vs 7.7%), reduced relative weight of abdominal fat (1.34% vs 1.78%) and higher CP in meat (22.1% vs 21.2%). The FA profile of meat was also significantly affected showing reduced palmitic acid, increased alpha-linolenic, eicosapentanoic, linoleic and dosahexanoic acids; higher total polyunsaturated fatty acids (PUFA), higher ratio of PUFA/SFA, both higher ω-3 and ω-6 FA with a reduced ω-6/ω-3 ratio and increased ratio of hypocholesterolaemic/hypercholesterolaemic FA. Dietary oxytetracycline supplementation at 500 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Deepa et al. (2018), a total of 432 one-day-old chickens for fattening (unspecified sex and strain) were allocated to six dietary treatments (72 birds/treatment), each including 12 replicates with six birds. The diets (pre-starter, starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted 42 days. Individual BW and replicate FI were recorded weekly and feed conversion ratio calculated. Nine birds per treatment were slaughtered for carcass characteristic (eviscerated yield, relative weight of heart, liver, gizzard, giblets, abdominal fat) and intestinal length recording. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher BW gain (1,817 vs 1,742 g), higher total FI (3,014 vs 2,531 g), but worse F:G (1.659 vs 1.487). Since conflicting results were identified for BW gain and F:G, no conclusions could be drawn on the growth-promoting effects of oxytetracycline in chickens for fattening.

In the study of Henry et al. (1987) a total of 144 one-day-old chickens for fattening (Peterson × Arbor Acres) were allocated to five groups, including a control and four dietary treatments, each including four or five replicates with six birds (24 birds/control; 30 birds/treatment). The diet was either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 200 mg/kg feed. The study lasted 21 days. The performance end points were weight gain, daily FI and feed conversion ratio. At the end of 21 days, experimental birds were weighed and killed for the evaluation of the relative weight of the small intestinal tract and biomarkers of deposition of essential elements (manganese and zinc in kidney and bone; copper and iron in kidney). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed lower relative weight of the small intestine (2.86% vs 3.34%) and higher manganese deposition in bone. Dietary oxytetracycline supplementation at 200 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Hong et al. (2012) a total of 240 one-day-old chickens for fattening (Arbor Acres) were allocated to three dietary treatments (80 birds/treatment), each including four replicates with ten males and ten females. The diets (starter, grower) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 100 mg/kg feed. The study lasted 42 days. BW was recorded individually on days 1, 21 and 42, total FI on days 21 and 42 to calculate BW gain and F:G for the respective period. At day 42, blood samples were harvested for the evaluation of humoral immunity (sheep red blood cells and Newcastle disease antibody titre), immunoglobulin G, lipoprotein profile, cholesterol, total polyphenol content and total flavonoids content. On 42 days of age all birds were killed for carcass evaluation (carcass weight, abdominal fat) and samples from breast and thigh muscles were taken for quality parameters analysis (colour, water holding capacity, DM and fat content, sensory characteristics). Intestinal contents were collected on day 42 from duodenum, jejunum, ileum and caeca of six birds per pen to determine the ileum microbiota (total bacteria count, *Salmonella*, coliforms, enterococci, lactobacilli), total caeca volatile fatty acids, intestinal pH, ileum ammonia concentration and histology of intestinal tissue (*villus* height, crypt depth). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher survival rate (97.5% vs 88.8%), total weight gain (2,291 vs 2,030 g), total FI (3,879 vs 3,466 g) and from carcass parameters reduced the water binding capacity of breast muscle (56.8% vs 65.7%). Faecal volatile fatty acids and ileum ammonia were both decreased compared to control. The serum lipid profile showed a reduction of cholesterol and VLDL lipoproteins. Supplemented chickens showed reduced antibody titres to Newcastle virus. Dietary oxytetracycline supplementation at 100 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Hossain et al. (2012d), a total of 140 1-day-old chickens for fattening (Ross, unspecified strain) were allocated to four dietary treatments (35 birds/treatment), each including five replicates with seven birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted 35 days. BW and FI were measured weekly and feed/gain ratio calculated. At the end of the experiment birds were slaughtered and samples were collected from breast and thigh muscles for the evaluation of moisture, CP, crude ash, CF, lipid profile and oxidative stability (TBARS). Blood samples were obtained at the end of trial for serum biochemistry (albumin, aspartate amino transferase, alanine amino transferase, creatinine, urea, bilirubin, cholesterol) and immunity (IgG, IL-2) evaluation. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed improved feed conversion ratio (1.60 vs 1.68) and higher CF content in breast (0.56% vs 0.27%). The lipid profile in thigh showed the following changes as percentage of fatty tissue: decrease of oleic acid, increase of palmitoleic, linoleic, eicosanoic and eicosapentaenoic acids, and increase of PUFA. Dietary oxytetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Hossain et al. (2012e), a total of 140 1-day-old chickens for fattening (Ross, unspecified strain) were allocated to four dietary treatments (35 birds/treatment), each including five replicates with seven birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted 35 days. BW and FI were measured weekly and feed/gain ratio calculated. At the end of the experiment all birds were slaughtered. Organ relative weight was determined and meat samples were analysed for moisture, total ash, CP, CF, lipid profile and oxidative stability (TBARS). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed improved feed conversion ratio (1.64 vs 1.69) and higher CF content in breast (0.56% vs 0.27%). The lipid profiles showed the following changes as percentage of fatty tissue: decrease of ω-3 and increase of the ω-6/ ω-3 ratio in breast; increase of linoleic acid, PUFA, ω-6 FA and of the ratio PUFA/SFA in thigh. The oxidative stability of breast and thigh muscle was improved on days 5 and 7 of storage. Dietary oxytetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Hossain and Yang (2014) a total of 140 one-day-old chickens for fattening (Ross, unspecified strain) were allocated to four dietary treatments (35 birds/treatment), each including five replicates with seven birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form, supplied by Hebei Guangren Pharmaceutical Technology Co., Ltd., Hebei, China) at a concentration of 50 mg/kg feed. The study lasted 35 days. BW and FI were measured weekly and feed/gain ratio calculated. At the end of the experiment all birds were slaughtered. Organ relative weight was determined and meat samples were analysed for moisture, total ash, CP, CF, lipid profile and oxidative stability (TBARS). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher BW (1,819 vs 1,773 g), BW gain (1,776 vs 1,730 g), improved F:G (1.66 vs 1.69), absolute and relative weight of breast meat (234 vs 200 g, 13.74% vs 12.22%, respectively) and lower CF content in thigh (0.40% vs 091%). The oxidative stability of breast and thigh muscles was improved at the second week of storage. Dietary oxytetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Kalavathy et al. (2008), a total of 270 1-day-old chickens for fattening (Hubbard) were allocated to three dietary treatments (90 birds/treatment), each including six replicates with 15 birds. The diets (starter, grower) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted 42 days. BW was recorded at 1, 21 and 42 days, FI was recorded daily and feed conversion was calculated. At 42 days two chickens from each replicate were sacrificed and blood was collected for serum biochemistry (cholesterol, total triglycerides, LDL cholesterol). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher final weight (1,962 vs 1,700 g), higher total weight gain (1,920 vs 1,659 g), improved F:G (1.94 vs 2.12) and increased serum total triglycerides and LDL cholesterol. Dietary oxytetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Khadem et al. (2014), a total of 900 1-day-old male chickens for fattening (Ross 308) were allocated to five dietary treatments (180 birds/treatment), each including 12 replicates with 15 birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline hydrochloride (crystalline, supplied by Sigma-Aldrich) at a concentration of 200 mg/kg feed (corresponding to 186 mg oxytetracycline/kg feed). The study lasted 35 days. BW, FI and G:F were determined on days 14, 21 and 35. Twelve chickens per treatment were euthanised at the end of the trial for the carcass characteristic (relative weights of liver, intestine, abdominal fat) and assessment of intestinal expression of the genes of inflammatory cytokines and inducible nitric oxide synthase. Blood samples were taken from 12 chickens per treatment on days 21 and 35 for plasma α1-acid glycoprotein measurement. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher final weight (2,010 vs 1,900 g), total FI (3,240 vs 3,110 g) and G:F (0.62 vs 0.61). Expression level of jejunal genes for inducible nitric oxide synthase was decreased in supplemented group. Dietary oxytetracycline hydrochloride supplementation at 200 mg/kg feed (corresponding to 186 mg oxytetracycline/kg feed) had a growth-promoting effect in chickens for fattening.

In the study of Lee et al. (2011a) a total of 640 one-day-old chickens for fattening (Arbor-Acres, both sexes) were allocated to four dietary treatments (160 birds/treatment), each including eight replicates with 20 birds. The diet was either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 55 mg/kg feed. The study lasted 21 days. FI and BW were measured weekly. Blood samples were taken on day 21 from two birds per pen (male and female) and birds were then sacrificed. The following parameters were examined in two birds per pen: relative weight of small intestine; histology of jejunum and ileum (*villus* length, crypt depth) and counting of IgA-positive cells in 100 μL mucosal cell suspensions of jejunum or ileum; digestive enzymes in proventriculus (pepsin), jejunum and ileum (maltase, sucrase); counts of rectal coliforms, enterococci and lactobacilli. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed only higher mean count of IgA-positive cells in the ileum. Dietary oxytetracycline supplementation at 55 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Mahmoud et al. (2020), a total of 336 7-day-old chickens for fattening (strain IR) were allocated to six dietary treatments (56 birds/treatment), each including seven replicates with eight birds. The diets (starter, grower) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 50 mg/kg feed. The study lasted 28 days. BW and FI were recorded weekly and feed conversion calculated. At 35 days two birds from each pen were sacrificed; carcass quality parameters (pH, dripping loss, texture, colour, overall acceptability, eviscerated yield, edible yield), intestinal microbial population (total bacteria, anaerobic, coliforms, lactobacilli) and blood serum biochemistry (glucose, total protein, albumin, globulins, HDL-cholesterol, triglycerides, uric acid, creatinine, Ca, P, Newcastle disease haemagglutination inhibition titre) were evaluated. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed improved feed conversion ratio (1.39 vs 1.48) and decreased gizzard size (1.12% vs 1.38%). With respect to blood parameters, results showed an increase in creatinine, a decrease in triglycerides and in the Newcastle disease haemagglutination inhibition titre. Dietary oxytetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Oguntona (1988a), a total of 400 one-day-old guinea fowls (*Numida meleagris*) were allocated to four dietary treatments (100 birds/treatment), each including four replicates with 15 birds. Three diets (starter, grower, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form, obtained from Agbenla Farms, Akure, Nigeria) at a concentration of 7.5 mg/kg feed. The study lasted 84 days. Feed intake and BW were recorded weekly and G:F calculated at 4, 8 and 12 weeks. At day 84, ten birds from each treatment were put in individual cages and used for nitrogen balance study. After the end of the trial carcass composition was assessed on five birds and nitrogen retention on ten birds per treatment. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher BW (998 vs 898 g), G:F (0.29 vs 0.26), DM (368 vs 352 g/kg) and fat (338 vs 309 g/kg) content in the carcass and also higher retention of nitrogen (49% vs 42%). Dietary oxytetracycline supplementation at 7.5 mg/kg feed had a growth-promoting effect in Guinea fowls.

In the study of Oguntona (1988a) two experiments are described. In Experiment 1 a total of 300 one-day-old male guinea fowls (*Numida meleagris*) were allocated to five dietary treatments (60 birds/treatment), each including four replicates with 15 birds. Three diets (starter, grower, finisher) were either not supplemented (control) or supplemented with oxytetracycline (unspecified form) at a concentration of 5, 10, 15 and 20 mg/kg feed. The study lasted 84 days. Feed intake and BW were recorded weekly and G:F calculated at 4, 8 and 12 weeks. After the end of the trial carcass composition was assessed on 20 animals per treatment. In Experiment 2 the same general procedure was used, the only difference was in the concentration of oxytetracycline which was supplemented at 5.0, 6.6, 8.2 and 10.0 mg/kg feed. At the end of the first trial, birds receiving oxytetracycline showed higher BW with every increase in oxytetracycline up to 10 mg/kg feed (909, 993, 1,098, 1,090, 1,091 g for 0, 5, 10, 15, 20 mg/kg, respectively) and F:G for all medicated groups was lower compared to the control group (3.29, 3.41, 3.44, 3.45 vs 3.94). The inclusion level of 20 mg/kg feed showed compared to control greater heart size (6.2 vs 4.0 g), lower relative weights of intestine (2.2% vs 2.92%) and liver (0.88% vs 0.94%). At the end of the second trial birds receiving oxytetracycline showed compared to control group higher BW (1,040, 1,260, 1,190, 1,220 vs 980 g) and F:G (3.39, 2.85, 2.93, 2.97 vs 3.75). The best BW and F:G were obtained at 6.6 mg/kg, higher levels of oxytetracycline did not produce further improvement. Dietary oxytetracycline supplementation at 5.0, 6.6, 8.2, 10.0, 15.0 and 20.0 mg/kg feed had a growth-promoting effect in Guinea fowls.

In the study of Oko et al. (2018), a total of 180 1-week-old Japanese quail chicks (Coturnix japonica) were allocated to six dietary treatments (30 birds/treatment), each including three replicates with ten birds. The diets (growing period, laying period) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 20 mg/kg feed. The study lasted 16 weeks. During growing phase (1–6 weeks of experiment) BW and FI were monitored weekly and carcass characteristics were evaluated at the sixth week. In the laying phase (7–16 weeks of experiment), layers were separated and quail eggs were collected twice daily and FI egg weight, egg size and egg quality were measured weekly. On a weekly basis three freshly laid eggs were randomly picked from each replicate and were used for egg quality determination. At the end of growing phase, birds receiving oxytetracycline showed compared to the control group, lower mortality (2.7% vs 4.0%), lower daily FI (15.03 vs 15.70 g) and better feed conversion ratio (5.57 vs 5.82). Carcass characteristics showed increased carcass yield (59.3% vs 58.9%) and abdominal fat (0.67% vs 0.34%), but reduced gizzard and intestine weight (2.75% vs 3.19% and 4.90% vs 5.69%, respectively). Regarding egg quantity and quality, birds supplemented with oxytetracycline compared to the control group, showed higher total number of laid eggs (1,453 vs 1,288) and hen day production (69.19% vs 61.33%), increased shell thickness (0.30 vs 0.29 mm), but reduced egg size (9.72 vs 9.99 g). Increased yolk weight (31.0% vs 30.7%), reduced shell weight (17.0% vs 21.6%) and increased yolk colour (4.31 vs 3.03) were also found. Dietary oxytetracycline supplementation at 20 mg/kg feed had a growth-promoting effect in growing and laying Japanese quails.

In the study of Sarker et al. (2010a), a total of 210 one-day-old chickens for fattening (Ross, unspecified strain) were allocated to six dietary treatments (35 birds/treatment), each including five replicates with seven birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 55 mg/kg feed. The study lasted five weeks. BW and FI were measured weekly and feed conversion calculated. Four chickens per treatment were slaughtered for the assessment of body composition (moisture, CP, CF, crude ash), organ relative weight and length of the intestines. Carcass rancidity was evaluated in fresh, after 1, 2 and 3 weeks of the storage in meat by measurement of TBARS. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed only increased length of the small and large intestines (182.5 vs 171.8 and 10.8 vs 8.2 cm, respectively) and lower moisture of the meat (73.7% vs 74.9%). With respect to carcass rancidity, TBARS in meat was reduced after one week. Dietary oxytetracycline supplementation at 55 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Sarker et al. (2010b), a total of 168 one-day-old chickens for fattening (Ross, unspecified strain) were allocated to six dietary treatments (28 birds/treatment), each including four replicates with seven birds. The diets (starter and finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 30 mg/kg feed. The study lasted five weeks. The following parameters were measured: FI, F:G, BW, weight of internal organs, body composition, lipid oxidation of meat (fresh, 1, 2 and 3 weeks of storage by measurement of TBARS), caecal microbiota. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed lower TBARS content in meat after one week. Dietary oxytetracycline supplementation at 30 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Sarker et al. (2010c), a total of 140 one-day-old chickens for fattening (Ross, unspecified strain) were allocated to four dietary treatments (35 birds/treatment), each including five replicates with seven birds. The diets (starter and finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 500 mg/kg feed. The study lasted five weeks. BW and FI were measured weekly and F:G calculated. Four chickens per treatment were slaughtered for the assessment of body composition (moisture, CP, CF, crude ash), organ relative weight and length of the intestines. Carcass rancidity was evaluated in fresh, after 1, 2 and 3 weeks of the storage in meat by measurement of TBARS. At the end of the trial, birds receiving oxytetracycline compared to the control group, had lower relative weight of large intestine (0.11% vs 0.17%) and lower fat content in the carcass (0.68% vs 1.04%). Dietary oxytetracycline supplementation at 500 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Shalaei et al. (2014), a total of 160 laying hens (Leghorn Hy-line W36), 32 weeks old, were allocated to five dietary treatments (32 birds/treatment), each including four replicates with eight birds. The diet was either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form, supplied by Damloran Co., Tehran, Iran) at a concentration of 150 mg/kg feed. The study lasted ten weeks. BW was determined at the beginning and end of the study. Egg production and egg weight were recorded daily, and feed conversion was calculated. At the end of the experiment three eggs from every replicate were selected and eggshell quality parameters measured (eggshell percentage, eggshell thickness, eggshell strength). Two hens from each replicate were slaughtered, internal organs of gastrointestinal tract removed, the pH of different parts was measured and histomorphology of small intestines was performed. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed only a lower pH in the duodenum (5.45 vs 5.67) and increased crypt depth (275 vs 205 μm) in ileum. Dietary oxytetracycline supplementation at 150 mg/kg feed did not affect performance in laying hens.

In the study of Shokaiyan et al. (2019), a total of 250 1-day-old chickens for fattening (Ross 308, males) were allocated to five dietary treatments (50 birds/treatment), each including five replicates with ten birds. The diets (starter, grower, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 200 mg/kg feed. The study lasted six weeks. BW gain and FI were obtained at 42 days of age. At the end of the experiment two birds per pen were slaughtered and used to evaluate carcass, internal organs and intestinal morphology. Blood samples were used for measurement of serum glucose, cholesterol (LDL, HDL), triglycerides, total protein, AST, ALT and ALP. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher BW gain (2,547 vs 2,416 g). With respect to intestinal morphology, *villus* height (1,203 vs 885 μm) and *villus* surface area (0.65 vs 0.40 mm^2) in the ileum were increased, crypt depth in the jejunum was decreased (175 vs 188 μm) and the ratio of *villus*/crypt depth in the jejunum was increased (8.34 vs 6.73). Dietary oxytetracycline supplementation at 200 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Singh et al. (2014a), a total of 100 1-day-old chickens for fattening (IBL-80) were allocated to five dietary treatments (20 birds/treatment), each including two replicates with ten birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 100 mg/kg feed. The study lasted 35 days. BW and FI were recorded weekly and feed conversion ratio calculated. At the end of the trial six birds (three male and three female) from each treatment were sacrificed for carcass characteristic (dressing percentage, relative weight of abdominal fat, heart, gizzard and liver) and sensory evaluation of meat (appearance and colour, tenderness, juiciness, flavour, overall acceptability). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed improved feed conversion ratio (2.07 vs 2.14) and higher relative weight of heart (0.67% vs 0.55%). Dietary oxytetracycline supplementation at 100 mg/kg feed had a growth-promoting effect in chickens for fattening.

In each of the studies conducted by Singh et al. (2014b, 2015), a total of 210 1-day-old chickens for fattening (IBL-80) were allocated to five dietary treatments (42 birds/treatment), including three replicates/treatment with 14 birds/replicate. A common control and a positive control using oxytetracycline (unspecified form) was used for both studies. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 100 mg/kg feed. The study lasted 5 weeks. BW and FI were recorded weekly and feed conversion calculated. On day 35 two birds (one male, one female) per replicate were slaughtered for the evaluation of carcass (dressing percentage, weight of liver, gizzard, hearth) and sensory evaluation of meat (8-point scale where 8 extremely desirable and 1 extremely undesirable). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed increased final body weight (1,369 vs 1,287 g), body weight gain (1,321 vs 1,239 g) and daily FI (72 vs 68 g). Sensory meat evaluation showed better appearance, flavour and juiciness. Dietary oxytetracycline supplementation at 100 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Singh et al. (2018) a total of 210 one-day-old chickens for fattening (unspecified strain) were allocated to five dietary treatments (42 birds/treatment), each including three replicates (equal sex ratio) with 14 birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 100 mg/kg feed. The study lasted 35 days. Body weight and FI were recorded weekly and feed conversion was calculated. At the end of the trial two birds (male and female) from each replicate were sacrificed for carcass characteristic (dressing percentage, relative weight of abdominal fat, heart, gizzard and liver), sensory evaluation of meat (appearance and colour, tenderness, juiciness, flavour, overall acceptability) and duodenum morphology (*villus* height, crypt depth). Fresh faecal samples were taken for microbial examination (total bacterial count, coliforms count). Blood samples of three birds from each treatment were taken on day 35 for haematology and serum biochemistry. Balance study was done with two birds (male and female) from each replicate at the age of five weeks to assess the digestibility of DM, CP, CF, crude fibre, calcium and phosphorus. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher daily FI (72.3 vs 67.6 g), higher digestibility of calcium (52.1% vs 43.2%), reduction of total bacteria (2.0 vs 12.0 × 1,010 CFU/mL) and coliforms (1.0 vs 8.50 × 108 cfu/mL) and lower serum cholesterol. Dietary oxytetracycline supplementation at 100 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Singh et al. (2019) a total of 210 one-day-old chickens for fattening (IBL-80) were allocated to five dietary treatments (42 birds/treatment), each including three replicates with 14 birds (seven male, seven female). The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and treatment consisting of oxytetracycline (unspecified form) at a concentration of 100 mg/kg feed. The study lasted five weeks. Body weight and FI were recorded weekly and feed conversion calculated. On day 35 two birds (one male, one female) per replicate were slaughtered for the evaluation of carcass (dressing percentage, weight of liver, gizzard, heart and abdominal fat), duodenum morphology (*villus* height, crypt depth) and sensory evaluation of meat (8-point scale where 8 = extremely desirable and 1 = extremely undesirable). A balance study was conducted at the age of five weeks with two birds (one male, one female) from each replicate for evaluation of digestibility (DM, CP, CF, DM of crude fibre, DM of calcium, DM of phosphorus). Blood samples of three birds from each treatment on 35 days were collected for determining haemoglobin, packed cell volume, glucose, triglycerides, cholesterol, total protein and albumin. Fresh faecal samples were collected on last day of the metabolic trial for evaluation of faecal microbial load (total bacteria, *E. coli*). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed lower relative heart weight (0.80% vs 1.09%), reduced crypt depth (92 vs 140 μm), increased the ratio of *villus* height/crypt depth (21 vs 15), decreased total bacteria (2.33 vs 5.50 105/mL) and blood haemoglobin. Sensory meat evaluation showed better appearance, flavour, tenderness and overall acceptability. Dietary oxytetracycline supplementation at 100 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Stutz and Lawton (1984), Experiment 3, a total of 192 two-day-old male chickens for fattening (Hubbard) were allocated to seven 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 oxytetracycline (unspecified form) supplementation at a concentration of 55 mg/kg feed. The experiment lasted eight days (from day 3 to day 11 of age). Body weight 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 (oxytetracycline treatment) were slaughtered for relative ileal weight determination, whereas ileal digesta from twelve chickens (control) or six chickens (oxytetracycline treatment) were used for enumeration of *C. perfringens*. At the end of the experiment, the birds treated with oxytetracycline at 55 mg/kg feed, compared to the control group, showed higher daily weight gain (135 vs 123 g/day), and an improved F:G (1.26 vs 1.34), and had decreased relative ileum weight (1.37 vs 1.64% BW) and lower *C. perfringens* count (2.3 vs 3.1 log10/g digesta). Dietary oxytetracycline supplementation at 55 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Zamora et al. (2017) a total of 162 one-day-old chickens for fattening (Ross, unspecified strain) were allocated to three dietary treatments (54 birds/treatment), each including 27 replicates with two birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form, supplied from Bayer Pfizer Inc, New York City, USA) at a concentration of 250 mg/kg feed. The study lasted 42 days. Body weight and FI were evaluated weekly and feed efficiency calculated. At day 42 blood samples (ten per treatment) were taken for biochemistry (cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, very low-density lipoproteins) and haematology parameters (erythrocytes, haemoglobin, heterophils, eosinophils mean corpuscular volume, lymphocytes, monocytes, mean corpuscular haemoglobin) and 20 birds per treatment were slaughtered for carcass evaluation (slaughter weight, hot carcass weight, cold carcass weight, hot carcass yield, cold carcass yield). At the end of the trial, birds receiving oxytetracycline compared to the control group, showed lower total FI (4,103 vs 4,287 g). Dietary oxytetracycline supplementation at 250 mg/kg feed did not have a growth-promoting effect in chickens for fattening.

In the study of Zulkifli et al. (2000), a total of 360 female day-old chickens for fattening (180 Shaver, 180 Hubbard) were allocated to three dietary treatments (120 birds/treatment), each including 12 replicates with ten birds. The diets (starter, finisher) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form, Terramycin LA-2000; Pfizer, New York, NY) at a concentration of 50 mg/kg feed. The study lasted 42 days. The chickens were exposed to 36 $\pm$ 1°C for 3 h daily from day 21 to 42. Live Newcastle disease vaccine was applied intraocularly on day 7 and 21. Body weight was evaluated at days 1, 21 and 42, FI was recorded weekly and feed efficiency was determined. At day 21 blood samples were taken from six chickens per strain-diet subgroup (12 per group) for serum concentration of Newcastle disease antibody titre. At the end of the trial, birds receiving oxytetracycline compared to the control group, showed higher body weight (1,487 vs 1,417 g) and weight gain (1,449 vs 1,379 g). Dietary oxytetracycline supplementation at 50 mg/kg feed had a growth-promoting effect in chickens for fattening.

3.3.3.3.4. Studies in fish

In the study of Adeniyi (2020), a total of 225 fingerlings of African catfish (*Clarias gariepinus*), BW 3.56 g, were allocated to five dietary treatments and distributed in three replicates per treatment in groups of 15 animals. Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with oxytetracycline at a concentration of 600 mg/kg feed (unspecified chemical form, OXY 200 WSP, Kepro, Deventer, Holland). The diets were provided in pellet/crumbles. The study lasted 70 days. Animal health was checked daily. Dead fish were removed and counted to calculate survival. Batch weights and the weight of randomly sampled fish were measured fortnightly and average daily growth, specific and relative growth rate and feed efficiency calculated. Four fish were sampled from each replicate, sacrificed on ice and measured to determine condition factor, hepato-somatic, gonado-somatic and spleen-somatic indexes. At the end of the trial, the fish treated with oxytetracycline showed, compared to the control group, higher final weight (36.51 vs 33.0 g) and weight gain (32.98 vs 29.47 g). Dietary oxytetracycline supplementation at 600 mg/kg feed showed growth-promoting effects in fingerlings of African catfish.

In the study of Adeniyi et al. (2018) a total of 900 fingerlings of African catfish (*Clarias gariepinus*), BW 5.75 g, were allocated to one of ten dietary treatments and distributed in three replicates per treatment in groups of 30 animals. Two were the relevant treatments obtained from a basal diet (containing chromium oxide as indigestible marker) which was either not supplemented (control) or supplemented with oxytetracycline (unspecified chemical form, OXY 200, WSP, Holland) at a concentration of 400 mg/kg feed. The study lasted 84 days. Animal health was checked daily. Dead fish were removed and counted to calculated survival rate. Twelve fish per treatment were randomly taken fortnightly to record weight gain to calculate average daily growth, specific growth rate and feed efficiency. Faeces were collected to determine the apparent digestibility of nutrients. At the end of the trial, the fish treated with oxytetracycline showed, compared to the control group, higher apparent digestibility of CP (62.83% vs 52.98%). Oxytetracycline dietary supplementation at 400 mg/kg feed showed a growth-promoting effect in fingerlings of African catfish.

In the study of Ebrahimi et al. (2020), a total of 180 young Beluga (*Huso huso*) fish with initial mean body weight of 130.94 g were randomly distributed to 18 tanks (six dietary treatments of three replicates, 10 fish per tank). Fish were acclimatised for two weeks and then fed with prepared diets based on 2% of their body weights. The experimental groups were either not supplemented (control) or supplemented with different feed additives. Two are the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 30 mg/kg feed. The study lasted eight weeks. Endpoints included growth performance and survival rate; immune-haematological parameters and serum metabolic products including cholesterol, glucose, total protein, albumin, globulin, triglyceride as well as some liver enzymes such as glutamic oxaloacetic transaminase

(GOT) and glutamic pyruvic transaminase (GPT) as well as differential count and muscle composition were examined. At the end of the experiment, the oxytetracycline supplementation compared to the control group showed a higher hepatosomatic index (2.91 vs 2.17). From the haematological parameters an increase was seen in albumin, total protein (TP) and globulin, whereas decreases were observed in glucose, GOT and GPT. The differential leukocyte count increased in lymphocytes and decreased in both eosinophil and neutrophil. Muscle proximal composition showed higher protein (14.58% vs 12.39%). Dietary supplementation with oxytetracycline at a concentration of 30 mg/kg feed did not show a growth-promoting effect in Beluga fish.

In the study of El-Sayed et al. (2014), a total of 200 Nile tilapias (*Oreochromis niloticus*, sex and strain unspecified), with average weight 43 g, were allocated to four different treatments via feed, that was either not supplemented (control) or supplemented with different feed additives. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline hydrochloride (supplemented via Muv-Oxytetracycline) at the concentration of 200 mg/kg feed (corresponding to 186 mg oxytetracycline/kg feed). Each treatment group comprised 50 fish, in two replicates of 25 fish. Fish were fed throughout the experiment at an amount corresponding to 3% of body weight. The study lasted eight weeks in regard of the assessment of performance parameters. A follow-up on 20 fishes/ group challenged by intraperitoneal injection with a pathogen bacterium is outside the scope of this review. Endpoints included final weight, FI F:G, weight (in g)/length (in cm) ratio, survival and a panel of immune parameters. At the end of the experiment oxytetracycline supplementation compared to the control group increased the FI (43.73% vs 41.65%) and improved the weight gain (32.92% vs 26.25%), the F:G (3.03 vs 3.69) and the weight to length ratio (1.78 vs 1.63). Dietary supplementation with oxytetracycline hydrochloride at a concentration of 200 mg/kg feed (corresponding to 186 mg oxytetracycline/kg feed) showed growth-promoting effects in Nile tilapia.

In the study of Lawal et al. (2019), a total of 150 juvenile African catfish (*Clarias garepinus*, strain unspecified), average weight 94.3 g were allocated to five different treatments via feed, that was either not supplemented (control) or supplemented with different feed additives. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 200 mg/kg feed. Each treatment group comprised 30 fish (2 tanks of 15 fish/ treatment). The study lasted eight weeks. Endpoints included total FI daily FI feed conversion ratio and protein efficiency ratio (based on protein content of feed, FI and weight gain); mortality; final weight, percentage of weight increase; haematological parameters (red and white blood cells); activities of serum enzymes (ALT, AST, alkaline phosphatase) and liver antioxidant enzymes (SOD, GSH, catalase). At the end of the experiment, only a decrease in serum ALT and an increase of liver superoxide dismutase (SOD) were observed. Dietary supplementation with oxytetracycline at a concentration of 200 mg/kg feed showed no growth-promoting effects in juvenile African catfish.

In the study of Olusola et al. (2020), a total of 400 juvenile African catfish (*Clarias gariepinus*) (3g BW) were distributed in 20 tanks of 20 animals and allocated to 10 treatments (2 replicates/ treatment). The fish were fed twice daily at 3% body weight. The diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 30 mg/kg diet. The study lasted eight weeks (feeding trial). The following parameters were measured in the feeding trial: body weight, specific growth rate, protein efficiency ratio, protein productive value. At the end of the trial, the animals treated with oxytetracycline, compared to the control group, showed only a lower protein intake. Proximate composition of the fish after the experiment showed in the oxytetracycline group increased moisture (16 vs 14), CP (66 vs 65), ash (16 vs 14) and decreased ether extract (4.4 vs 5.4) and nitrogen free extract (3.2 vs 6.0). Dietary supplementation with oxytetracycline at a concentration of 30 mg/kg feed showed a growth-promoting effect in African catfish.

In the study of Park et al. (2016a), a total of 300 juvenile rainbow trout (*Oncorhynchus mykiss*) (5.8 g BW) were distributed in 15 tanks in groups of 20 animals and allocated to 5 treatments (3 replicates/ treatment). Fish were fed two times a day at the rate of 3.89% of wet BW per day. The diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 5,000 mg/kg diet. The study lasted eight weeks. The following parameters were measured: survival rate, weight gain, feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), whole body proximate composition (on 3 fish/tank). Blood parameters (glutamic oxaloacetic transaminase, glutamate pyruvate transaminase, glucose and cholesterol), respiratory burst activity, serum lysozyme, myeloperoxidase activity and superoxide dismutase activity were also assessed on 5 fish/tank. At the end of the trial, the animals treated with oxytetracycline, compared to the control group, showed increased weight gain (235% vs 210%) and specific growth rate (2.69% vs 2.52%/day). From the parameters of the non-specific immune responses of juvenile rainbow trout, serum lysozyme was increased and myeloperoxidase activity was decreased. Dietary supplementation with oxytetracycline at a concentration of 5,000 mg/kg feed showed growth-promoting effects in juvenile rainbow trout.

In the study of Park et al. (2016b), a total of 270 starry flounders (*Platichthys stellatus*) (47g BW) were distributed in 18 tanks in groups of 15 animals and allocated to 6 treatments (3 replicates/treatment). Fish were fed two times daily at a rate of 2.0% of wet body weight per day. Two diets (starter and grower) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 5,000 mg/kg diet. The study lasted eight weeks (feeding trial). The following parameters were measured: weight gain, feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), survival rate, whole body proximate composition (on 3 fish/tank). Blood parameters (GOT, GPT, glucose and total proteins), respiratory burst activity (NBT), serum lysozyme, myeloperoxidase activity and superoxide dismutase activity were also assessed on 5 fish/tank. At the end of the trial, no effects were shown in the parameters measured. Dietary supplementation with oxytetracycline at a concentration of 5,000 mg/kg feed had no growth-promoting effects in starry flounders.

In the study of Reda et al. (2016), a total of 720 fingerlings of Nile tilapia (*Oreochromis niloticus*) (29 g BW) were distributed in 24 tanks in groups of 30 animals and allocated to 4 treatments (2 subgroups, A and B, each with 3 replicates /treatment). Subgroup B is not further considered. Feed was provided twice daily at the rate of 5% of fish live body weight. The diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form, Oxyvet 20%) at a concentration of 100 mg/kg diet. The study lasted 60 days. The following parameters were measured: F:G, specific growth rate (SGR), body weight, weight gain, body composition, haematological parameters. At the end of the trial, the animals treated with oxytetracycline, compared to the control group, showed higher body weight (40 vs 37 g) and weight gain (12 vs 8 g), improved F:G (6.3 vs 8.4) and specific growth rate (0.54 vs 0.41); in terms of body composition decreased ash (5.9% vs 6.5%); in terms of haematological parameters: lower haematocrit (19% vs 25 %) and increased platelets (15 vs 13 $\times$ 103/μL). Dietary supplementation with oxytetracycline at a concentration of 100 mg/kg feed showed growth-promoting effects in fingerlings of Nile tilapia.

In the study of Rhee et al. (2020), a total of 300 juvenile olive flounder (*Paralichthys olivaceus*), 27 g BW, were distributed in 15 thanks in groups of 20 animals and allocated to 5 treatments (3 replicates/treatment). The diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 5,000 mg/kg diet. The study lasted eight weeks. The following parameters were measured: WG, FE, SGR, PER, SR (survival rate), condition factor. Whole body proximate composition, *VS*I (viscero-somatic index), microbial community analysis, blood lysozyme, SOD (superoxide dismutase) and respiratory burst activity (NBT) were assessed on three fish/replicate. At the end of the trial, the animals treated with oxytetracycline, compared to the control group, showed increased weight gain (80 vs 63 g), G:F (FE) (52% vs 42%), SGR (1.05% vs 0.87%/day) and gain to protein ratio (PER) (0.98 vs 0.77). Dietary supplementation with oxytetracycline at a concentration of 5,000 mg/kg feed showed growth-promoting effects in juvenile olive flounder.

In the study of Sanchez-Martínez et al. (2008), a total of 210 juvenile channel catfish (*Ictalurus punctatus*), 10 g BW, were distributed in six tanks in groups of 35 animals and allocated to three treatments (two replicates/treatment). The diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 2,500 mg/kg diet. The study lasted 11 weeks. The following parameters were measured: feed conversion index, specific growth rate, feed consumption, body weight, mean weight, fork length, condition index and growth curves. At the end of the trial, the mean weight for the oxytetracycline-treated catfish (33.23 g) was 11.7% greater than the control group (29.35 g). The catfish treated with oxytetracycline, compared to the control group, showed increased condition index (K, weight/length) (1.08 vs 0.98). Dietary supplementation with oxytetracycline at a concentration of 2,500 mg/kg feed showed growth-promoting effects in juvenile channel catfish.

In the study of Trushenski et al. (2018), a total of 200 fish incl. 80 Nile Tilapia (*Oreochromis niloticus*, 53.5 g BW) and 40 fish for each of the following taxa: channel catfish (*Ictalurus punctatus*, 5.4 g BW), hybrid striped bass (*Morone chrysops* × *M. saxatilis*, 27 g BW) and, rainbow trout (*Oncorhynchus mykiss*, 34 g BW) were distributed in 16 tanks in groups of 20 (Nile Tilapia) or 10 (all other taxa) fish and allocated to 3 treatments (4 replicates for each of the 4 fish species and treatment). Dietary treatments were offered once daily at a rate of 3% of BW. The diets were either not supplemented (control) or supplemented with different treatments. Three were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified chemical form, Liquamycin LA-200) at concentrations of 240 and 1,200 mg/kg diet. The study lasted eight weeks. The following parameters were measured: performance (weight gain, specific growth rate, feed conversion ratio, FI). On 4 fish from each tank the following parameters were also assessed: for individual weighing, health evaluation, necropsies to assess external (i.e. body surface, fins, gills and eyes) and internal (i.e. liver, kidney, spleen, adipose tissue, gall bladder, alimentary canal and musculature) tissues and structures. Hepatosomatic index and viscero-somatic index were also calculated. At the end of the trial, no differences were seen in the fish supplemented with the two doses of oxytetracycline compared to control group, with the exception of an increased hepatosomatic index in Hybrid striped bass (3.2 vs 2.8), in the group supplemented with the lower oxytetracycline concentration used. Necropsies identified only a reduced frequency of 'normal skin and body surface' of channel catfish. Dietary supplementation with oxytetracycline at a concentration of 240 or 1,200 mg/kg feed did not promote growth in Nile tilapia and the other species.

In the study of Won et al. (2020) a total of 480 juvenile Nile tilapias (*Oreochromis niloticus*) (2.8 g BW) were distributed in 24 tanks in groups of 20 fish and allocated to eight treatments (six dietary plus 2 additional for challenge test; three replicates/treatment). Fish were fed two times at 3–4% of wet body weight/day. The diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 4,000 mg/kg diet. The study lasted 8 weeks. The following parameters were measured on nine animals per treatment: performance (survival growth rate (SGR), final body weight (BW), weight gain (WG), feed efficiency (FE), carcass parameters, hepatosomatic index, viscero-somatic index, condition factor and whole-body proximate composition. The following parameters were assessed on three animals per treatment group: superoxide dismutase (SOD), myeloperoxidase (MPO), lysozyme, aspartate transaminase (AST) and alanine transaminase, TP and glucose. Gene expression was assessed on five animals per treatment. Intestines were used for the evaluation of histomorphology (*villi* length (VL) and muscular thickness (MT)) and enzyme activities (trypsin, lipase and amylase). At the end of the trial, the animals treated with oxytetracycline, compared to the control group, showed increased final BW (10.6 vs 9.77 g), weight gain (276% vs 242%), better G:F (FE, 93.5 vs 81.9), SGR (2.54 vs 2.37) and gain to protein ratio (PER, 2.58 vs 2.31). Intestines histomorphology showed increased *villi* length (235 vs 202 μm) and muscular thickness (46 vs 36 μm). Non-specific immune response indexes (SOD, MPO and lysozyme) were increased (positive effects) in the group treated with oxytetracycline. With respect to blood parameters, only AST was increased in the oxytetracycline group. Dietary supplementation with oxytetracycline at a concentration of 4,000 mg/kg feed showed growth-promoting effects in juvenile Nile tilapia.

In the study of Won et al. (2017), two experiments were carried out. In Experiment 1 a total of 360 juvenile Rainbow trout (*Oncorhynchus mykiss*) (2.7 g BW) were distributed in 18 pens in groups of 20 animals and allocated to six treatments (three replicates/treatment). Fish were fed twice daily at satiation rate of 3.89% of wet body weight per day. In Experiment 2 a total of 1,300 sub adult rainbow trout (*Oncorhynchus mykiss*) (262 g BW) were distributed in eight ponds in groups of 163 animals and allocated to four treatments (two replicates/ treatment). Fish were fed twice daily at satiation rate of 1~1.5% of wet body weight per day. In both experiments, the diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of oxytetracycline (unspecified form) at a concentration of 4,000 mg/kg diet. The studies lasted eight weeks (Experiment 1) and 22 weeks (Experiment 2). In Experiment 1 the following parameters were measured: survival, performance (final BW, weight gain), feed efficiency, survival growth rate and protein efficiency rate; carcass parameters (on three fish/tank: condition factor, hepatosomatic index, viscero-somatic index, whole body proximate composition); blood parameters (on 5 fish/tank: oxidative radical production (nitroblue tetrazolium assay), superoxide dismutase, myeloperoxidase, lysozyme, aspartate transaminase and alanine transaminase, total protein and glucose). In Experiment 2 fish growth performance and biochemical parameters were analysed in duplicates as followed in the Experiment 1. At the end of the Experiment 1 the animals treated with oxytetracycline did not show any difference in the parameters measured compared to the control group; concerning Experiment 2, the only effect seen was on aspartate transaminase which was higher in the treated group (140 vs 112 U/L). Dietary supplementation with oxytetracycline at a concentration of 4,000 mg/kg diet showed no growth-promoting effects in sub-adult rainbow trout.

3.3.3.4. Discussion

From the studies examined, the test item has been described as (i) 'oxytetracycline hydrochloride' (two studies), (ii) an oxytetracycline commercial preparation (unspecified chemical form; ten studies) or (iii) 'oxytetracycline' (unspecified form; 36 studies, corresponding to 37 publications). Therefore, for the cases (ii) and (iii), an uncertainty on the exact product used/concentration applied has been identified.

A detailed analysis of the uncertainties for oxytetracycline is included in Appendix A.2 (Table A.4) of this document, and Section 5 of the Scientific Opinion Part 1 (see also the Virtual Issue).

3.3.3.4.1. Ruminants

Only one study in calves was identified as suitable for the assessment (Yuangklang et al., 2005). Oxytetracycline at the concentration of 80 mg/kg milk replacer, added to the basal diet for 35 days (between 19 and 32 experimental weeks) did not change zootechnical (FI, final BW, BW gain) and other controlled parameters (faecal bile acid excretion and digestibility coefficient of DM, CP, total fat, ash, calcium, phosphorus). However, the coefficient of digestibility of magnesium was increased in the treated group (0.41 vs 0.32).

3.3.3.4.2. Pigs

Two studies in pigs were identified as suitable for the assessment, one in weaned piglets (Han et al., 2014) and one in pigs for fattening (Akinfala and Tewe, 2004). In the assessed studies, treatments contained groups of animals treated with only one oxytetracycline concentration and did not allow dose-related effects to be assessed.

Dietary oxytetracycline supplementation in weaned piglets at 500 mg oxytetracycline/kg feed (Han et al., 2014) and in pigs for fattening at 450 mg oxytetracycline/kg feed (Akinfala and Tewe, 2004) showed growth-promoting effects.

3.3.3.4.3. Poultry

Thirty-two publications (30 studies) considered as suitable for the assessment covered chickens for fattening (25), hens (2), Guinea fowls (2) and Japanese quail (1). The majority of these studies used oxytetracycline as positive control and thus only one concentration was tested, which preclude the possibility to correlate the observed effects with the level administered. Different doses were tested only in one study in Guinea fowls (Oguntona, 1988a).

In fourteen studies (15 publications) in chickens for fattening, dietary oxytetracycline supplementation at 50 to 500 mg/kg feed improved growth performance of chickens. Individual publications reported the following doses of oxytetracycline: 50 mg oxytetracycline/kg feed (Zulkifli et al. (2000), Kalavathy et al. (2008), Hossain and Yang (2014), Hossain et al. (2012d,e) and Mahmoud et al. (2020)); 55 mg oxytetracycline/kg feed (Stutz and Lawton (1984)); 100 mg oxytetracycline/kg feed (Hong et al. (2012) and Singh et al. (2014a,b, 2015)); 200 mg oxytetracycline hydrochloride, corresponding to 186 mg oxytetracycline/kg feed (Khadem et al. (2014)); 200 mg oxytetracycline/kg feed (Attia et al. (2017) and Shokaiyan et al. (2019)); 500 mg oxytetracycline/kg feed (Bostami et al. (2017)).

Other ten studies in chickens for fattening showed that dietary oxytetracycline supplementation at similar levels, 30–600 mg/kg feed, did not affect growth performance of chickens for fattening: 30 mg oxytetracycline/kg feed (Sarker et al. (2010b)); 50 mg oxytetracycline/kg feed (Ahmed et al. (2014)); 55 mg oxytetracycline/kg feed (Sarker et al. (2010a) and Lee et al. (2011a)); 100 mg oxytetracycline/kg feed (Singh et al. (2018, 2019)); 200 mg oxytetracycline/kg feed (Henry et al. (1987)); 250 mg oxytetracycline/kg feed (Zamora et al. (2017)); 500 mg oxytetracycline/kg feed (Sarker et al. (2010c)); 600 mg oxytetracycline/kg feed (Alonge et al. (2017a)). No conclusions could be drawn on the growth-promoting effects in chickens for fattening at 50 mg oxytetracycline/kg feed in one study since conflicting results were identified for body weight gain and F:G (Deepa et al., 2018).

In two studies in laying hens, dietary oxytetracycline supplementation at 150 mg oxytetracycline/kg feed (Shalaei et al. (2014)) and 500 mg oxytetracycline/kg feed (Aalaei et al. (2018, 2019)) did not affect performance.

Three studies in other poultry species, reported that dietary oxytetracycline supplementation from 5 to 20 mg/kg feed improved growth/yield performance. In growing Guinea fowls, the tested levels of oxytetracycline ranged from 5 to 20 mg oxytetracycline/kg feed (Oguntona, 1988a,b) that allows the assessment of a dose-related effect; the best feed efficiency was obtained at 6.6 mg oxytetracycline/kg feed. In growing/laying Japanese quail, dietary supplementation at 20 mg oxytetracycline/kg feed (Oko et al., 2018) improved growth and yield performance of birds.

3.3.3.4.4. Aquatic animals

A total of 14 studies in aquatic animals were identified as suitable for the assessment. In all these studies oxytetracycline was added to feed. Dietary addition of oxytetracycline in five of the studies were performed with channel catfish (mainly African, *Clarias gariepinus* and *Ictalurus punctatus*) (Sanchez-Martínez et al. (2008), Adeniyi et al. (2018), Lawal et al. (2019), Olusola et al. (2020) Adeniyi (2020)). Three other studies (El-Sayed et al. (2014), Reda et al. (2016) and Won et al. (2020)) were conducted with Nile tilapia. Only two studies reported results on the effects of the oral administration of oxytetracycline in rainbow trout (Park et al. (2016a) and Won et al. (2017)); and one study in Beluga (Ebrahimi et al. (2020)), starry flounder (Park et al. (2016b)) and olive flounder (Rhee et al. (2020)). Another study (Trushenski et al., 2018) used four species of aquatic animals – channel catfish, hybrid striped bass, Nile tilapia and rainbow trout.

Regarding the tested levels of oxytetracycline, the supplementation was made at concentrations within a wide range. The lowest concentration used was 30 mg oxytetracycline/kg feed in the studies with Beluga (Ebrahimi et al. (2020)) and with channel catfish (Olusola et al. (2020)), followed by 100 mg oxytetracycline/kg feed and 200 mg oxytetracycline hydrochloride/kg feed (corresponding to 186 mg oxytetracycline/kg feed) in studies with Nile tilapia (Reda et al. (2016) and El-Sayed et al. (2014)), 200 mg oxytetracycline/kg feed in study with channel catfish (Lawal et al. (2019)) and 240 mg oxytetracycline/kg feed in the study by Trushenski et al. (2018) with four species of fish. Doses of 400 and 600 mg oxytetracycline/kg feed were tested in two studies with African catfish (Adeniyi et al. (2018) and Adeniyi (2020)). In all other studies the concentrations tested were higher: 2,500 mg oxytetracycline/kg feed for African catfish (Sanchez-Martínez et al. (2008)), 4,000 mg oxytetracycline/kg feed for Nile tilapia (Won et al. (2017)) and for rainbow trout (Won et al. (2020); and 5,000 mg oxytetracycline/kg feed for rainbow trout (Park et al. (2016a)), for starry flounder (Park et al. (2016b)) and for olive flounder (Rhee et al. (2020)).

The effect of oxytetracycline as a growth promoter or yield-enhancer was confirmed in nine studies. In more detail, the following levels revealed a positive effect: with African and channel catfish, 30 mg oxytetracycline/kg feed (Olusola et al. (2020)), 400 mg oxytetracycline/kg feed (Adeniyi et al. (2018)), 600 mg oxytetracycline/kg feed (Adeniyi (2020)) and 2,500 mg oxytetracycline/kg feed (Sanchez-Martínez et al. (2008)); with Nile tilapia, 100 mg oxytetracycline/kg feed (Reda et al. (2016)), 200 mg oxytetracycline hydrochloride/kg feed (corresponding to 186 mg oxytetracycline/kg feed) (El-Sayed et al. (2014)) and 4,000 mg oxytetracycline/kg feed (Won et al. (2020)); with rainbow trout, 5,000 mg oxytetracycline/kg feed (Park et al. (2016a)); and with olive flounder, 5,000 mg oxytetracycline/kg feed (Rhee et al. (2020)). Thus, the levels that had promoting effects on performance parameters were very variable and no specific conclusion can be drawn on dose-related effects.

The other five studies in which oxytetracycline did not show growth-promoting effects were the following: Ebrahimi et al. (2020) with Beluga and 30 mg oxytetracycline/kg feed; Lawal et al. (2019) with African catfish and 200 mg oxytetracycline/kg feed; Park et al. (2016b) with starry flounder and 5,000 mg oxytetracycline/kg feed; Won et al. (2017) with rainbow trout and 4,000 mg oxytetracycline/kg feed; Trushenski et al. (2018) with four fish species and 240 and 1,200 mg oxytetracycline/kg feed.

3.3.3.5. Concluding remarks

It is judged 50–66% certain that oxytetracycline has growth-promoting/increase yield effects in chickens for fattening at concentrations ranging from 50 to 500 mg/kg complete feed (14 studies).

It is judged 33–66% certain ('about as likely as not') that oxytetracycline has growth-promoting/increased yield effects: in weaned piglets at a concentration of 500 mg/kg complete feed (one study), in pigs for fattening at a concentration of 450 mg/kg complete feed (one study), in growing Guinea fowls at concentrations ranging from 5 to 20 mg/kg complete feed (two studies), in growing/laying Japanese quail at a concentration of 20 mg/kg complete feed (one study), in Nile tilapia at concentrations ranging from 100 to 4,000 mg/kg complete feed (three studies), in African and Channel catfish at concentrations ranging from 30 to 2,500 mg/kg diet (four studies), in rainbow trout at a concentration of 5,000 mg/kg diet (one study) and in olive flounder at a concentration of 5,000 mg/kg diet (one study).

No data are available in the scientific literature showing effects of oxytetracycline on growth promotion/increased yield when added (i) to weaned piglets feed at concentrations below 500 mg/kg, (ii) to pigs for fattening feed at concentrations below 450 mg/kg, (iii) to chickens for fattening feed at concentrations below 50 mg/kg, (iv) to growing Guinea fowls feed at concentrations below 5 mg/kg, (v) to growing/laying Japanese quail feed at concentrations below 20 mg/kg, (vi) to Nile tilapia feed at concentrations below 100 mg/kg, (vii) to African and Channel catfish feed at concentrations below 30 mg/kg, (viii) to rainbow trout and olive flounder feed at concentrations below 5,000 mg/kg or (ix) to feed of any other food-producing animal species or categories.

3.3.4. Doxycycline

3.3.4.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion Part 1; see also the Virtual Issue) resulted in 548 papers mentioning doxycycline 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 doxycycline.[4] After removing the reports not matching the eligibility criteria, 15 publications were identified.

3.3.4.2. Evaluation of the studies

The 15 publications identified in the literature search were appraised for suitability for the assessment of the effects of doxycycline 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; see also the Virtual Issue).[5] None the publications was 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 B.4 (Table B.4).

3.3.4.3. Concluding remark

Owing to the lack of suitable data, levels of doxycycline 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 chlortetracycline, oxytetracycline, tetracycline and doxycycline in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/rumen?

With regards to tetracycline, chlortetracycline, oxytetracycline:

- The Feed Antimicrobial Resistance Selection Concentration (FARSC, for large intestine and/or rumen in the case of adult ruminants after weaning) corresponding to the concentration of chlortetracycline, oxytetracycline and tetracycline 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 ranges, for the different animal species, from 0.13 to 2.4 μg/kg feed. No FARSC was determined for horses and rabbits.
- For each animal species, the FARSC obtained ranged:
  - [0.30–1.56] μg/kg feed for lactating sows
  - [0.21–1.07] μg/kg feed for piglets
  - [0.25–1.28] μg/kg feed for pigs for fattening
  - [0.26–2.00] μg/kg feed for veal calves

- [0.72–1.44] μg/kg feed for dairy cows ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined)
- [1.20–2.40] μg/kg feed for cattle for fattening, adult sheep and goats ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined)
- [0.17–0.84] μg/kg feed for chickens for fattening
- [0.30–1.51] μg/kg feed for laying hens
- [0.13–0.62] μg/kg feed for turkeys for fattening
- [0.23–0.93] μg/kg feed for salmons

The values for dairy cows, cattle for fattening, sheep and goats only correspond to $FARSC_{rumen}$, because the absence of data on bioavailability for ruminants after weaning prevents the calculation of $FARSC_{intestine}$.

With regards to doxycycline:

- The FARSC corresponding to the concentration of doxycycline ranges, for the different species, from 0.12 to 3.3 μg/kg feed. No FARSC was determined for rabbits.
- For each animal species, the FARSC obtained ranged:
  - [0.25–1.30] $\times 10^{-3}$ mg /kg feed for lactating sows
  - [0.17–0.89] $\times 10^{-3}$ mg /kg feed for piglets
  - [0.20–1.07] $\times 10^{-3}$ mg /kg feed for pigs for fattening
  - [0.40–3.33] $\times 10^{-3}$ mg /kg feed for veal calves
  - [0.50–2.97] $\times 10^{-3}$ mg /kg feed for dairy cows ($FARSC_{intestine}$ and $FARSC_{rumen}$)
  - [0.42–2.52] $\times 10^{-3}$ mg /kg feed for cattle for fattening ($FARSC_{intestine}$ and $FARSC_{rumen}$)
  - [0.26–2.40] $\times 10^{-3}$ mg /kg feed for goats ($FARSC_{intestine}$ and $FARSC_{rumen}$)
  - [0.22–2.40] $\times 10^{-3}$ mg /kg feed for sheep ($FARSC_{intestine}$ and $FARSC_{rumen}$)
  - [0.17–1.12] $\times 10^{-3}$ mg /kg feed for chickens for fattening
  - [0.30–2.01] $\times 10^{-3}$ mg /kg feed for laying hens
  - [0.12–0.83] $\times 10^{-3}$ mg /kg feed for turkeys for fattening
  - [0.17–0.65] $\times 10^{-3}$ mg /kg feed for horses
  - [0.23–1.21] $\times 10^{-3}$ mg /kg feed for salmons

For all substances:

The probability that tetracycline, chlortetracycline, oxytetracycline and/or doxycycline concentrations below the lowest FARSC value for an animal species will confer any enrichment of, and/or selection for, resistant bacteria in the intestine and/or rumen is estimated to be 1–5% (extremely unlikely).

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

AQ2. Which are the specific concentrations of chlortetracycline, oxytetracycline, tetracycline and doxycycline in feed of food-producing animals that have an effect in terms of growth promotion/increased yield?

With regards to chlortetracycline:

- It is judged 66–90% certain ('likely') that chlortetracycline has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 40 to 500 mg/kg complete feed (19 studies).
- It is judged 50–66% certain that chlortetracycline has growth-promoting/increase yield effects in pigs for fattening at concentrations ranging from 11 to 1,000 mg/kg complete feed (nine studies) and in chickens for fattening at concentrations ranging from 10 to 929.3 mg/kg complete feed (17 studies).
- It is judged 33–66% certain ('about as likely as not') that chlortetracycline
  - has growth-promoting/increase yield effects: in calves at concentrations ranging from 45 to 80 mg/kg DM (three studies), in cattle for fattening at concentrations ranging from 35 to 40 mg/kg DM (two studies), in lambs for fattening at the concentration of 25 mg/kg DM (one study), in sows at concentrations ranging from 110 to 2,000 mg/kg complete feed (four studies) and in fish at a concentration of 50 mg/kg complete feed (one study).

- o has negative effects on performance of lambs for fattening at a concentration of 11 mg/kg DM (one study) and on performance and feed utilisation of pigs for fattening at a concentration of 150 mg/kg complete feed (one study).

- No data are available in the scientific literature showing effects of chlortetracycline on growth promotion/increased yield when added (i) to calves feed at concentrations below 45 mg/kg DM, (ii) to cattle for fattening feed at concentrations below 35 mg/kg DM, (iii) to lambs for fattening feed at concentrations below 25 mg/kg DM, (iv) to weaned piglets feed at concentrations below 40 mg/kg, (v) to pigs for fattening feed at concentrations below 11 mg/kg, (vi) to sows feed at concentrations below 110 mg/kg, (vii) to chickens for fattening feed at concentrations below 10 mg/kg, (viii) to fish feed at concentrations below 50 mg/kg, or (ix) to feed of any other food-producing animal species or categories

With regards to oxytetracycline:

- It is judged 50–66% certain that oxytetracycline has growth-promoting/increase yield effects in chickens for fattening at concentrations ranging from 50 to 500 mg/kg complete feed (14 studies).
- It is judged 33–66 % certain ('about as likely as not') that oxytetracycline has growth-promoting/increase yield effects: in weaned piglets at a concentration of 500 mg/kg complete feed (one study), in pigs for fattening at a concentration of 450 mg/kg complete feed (one study), in growing Guinea fowls at concentrations ranging from 5 to 20 mg/kg complete feed (two studies), in growing/laying Japanese quail at a concentration of 20 mg/kg complete feed (one study), in Nile tilapia at concentrations ranging from 100 to 4,000 mg/kg complete feed (three studies), in African and Channel catfish at concentrations ranging from 30 to 2,500 mg/kg diet (four studies), in rainbow trout at a concentration of 5,000 mg/kg diet (one study) and in olive flounder at a concentration of 5,000 mg/kg diet (one study).
- No data are available in the scientific literature showing effects of oxytetracycline on growth promotion/increased yield when added (i) to weaned piglets feed at concentrations below 500 mg/kg, (ii) to pigs for fattening feed at concentrations below 450 mg/kg, (iii) to chickens for fattening feed at concentrations below 50 mg/kg, (iv) to growing Guinea fowls feed at concentrations below 5 mg/kg, (v) to growing/laying Japanese quail feed at concentrations below 20 mg/kg, (vi) to Nile tilapia feed at concentrations below 100 mg/kg, (vii) to African and Channel catfish feed at concentrations below 30 mg/kg, (viii) to rainbow trout and olive flounder feed at concentrations below 5,000 mg/kg or (ix) to feed of any other food-producing animal species or categories

With regards to tetracycline:

- It is judged 33–66% certain ('about as likely as not') that tetracycline
  - o has growth-promoting/increase yield effects in chickens for fattening at a concentration of 500 mg/kg complete feed (one study).
  - o has negative effects at a concentration of 55 mg/kg complete feed on growth performance in pigs for fattening (one study).

- No data are available in the scientific literature showing effects of tetracycline on growth promotion/increased yield when added (i) to chickens for fattening feed at concentrations below 500 mg/kg, or (ii) to feed of any other food-producing animal species or categories.

With regards to doxycycline:

- Owing to the lack of suitable data, levels of doxycycline 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. Recommendations

To perform further studies to supply more diverse and complete data to reduce uncertainties around the calculation of the FARSC for the tetracyclines under assessment (tetracycline, chlortetracycline, oxytetracycline, and doxycycline).

References

Aalaei M, Khatibjoo A, Zaghari M, Taherpour K, Akbari Gharaei M and Soltani M, 2018. Comparison of single- and multi-strain probiotics effects on broiler breeder performance, egg production, egg quality and hatchability. British Poultry Science, 59, 531–538. https://doi.org/10.1080/00071668.2018.1496400

Aalaei M, Khatibjoo A, Zaghari M, Taherpou K, Akbari-Gharaei M and Soltani M, 2019. Effect of single- and multi-strain probiotics on broiler breeder performance, immunity and intestinal toll-like receptors expression. Journal of Applied Animal Research, 47, 236–242. https://doi.org/10.1080/09712119.2019.1618311

Abdelhamid AM, Mehrim AI, El-Barbary MI, Ibrahim SM and El-Wahab AIA, 2009. Evaluation of a new Egyptian probiotic by African catfish fingerlings. Journal of Environmental Science and Technology, 2, 133–145. https://doi.org/10.3923/jest.2009.133.145

Abdul-Aziz TA and Weber LJ, 1999. *Ornithobacterium rhinotracheale* infection in a turkey flock in Ontario. The Canadian veterinary journal = La revue veterinaire canadienne, 40, 349–350.

Abu-Ruwaida AS, Husseini M and Banat IM, 1995. *Salmonella* exclusion in broiler chicks by the competitive action of adult gut microflora. Microbios, 83, 59–69.

Adeniyi OV, 2020. Growth performance of *Clarias gariepinus* (Burchell, 1822) fed diets fortified with lemongrass (*Cymbopogon citratus*). Acta Veterinaria Eurasia, 46, 15–23. https://doi.org/10.5152/actavet.2020.19023

Adeniyi OV, Olaifa FE and Emikpe BO, 2018. Growth performance and nutrient digestibility of *Clarias gariepinus* (Burchell 1822) fed diets fortified with *Tamarindus indica* pulp and leaf meal. Asian Fisheries Science, 31, 17–31.

Aguirre ATA, Acda SP, Angeles AA, Oliveros MCR, Merca FE and Cruz FA, 2015. Effect of bovine lactoferrin on growth performance and intestinal histologic features of broilers. Philippine Journal of Veterinary and Animal Sciences, 41, 12–20.

Agunos A, Carson C and Leger D, 2013. Antimicrobial therapy of selected diseases in turkeys, laying hens, and minor poultry species in Canada. The Canadian Veterinary Journal La revue veterinaire canadienne, 54, 1041–1052.

Agwuh K and Macgowan A, 2006. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. The Journal of Antimicrobial Chemotherapy, 58, 256–265. https://doi.org/10.1093/jac/dkl224

Ahmed ST, Mun H-S, Islam MM and Yang C-J, 2014. Effects of fermented corni fructus and fermented kelp on growth performance, meat quality, and emission of ammonia and hydrogen sulphide from broiler chicken droppings. British Poultry Science, 55, 745–751. https://doi.org/10.1080/00071668.2014.960804

Ahmed ST, Mun H-S, Son S-B and Yang C-J, 2018. Effects of fermented bamboo vinegar liquid on growth performance, nutrient digestibility, faecal *Escherichia coli* concentration and ammonia emissions in growing pigs. South African Journal of Animal Science, 48, 621–626. https://doi.org/10.4314/sajas.v48i4.3

Ahn Y, Jung JY, Veach BT, Khare S, Gokulan K, Piñeiro SA and Cerniglia CE, 2018. *In vitro* test systems to determine tetracycline residue binding to human feces. Regulatory Toxicology and Pharmacology, 99, 105–115. https://doi.org/10.1016/j.yrtph.2018.09.013

Akinfala EO and Tewe OO, 2004. Supplemental effects of feed additives on the utilization of whole cassava plant by growing pigs in the tropics. Livestock Research for Rural Development, 16.

Alexopoulos C, Fthenakis GC, Burriel A, Bourtzi-Hatzopoulou E, Kritas SK, Sbiraki A and Kyriakis SC, 2003. The effects of the periodical use of in-feed chlortetracycline on the reproductive performance of gilts and sows of a commercial pig farm with a history of clinical and subclinical viral and bacterial infections. Reproduction in Domestic Animals, 38, 187–192. https://doi.org/10.1046/j.1439-0531.2003.00415.x

Alonge EO, Eruvbetine D, Idowu O, Obadina AO, Olukomaiya OO, Omotainse OS, Ahmad FH and Abiola YO, 2017a. Effects of antibiotic, probiotic and prebiotic supplementation in broiler diets on performance characteristics and apparent nutrient digestibility. Journal of Applied Sciences and Environmental Management, 21, 1297–1300.

Alonge EO, Eruvbetine D, Idowu OMO, Obadina AO and Olukomaiya OO, 2017b. Comparing the effects of supplementary antibiotic, probiotic, and prebiotic on carcass composition, *Salmonella* counts and serotypes in droppings and intestine of broiler chickens. Poultry Science Journal, 5, 41–50. https://doi.org/10.22069/psj.2017.11979.1214

Aluko K, Velayudhan DE, Khafipour E, Li A, Yin Y and Nyachoti M, 2017. Combined effects of chitosan and microencapsulated *Enterococcus faecalis* CG1.0007 probiotic supplementation on performance and diarrhea incidences in enterotoxigenic *Escherichia coli* K88+ challenged piglets. Animal Nutrition, 3, 366–371. https://doi.org/10.1016/j.aninu.2017.09.003

Alvares AP, Harbers LH and Visek WJ, 1964. Effect of barbituric acid, chlortetracycline and carbohydrates upon growth and gastrointestinal urease activity of chicks. The Journal of Nutrition, 82, 93–98. https://doi.org/10.1093/jn/82.1.93

Amachawadi RG, Shelton NW, Tokach MD, Scott HM, Dritz SS, Goodband RD, DeRouchey JM, Nelssen JL and Nagaraja TG, 2011. Effects of feeding copper and feed-grade antimicrobials on the growth performance of weanling pigs. In: Kansas State University Swine Day 2011. Report of Progress 1056, Kansas State University, Kansas, pp. 57–61.
Anadón A, Martínez-Larrañaga MR, Diaz MJ, Bringas P, Fernández MC, Fernández-Cruz ML, Iturbe J and Martínez MA, 1994. Pharmacokinetics of doxycycline in broiler chickens. Avian Pathology, 23, 79–90. https://doi.org/10.1080/03079459408418976
Anadón A, Gamboa F, Martínez MA, Castellano V, Martínez M, Ares I, Ramos E, Suarez FH and Martínez-Larrañaga MR, 2012. Plasma disposition and tissue depletion of chlortetracycline in the food producing animals, chickens for fattening. Food and Chemical Toxicology, 50, 2714–2721. https://doi.org/10.1016/j.fct.2012.05.007
al-Ankari AS and Homeida AM, 1996. Effect of antibacterial growth promoters on the immune system of broiler chicks. Veterinary Immunology and Immunopathology, 53, 277–283. https://doi.org/10.1016/S0165-2427(96)05609-7
Antonelli A, D'Andrea MM, Brenciani A, Galeotti C, Morroni G, Pollini S, Varaldo P and Rossolini G, 2018. Characterization of *poxt*A, a novel phenicol–oxazolidinone–tetracycline resistance gene from an MRSA of clinical origin. Journal of Antimicrobial Chemotherapy, 73. https://doi.org/10.1093/jac/dky088
Attia G, El-Eraky W, Hassanein E, El-Gamal M, Farahat M and Hernandez-Santana A, 2017. Effect of dietary inclusion of a plant extract blend on broiler growth performance, nutrient digestibility, caecal microflora and intestinal histomorphology. International Journal of Poultry Science, 16, 344–353. https://doi.org/10.3923/ijps.2017.344.353
Azam K and Narayan P, 2013. Safe usage of antibiotic (oxytetracycline) in larval rearing of mud crab, *Scylla serrata* (Forsskål, 1775) in Fiji. World Journal of Fish and Marine Sciences, 5, 209–213.
Baert K, Croubels S, Gasthuys F, De Busser J and De Backer P, 2000. Pharmacokinetics and oral bioavailability of a doxycycline formulation (doxycycline 75%) in nonfasted young pigs. Journal of Veterinary Pharmacology and Therapeutics, 23, 45–48. https://doi.org/10.1046/j.1365-2885.2000.00235.x
Bagal VL, Khatta VK, Tewatia BS, Sangwan SK and Raut SS, 2016. Relative efficacy of organic acids and antibiotics as growth promoters in broiler chicken. Veterinary World, 9, 377–382. https://doi.org/10.14202/vetworld.2016.377-382
Bahl MI, Sørensen SJ, Hansen LH and Licht TR, 2004. Effect of tetracycline on transfer and establishment of the tetracycline-inducible conjugative transposon TN*916* in the guts of gnotobiotic rats. Applied and Environmental Microbiology, 70, 758. https://doi.org/10.1128/AEM.70.2.758-764.2004
Bai SP, Wu AM, Ding XM, Lei Y, Bai J, Zhang KY and Chio JS, 2013. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry Science, 92, 663–670. https://doi.org/10.3382/ps.2012-02813
Bains BS, 1974. The economic appraisal of the control of chronic respiratory disease in meat chickens. Poultry Science, 53, 2059–2065. https://doi.org/10.3382/ps.0532059
Baldwin RL, McLeod KR, Elsasser TH, Kahl S, Rumsey TS and Streeter MN, 2000. Influence of chlortetracycline and dietary protein level on visceral organ mass of growing beef steers. Journal of Animal Science, 78, 3169–3176. https://doi.org/10.2527/2000.78123169x
Banerjee S, Sar A, Misra A, Pal S, Chakraborty A and Dam B, 2018. Increased productivity in poultry birds by sub-lethal dose of antibiotics is arbitrated by selective enrichment of gut microbiota, particularly short-chain fatty acid producers. Microbiology (United Kingdom), 164, 142–153. https://doi.org/10.1099/mic.0.000597
Battaglene SC, Morehead DT, Cobcroft JM, Nichols PD, Brown X and Carson J, 2006. Combined effects of feeding enriched rotifers and antibiotic addition on performance of striped trumpeter (*Latris lineata*) larvae. Aquaculture, 251, 456–471. https://doi.org/10.1016/j.aquaculture.2005.06.021
Beacom SE, Mir Z, Korsrud GO, Yates WDG and MacNeil JD, 1988. Effect of the feed additives chlortetracycline, monensin and lasalocid on feedlot performance of finishing cattle, liver lesions and tissue levels of chlortetracycline. Canadian Journal of Animal Science, 68, 1131–1141. https://doi.org/10.4141/cjas88-129
Begin JJ, 1971. The effect of antibiotic supplementation on growth and energy utilization of chicks. Poultry Science, 50, 1496–1500. https://doi.org/10.3382/ps.0501496
Bengtsson-Palme J and Larsson DG, 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environment International, 86, 140–149. https://doi.org/10.1016/j.envint.2015.10.015
Berge ACB, Lindeque P, Moore DA and Sischo WM, 2005. A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of preweaned calves. Journal of Dairy Science, 88, 2166–2177. https://doi.org/10.3168/jds.S0022-0302(05)72892-7
Berge ACB, Moore DA, Besser TE and Sischo WM, 2009. Targeting therapy to minimize antimicrobial use in preweaned calves: effects on health, growth, and treatment costs. Journal of Dairy Science, 92, 4707–4714. https://doi.org/10.3168/jds.2009-2199
Bergstrom JR, Nelssen JL, Tokach MD, Dritz SS, DeRouchey JM, Goodband RD and Woodworth JC, 2007a. An evaluation of arabinogalactan (Larafeed® ag) as a nutraceutical growth promoter in starter diets for weanling pigs. In: Kansas State University Swine Day 2007a. Report of Progress 985, Kansas State University, Kansas, pp. 77–82.

Bergstrom JR, Nelssen JL, Tokach MD, Dritz SS, DeRouchey JM and Goodband RD, 2007b. An evaluation of astaxanthin as a nutraceutical growth promoter in starter diets for weanling pigs. https://doi.org/10.4148/2378-5977.6963
Bergstrom JR, Tokach MD, Nelssen JL, Dritz SS, DeRouchey JM and Goodband RD, 2007c. An evaluation of an enzyme blend (Natuzyme®) in diets for weanling pigs. In: Kansas State University Swine Day 2007. Report of Progress 985, Kansas State University, Kansas, pp. 66–76.
Betancourt L, Rodriguez F, Phandanouvong V, Ariza-Nieto C, Hume M, Nisbet D, Afanador-Téllez G, van Kley AM and Nalian A, 2014. Effect of *Origanum chemotypes* on broiler intestinal bacteria. Poultry Science, 93, 2526–2535. https://doi.org/10.3382/ps.2014-03944
Bhandari SK, Xu B, Nyachoti CM, Giesting DW and Krause DO, 2008. Evaluation of alternatives to antibiotics using an *Escherichia coli* K88(+) model of piglet diarrhea: effects on gut microbial ecology. Journal of Animal Science, 86, 836–847. https://doi.org/10.2527/jas.2006-822
Bónai A, Szendrő Z, Maertens L, Matics Z, Fébel H, Kametler L, Tornyos G, Horn P, Kovács F and Kovács M, 2008. Effect of inulin supplementation on caecal microflora and fermentation in rabbits. World Rabbit Science Association, Castanet-Tolosan. pp. 555–560.
Bónai A, Szendrö Z, Matics Z, Fébel H, Kametler L, Tornyos G, Horn P, Kovács F and Kovács M, 2010. Effect of inulin supplementation and age on growth performance and digestive physiological parameters in weaned rabbits. World Rabbit Science, 18, 121–129. https://doi.org/10.4995/wrs.2010.5883
Bosi P, Merialdi G, Scandurra S, Messori S, Bardasi L, Nisi I, Russo D, Casini L and Trevisi P, 2011. Feed supplemented with 3 different antibiotics improved food intake and decreased the activation of the humoral immune response in healthy weaned pigs but had differing effects on intestinal microbiota. Journal of Animal Science, 89, 4043–4053. https://doi.org/10.2527/jas.2010-3311
Bostami ABMR, Ahmed ST, Mun HS, Hong SB and ChulJu Y, 2016. Efficacy of *Rhodopseudomonas* containing multi-microbe probiotic on growth performance, mortality and cecal microflora in broilers. African Journal of Microbiology Research, 10, 985–993.
Bostami ABMR, Sarker MSK and Yang C-J, 2017. Performance and meat fatty acid profile in mixed sex broilers fed diet supplemented with fermented medicinal plant combinations. Journal of Animal and Plant Sciences, 27, 360–372.
Bousquet E, Pommier P, Wessel-Robert S, Morvan H, Benoit-Valiergue H and Laval A, 1998. Efficacy of doxycycline in feed for the control of pneumonia caused by *Pasteurella multocida* and *Mycoplasma hyopneumoniae* in fattening pigs. Veterinary Record, 143, 269–272. https://doi.org/10.1136/vr.143.10.269
Bovera F, Nizza S, Marono S, Mallardo K, Piccolo G, Tudisco R, Martino L and Nizza A, 2010. Effect of mannan oligosaccharides on rabbit performance, digestibility and rectal bacterial anaerobic populations during an episode of epizootic rabbit enteropathy. World Rabbit Science, 18, 9–16. https://doi.org/10.4995/wrs.2010.18.02
Braude R and Johnson BC, 1953. Effect of aureomycin on nitrogen and water metabolism in growing pigs. The Journal of Nutrition, 49, 505–512. https://doi.org/10.1093/jn/49.3.505
Bray WA, Williams RR, Lightner DV and Lawrence AL, 2006. Growth, survival and histological responses of the marine shrimp, *Litopenaeus vannamei*, to three dosage levels of oxytetracycline. Aquaculture, 258, 97–108. https://doi.org/10.1016/j.aquaculture.2006.04.018
Bridge R, Owen BD and Beames RM, 1982. The effectiveness of adding penicillin to a mixture of chlortetracycline and sulfamethazine to improve growth of weanling pigs. Canadian Journal of Animal Science, 62, 967–969. https://doi.org/10.4141/cjas82-119
Brown PB, Becker DE, Terrill SW and Card LE, 1952. The effect of aureomycin on the growth and metabolism of the pig. Archives of Biochemistry and Biophysics, 41, 378–382. https://doi.org/10.1016/0003-9861(52)90466-9
Brown LD, Jacobson DR, Everett Jr JP, Seath DM and Rust JW, 1960. Urea utilization by young dairy calves as affected by chlortetracycline supplementation. Journal of Dairy Science, 43, 1313–1321. https://doi.org/10.3168/jds.S0022-0302(60)90318-0
Brown H, Bing RF, Grueter HP, McAskill JW, Cooley CO and Rathmacher RP, 1975. Tylosin and chloretetracycline for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. Journal of Animal Science, 40, 207–213. https://doi.org/10.2527/jas1975.402207x
Brumm MC and Peo Jr ER, 1985. Effect of receiving diets containing alfalfa and certain feed additives on performance of feeder pigs transported long distances. Journal of Animal Science, 61, 9–17. https://doi.org/10.2527/jas1985.6119
Burch DG, Jones GT, Heard TW and Tuck RE, 1986. The synergistic activity of tiamulin and chlortetracycline: in-feed treatment of bacterially complicated enzootic pneumonia in fattening pigs. Veterinary Record, 119, 108–112. https://doi.org/10.1136/vr.119.5.108
Burnell TW, Cromwell GL and Stahly TS, 1988. Effects of dried whey and copper sulfate on the growth responses to organic acid in diets for weanling pigs. Journal of Animal Science, 66, 1100–1108. https://doi.org/10.2527/jas1988.6651100x
Bush LJ, Allen RS and Jacobson NL, 1959. Effect of chlortetracycline on nutrient utilization by dairy calves. Journal of Dairy Science, 42, 671–678. https://doi.org/10.3168/jds.S0022-0302(59)90634-4

Cabral RG, Erickson PS, Guindon NE, Kent EJ, Chapman CE, Aragona KM, Cabral MD, Massa EC, Antaya NT, Muir CC, O'Donnell B and Branine ME, 2013. Effects of lasalocid and intermittent feeding of chlortetracycline on the growth of prepubertal dairy heifers. Journal of Dairy Science, 96, 4578–4585. https://doi.org/10.3168/jds.2013-6557

Cantas L, Midtlyng PJ and Sørum H, 2012. Impact of antibiotic treatments on the expression of the R plasmid *tra* genes and on the host innate immune activity during pRAS1 bearing *Aeromonas hydrophila* infection in zebrafish (*Danio rerio*). BMC Microbiology, 12, 37. https://doi.org/10.1186/1471-2180-12-37

Capps KM, Amachawadi RG, Menegat MB, Woodworth JC, Perryman K, Tokach MD, Dritz SS, DeRouchey JM, Goodband RD, Bai J, Apley MD, Lubbers BV and Nagaraja TG, 2020. Impact of added copper, alone or in combination with chlortetracycline, on growth performance and antimicrobial resistance of fecal *Enterococci* of weaned piglets. Journal of Animal Science, 98. https://doi.org/10.1093/jas/skaa003

Caryl JA, Cox G, Trimble S and O'Neill AJ, 2012. "tet(U)" is not a tetracycline resistance determinant. Antimicrobial Agents and Chemotherapy, 56, 3378–3379. https://doi.org/10.1128/AAC.05957-11

Castro LJ, Sahagún AM, Diez MJ, Fernández N, Sierra M and García JJ, 2009. Pharmacokinetics of doxycycline in sheep after intravenous and oral administration. Veterinary Journal, 180, 389–395. https://doi.org/10.1016/j.tvjl.2008.02.001

Çelýk K, Denlý M and Savas T, 2003. Reduction of toxic effects of aflatoxin B1 by using baker yeast (*Saccharomyces cerevisiae*) in growing broiler chicks diets. Revista Brasileira de Zootecnia, 32, 615–619. https://doi.org/10.1590/S1516-35982003000300013

Cernicchiaro N, Corbin M, Quinn M, Prouty F, Branine M and Renter DG, 2016. Meta-analysis of the effects of laidlomycin propionate, fed alone or in combination with chlortetracycline, compared with monensin sodium, fed alone or in combination with tylosin, on growth performance, health, and carcass outcomes in finishing steers in North America. Journal of Animal Science, 94, 1662–1676. https://doi.org/10.2527/jas.2015-0086

Cha C-N, Yu E-A, Park E-K, Kim S and Lee H-J, 2013. Effects of dietary supplementation with *Galla Rhois* on growth performance and diarrhea incidence in postweaning piglets. Journal of Veterinary Clinics, 30, 353–358.

Chansiripornchai N, 2009. Comparative efficacy of enrofloxacin and oxytetracycline by different administration methods in broilers after experimental infection with avian pathogenic *Escherichia coli*. Thai Journal of Veterinary Medicine, 39, 231–236.

Che L, Xu Q, Wu C, Luo Y, Huang X, Zhang B, Auclair E, Kiros T, Fang Z, Lin Y, Xu S, Feng B, Li J and Wu D, 2017. Effects of dietary live yeast supplementation on growth performance, diarrhoea severity, intestinal permeability and immunological parameters of weaned piglets challenged with enterotoxigenic *Escherichia coli* K88. British Journal of Nutrition, 118, 949–958. https://doi.org/10.1017/S0007114517003051

Chen YJ, Kwon OS, Min BJ, Son KS, Cho JH, Hong JW and Kim IH, 2005. The effects of dietary Biotite V supplementation as an alternative substance to antibiotics in growing pigs. Asian-Australasian Journal of Animal Sciences, 18, 1642–1645. https://doi.org/10.5713/ajas.2005.1642

Chen YJ, Min BJ, Cho JH, Kwon OS, Son KS, Kim IH and Kim SJ, 2006. Effects of dietary *Enterococcus faecium* SF68 on growth performance, nutrient digestibility, blood characteristics and faecal noxious gas content in finishing pigs. Asian-Australasian Journal of Animal Sciences, 19, 406–411. https://doi.org/10.5713/ajas.2006.406

Chen Y, Wen C and Zhou Y, 2018. Dietary synbiotic incorporation as an alternative to antibiotic improves growth performance, intestinal morphology, immunity and antioxidant capacity of broilers. Journal of the Science of Food and Agriculture, 98, 3343–3350. https://doi.org/10.1002/jsfa.8838

Cheng C, Xia M, Zhang X, Wang C, Jiang S and Peng J, 2018. Supplementing oregano essential oil in a reduced-protein diet improves growth performance and nutrient digestibility by modulating intestinal bacteria, intestinal morphology, and antioxidative capacity of growing-finishing pigs. Animals, 8. https://doi.org/10.3390/ani8090159

Choi JY, Kim JS, Ingale SL, Kim KH, Shinde PL, Kwon IK and Chae BJ, 2011a. Effect of potential multimicrobe probiotic product processed by high drying temperature and antibiotic on performance of weanling pigs. Journal of Animal Science, 89, 1795–1804. https://doi.org/10.2527/jas.2009-2794

Choi JY, Shinde PL, Ingale SL, Kim JS, Kim YW, Kim KH, Kwon IK and Chae BJ, 2011b. Evaluation of multi-microbe probiotics prepared by submerged liquid or solid substrate fermentation and antibiotics in weaning pigs. Livestock Science, 138, 144–151. https://doi.org/10.1016/j.livsci.2010.12.015

Chopra I and Roberts M, 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and Molecular Biology Reviews: MMBR, 65, 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001

Chow L, Waldron L and Gillings MR, 2015. Potential impacts of aquatic pollutants: sub-clinical antibiotic concentrations induce genome changes and promote antibiotic resistance. Frontiers in Microbiology, 6, 803. https://doi.org/10.3389/fmicb.2015.00803

Clawson AJ, Sheffy BE and Reid JT, 1955. Some effects of feeding chlortetracycline upon the carcass characteristics and the body composition of swine and a scheme for the resolution of the body composition. Journal of Animal Science, 14, 1122–1132. https://doi.org/10.2527/jas1955.1441122x

Colby RW, Rau FA and Dunn RC, 1950. Effect of feeding aureomycin to fattening lambs. Proceedings of the Society for Experimental Biology and Medicine, 75, 234–236. https://doi.org/10.3181/00379727-75-18155

Conejos JRV, Acda SP, Capitan SS, Agbisit EM and Merca FE, 2012. Mannan oligosaccharides from yeast (*Saccharomyces cerevisiae*) cell wall improves nutrient digestibility and intestinal morphology of growing pigs [*Sus domesticus* (Erxleben)]. Philippine Agricultural Scientist, 95, 305–311.
Connor JK and Neill AR, 1971. Effects of strain, antibiotic supplement and diet calcium level on chick growth and feed conversion and on utilization of dietary energy and nitrogen. Australian Journal of Experimental Agriculture, 11, 383–386. https://doi.org/10.1071/EA9710383
Cunha S, Mendes Â, Rego D, Meireles D, Fernandes R, Carvalho A and Martins da Costa P, 2017. Effect of competitive exclusion in rabbits using an autochthonous probiotic. World Rabbit Science, 25, 123–134. https://doi.org/10.4995/wrs.2017.4533
Cusack PMV, 2004. Effect of mass medication with antibiotics at feedlot entry on the health and growth rate of cattle destined for the Australian domestic market. Australian Veterinary Journal, 82, 154–156. https://doi.org/10.1111/j.1751-0813.2004.tb12644.x
Dabrowski K and Poczyczyński P, 1988. Comparative experiments on starter diets for grass carp and common carp. Aquaculture, 69, 317–332. https://doi.org/10.1016/0044-8486(88)90339-0
Das KK, 2004. A trial on the commonly available drugs and C-Flox IU for the treatment of repeat breeding condition in cattle. Intas Polivet, 5, 199–203.
Dean WF, Price JI and Leibovitz L, 1973. Effect of feed medicaments on bacterial infections in ducklings. Poultry Science, 52, 549–558. https://doi.org/10.3382/ps.0520549
Decundo JM, Diéguez SN, Martínez G, Romanelli A, Fernández Paggi MB, Pérez Gaudio DS, Amanto FA and Soraci AL, 2019. Impact of water hardness on oxytetracycline oral bioavailability in fed and fasted piglets. Veterinary Medicine and Science, 5, 517–525. https://doi.org/10.1002/vms3.185
Deepa K, Purushothaman MR, Vasanthakumar P and Sivakumar K, 2018. Effect of sodium butyrate as an antibiotic substitute on production performance, carcass characteristics and economics in broiler chicken. Animal Nutrition and Feed Technology, 18, 377–387. https://doi.org/10.5958/0974-181X.2018.00035.5
Del Castillo JRE, Beauchamp G, Martineau GP, Besner JG, del Castillo JRE, Beauchamp G, Martineau GP and Besner J-G, 2002. Short-term effects of in-feed supplementation of tetracyclines for disease control on feed intake pattern and growth in weaned pigs. Livestock Production Science, 76, 115–124. https://doi.org/10.1016/S0301-6226(02)00011-8
Dennis TS, Suarez-Mena FX, Hu W, Hill TM, Quigley JD and Schlotterbeck RL, 2019. Effects of milk replacer feeding rate and long-term antibiotic inclusion in milk replacer on performance and nutrient digestibility of Holstein dairy calves up to 4 months of age. Journal of Dairy Science, 102, 2094–2102. https://doi.org/10.3168/jds.2018-15652
Dobsikova R, Blahova J, Mikullkova I, Modra H, Praskova E, Svobodova Z, Skorie M, Jarkovsky J and Siwicki A-K, 2013. The effect of oyster mushroom beta-1.3/1.6-D-glucan and oxytetracycline antibiotic on biometrical, haematological, biochemical, and immunological indices, and histopathological changes in common carp (*Cyprinus carpio* L.). Fish & Shellfish Immunology, 35, 1813–1823. https://doi.org/10.1016/j.fsi.2013.09.006
Dong XF, Gao WW, Su JL, Tong JM and Zhang Q, 2011. Effects of dietary polysavone (Alfalfa extract) and chlortetracycline supplementation on antioxidation and meat quality in broiler chickens. British Poultry Science, 52, 302–309. https://doi.org/10.1080/00071668.2011.569008
Donovan DC, Franklin ST, Chase CCL and Hippen AR, 2002. Growth and health of Holstein calves fed milk replacers supplemented with antibiotics or Enteroguard. Journal of Dairy Science, 85, 947–950. https://doi.org/10.3168/jds.S0022-0302(02)74153-2
Dritz SS, Tokach MD, Goodband RD, Nelssen JL and Owen KQ, 1993. Comparison of feed-grade antibiotics in starter diets containing spray-dried blood products. In: Kansas State University Swine Day 1993. Report of Progress 695, Kansas State University, Kansas, pp. 75–77.
Dritz SS, Tokach MD, Goodband RD and Nelssen JL, 2002. Effects of administration of antimicrobials in feed on growth rate and feed efficiency of pigs in multisite production systems. Journal of the American Veterinary Medical Association, 220, 1690–1695. https://doi.org/10.2460/javma.2002.220.1690
Duff GC, Walker DA, Malcolm-Callis KJ, Wiseman MW and Hallford DM, 2000. Effects of preshipping *vs* arrival medication with tilmicosin phosphate and feeding chlortetracycline on health and performance of newly received beef cattle. Journal of Animal Science, 78, 267–274. https://doi.org/10.2527/2000.782267x
Duttlinger AW, Kpodo KR, Lay DC, Richert BT and Johnson JS, 2019. Replacing dietary antibiotics with 0.20% l-glutamine in swine nursery diets: impact on health and productivity of pigs following weaning and transport. Journal of Animal Science, 97, 2035–2052. https://doi.org/10.1093/jas/skz098
Dyer DC, 1989. Pharmacokinetics of oxytetracycline in the turkey: evaluation of biliary and urinary excretion. American Journal of Veterinary Research, 50, 522–524.
Ebrahimi E, Haghjou M, Nematollahi A and Goudarzian F, 2020. Effects of rosemary essential oil on growth performance and hematological parameters of young great sturgeon (*Huso huso*). Aquaculture, 521. https://doi.org/10.1016/j.aquaculture.2019.734909
Eckerman SE, Lardy GP, Thompson MM, Neville BW, van Emon ML, Berg PT, Luther JS and Schauer CS, 2011. Growth and carcass characteristics of conventionally raised lambs versus naturally raised lambs. Sheep & Goat Research Journal, 26, 1–7.

Edmonds MS, Izquierdo OA and Baker DH, 1985. Feed additive studies with newly weaned pigs: efficacy of supplemental copper, antibiotics and organic acids. Journal of Animal Science, 60, 462–469. https://doi.org/10.2527/jas1985.602462x

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021a. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 1: Methodology, general data gaps and uncertainties. EFSA Journal 2021;19(10):6852, 57 pp. https://doi.org/10.2903/j.efsa.2021.6852

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021b. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 2: Aminoglycosides/aminocyclitols: apramycin, paromomycin, neomycin, and spectinomycin. EFSA Journal 2021;19(10):6853, 40 pp. https://doi.org/10.2903/j.efsa.2021.6853

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021c. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 3: Amprolium. EFSA Journal 2021;19(10):6854, 20 pp. https://doi.org/10.2903/j.efsa.2021.6854

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021d. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 4: β-Lactams: amoxicillin and penicillin V. EFSA Journal 2021;19(10):6855, 26 pp. https://doi.org/10.2903/j.efsa.2021.6855

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021e. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 5: Lincosamides: lincomycin. EFSA Journal 2021;19(10):6856, 21 pp. https://doi.org/10.2903/j.efsa.2021.6856

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021f. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 6: Macrolides: tilmicosin, tylosin and tylvalosin. EFSA Journal 2021;19(10):6858, 52 pp. https://doi.org/10.2903/j.efsa.2021.6858

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021g. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 7: Amphenicols: florfenicol and thiamphenicol. EFSA Journal 2021;19(10):6859, 27 pp. https://doi.org/10.2903/j.efsa.2021.6859

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021h. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 8: Pleuromutilins: tiamulin and valnemulin. EFSA Journal 2021;19(10):6860, 27 pp. https://doi.org/10.2903/j.efsa.2021.6860

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021i. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 9: Polymyxins: colistin. EFSA Journal 2021;19(10):6861, 33 pp. https://doi.org/10.2903/j.efsa.2021.6861

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021j. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 10: Quinolones: flumequine and oxolinic acid. EFSA Journal 2021;19(10):6862, 18 pp. https://doi.org/10.2903/j.efsa.2021.6862

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021k. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 11: Sulfonamides. EFSA Journal 2021;19(10):6863, 26 pp. https://doi.org/10.2903/j.efsa.2021.6863

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021l. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 13: Diaminopyrimidines: trimethoprim. EFSA Journal 2021;19(10):6865, 19 pp. https://doi.org/10.2903/j.efsa.2021.6865

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos MdL, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

Ekperigin HE, Jang S and McCapes RH, 1983. Effective control of a gentamicin-resistant *Salmonella arizonae* infection in turkey poults. Avian Diseases, 27, 822–829. https://doi.org/10.2307/1590326

El-Deek AA, Al-Harthi MA, Osman M, Al-Jassas F and Nassar R, 2012a. Hot pepper (*Capsicum Annum*) as an alternative to oxytetracycline in broiler diets and effects on productive traits, meat quality, immunological responses and plasma lipids. Archiv für Geflügelkunde, 76, 73–80.

El-Deek AA, Al-Harthi MA, Osman M, Al-Jassas F and Nassar R, 2012b. Effect of different levels of green tea (*Camellia sinensis*) as a substitute for oxytetracycline as a growth promoter in broilers diets containing two crude protein levels. Archiv für Geflügelkunde, 76, 88–98.

Elema MO, Hoff KA and Kristensen HG, 1996. Bioavailability of oxytetracycline from medicated feed administered to Atlantic salmon (*Salmo salar* L.) in seawater. Aquaculture, 143, 7–14. https://doi.org/10.1016/0044-8486(96)01253-7

El-Sayed SAA, Ahmed SYA and Abdel-Hamid NR, 2014. Immunomodulatory and growth performance effects of ginseng extracts as a natural growth promoter in comparison with oxytetracycline in the diets of Nile tilapia (*Oreochromis niloticus*). International Journal of Livestock Research, 4, 130–142. https://doi.org/10.5455/ijlr.20140109084346

El-Tahawy AS and El-Shafey HA, 2015. The influence of different feed additives on the profitability of Nile tilapia (*Oreochromis niloticus*) fingerlings reared in hapas. Global Journal of Fisheries and Aquaculture Researches, 2, 1–28.

EMEA/CVMP (European Agency for the Evaluation of Medicinal Products - Committee for Veterinary Medicinal Products), 1995. Oxytetracycline, tetracycline, chlortetracycline. Summary Report 3. EMEA/MRL/023/95. Available online: https://www.ema.europa.eu/en/documents/mrl-report/oxytetracycline-tetracycline-chlortetracycline-summary-report-3-committee-veterinary-medicinal_en.pdf

EMEA/CVMP (European Agency for the Evaluation of Medicinal Products - Committee for Veterinary Medicinal Products), 1997. Doxycycline. Summary Report (2). EMEA/MRL/270/97-FINAL. October 1997. Available online: https://www.ema.europa.eu/en/documents/mrl-report/doxycycline-summary-report-2-committee-veterinary-medicinal-products_en.pdf

Feldpausch JA, DeJong JA, Tokach MD, Dritz SS, Woodworth JC, Amachawadi RG, Scott HM, Nelssen JL and Goodband RD, 2014a. Comparative effects of dietary copper, zinc, essential oils, and chlortetracycline on nursery pig growth performance. In: Kansas State University Swine Day 2014. Report of progress 1110, Manhattan, Kansas, USA, 20 November, 2014, Kansas State University, Kansas, pp. 8–16.

Feldpausch JA, DeJong JA, Tokach MD, Dritz SS, Woodworth JC, Amachawadi RG, Scott HM, Nelssen JL and Goodband RD, 2014b. Effects of dietary zinc oxide and chlortetracycline on nursery pig growth performance. In: Kansas State University Swine Day 2014. Report of progress 1110, Manhattan, Kansas, USA, 20 November, 2014, Kansas State University, Kansas, pp. 1–7.

Feldpausch JA, Amachawadi RG, Tokach MD, Scott HM, Dritz SS, Goodband RD, Woodworth JC and DeRouchey JM, 2018. Effects of dietary chlortetracycline, Origanum essential oil, and pharmacological Cu and Zn on growth performance of nursery pigs. Translational Animal Science, 2, 62–73. https://doi.org/10.1093/tas/txx004

Fomenky BE, Chiquette J, Bissonnette N, Talbot G, Chouinard PY and Ibeagha-Awemu EM, 2017. Impact of *Saccharomyces cerevisiae* boulardii CNCMI-1079 and *Lactobacillus acidophilus* BT1386 on total lactobacilli population in the gastrointestinal tract and colon histomorphology of Holstein dairy calves. Animal Feed Science and Technology, 234, 151–161. https://doi.org/10.1016/j.anifeedsci.2017.08.019

Fonseca AP, Falcão L, Kocher A and Spring P, 2005. Effects of Dietary Mannan Oligosaccharide in Comparison to Oxytetracyclin on Performance of Growing Rabbits. World Rabbit Science Association (WRSA), Corronsac, Pueblo, Mexico. pp. 829–833.

Foreman CF, Freeman AE and Jacobson NL, 1961. Effects of long-time feeding of chlortetracycline to dairy cattle. Journal of Dairy Science, 44, 141. https://doi.org/10.3168/jds.S0022-0302(61)89704-X

Fox MW, 1980. Antibiotics in animal feeds. Science, 210, 376. https://doi.org/10.1126/science.6159681

Franti CE, Adler HE and Julian LM, 1971. Antibiotic growth promotion: effects of bacitracin and oxytetracycline on intestines and selected lymphoid tissues of New Hampshire cockerels. Poultry Science, 50, 94–99. https://doi.org/10.3382/ps.0500094

Frantz NZ, Nelssen JL, DeRouchey JM, Goodband RD, Tokach MD and Dritz SS, 2004. The effect of a probiotic, KE-01, and Neoterramycin on nursery pig growth performance. In: Kansas State University Swine Day 2004. Report of Progress 940, Kansas State University, Kansas, pp. 36–40.

Freeman BM, Manning AC, Harrison GF and Coates ME, 1975. Dietary aureomycin and the response of the fowl to stressors. British Poultry Science, 16, 395–404. https://doi.org/10.1080/00071667508416203

Fuoco D, 2012. Classification framework and chemical biology of tetracycline-structure-based drugs. Antibiotics (Basel), 1, 1–13. https://doi.org/10.3390/antibiotics1010001

Furr RD, Sherrod LB, Carpente JA and Hansen KR, 1968. Effect of feeding either chlortetracycline or sulfamethazine or a combination to stressed feeder cattle. Journal of Animal Science, 27, 1110–1111.

Furusawa N, 2001. Transference of dietary veterinary drugs into eggs. Veterinary Research Communications, 25, 651–662. https://doi.org/10.1023/A:1012743230180

Gadberry MS, Hubbell III DS, Tucker JD, Hess T, Beck PA, Jennings J, Powell JG and Backes EB, 2014. Forage utilization and beef cow weight, body condition, and body temperature response to a continuous or strip-graze forage allocation with or without chlortetracycline added to the free choice mineral supplement. Research Series - Arkansas Agricultural Experiment Station, 21–25.

Gaikowski MP, Wolf JC, Schleis SM and Gingerich WH, 2003. Safety of oxytetracycline (Terramycin TM-100F) administered in feed to hybrid striped bass, walleyes, and yellow perch. Journal of Aquatic Animal Health, 15, 274–286. https://doi.org/10.1577/H03-042.1

Gallo GF and Berg JL, 1995. Efficacy of a feed-additive antibacterial combination for improving feedlot cattle performance and health. Canadian Veterinary Journal, 36, 223–229.

Gazdzinski P and Julian RJ, 1992. Necrotic enteritis in Turkeys. Avian Diseases, 36, 792–798. https://doi.org/10.2307/1591787

Gebru E, Lee JS, Son JC, Yang SY, Shin SA, Kim B, Kim MK and Park SC, 2010. Effect of probiotic-, bacteriophage-, or organic acid-supplemented feeds or fermented soybean meal on the growth performance, acute-phase response, and bacterial shedding of grower pigs challenged with *Salmonella enterica* serotype typhimurium. Journal of Animal Science, 88, 3880–3886. https://doi.org/10.2527/jas.2010-2939

Geidam YA, Ambali AG, Onyeyili PA, Tijjani MB, Gambo HI and Gulani IA, 2015. Antibacterial efficacy of ethyl acetate fraction of *Psidium guajava* leaf aqueous extract on experimental *Escherichia coli* (O78) infection in chickens. Veterinary World, 8, 358–362. https://doi.org/10.14202/vetworld.2015.358-362

George BA, Fagerberg DJ, Quarles CL and Fenton JM, 1977. Comparison of therapeutic efficacy of doxycycline, chlortetracycline and lincomycin-spectinomycin on *E. coli* infection of young chickens. Poultry Science, 56, 452–458. https://doi.org/10.3382/ps.0560452

Gibb DJ, Schwartzkopf-Genswein KS, McAllister TA, Genswein BMA and Streeter M, 2006. Effect of sub-therapeutic antibiotics and auction exposure on health, performance, and feeding behavior of weaned calves. Canadian Journal of Animal Science, 86, 457–460. https://doi.org/10.4141/A06-024

Gibb DJ, Streeter M, Schwartzkopf-Genswein KS and McAllister TA, 2008. Performance and bunk attendance of cattle fed steam-rolled or ground corn supplemented with laidlomycin and chlortetracycline or monensin and tylosin. Canadian Journal of Animal Science, 88, 499–506. https://doi.org/10.4141/CJAS07069

Giguère S, Prescott JF and Dowling PM, 2013. Antimicrobial Therapy in Veterinary Medicine, 5th Edition. Blackwell Scientific Publications. 683 pp.

Glisson JR, Hofacre CL and Mathis GF, 2004. Comparative efficacy of enrofloxacin, oxytetracycline, and sulfadimethoxine for the control of morbidity and mortality caused by *Escherichia coli* in broiler chickens. Avian Diseases, 48, 658–662. https://doi.org/10.1637/7166

Goma AA, Abumandour MMA and Tohamy HG, 2018. Evaluation of doxycycline toxicological effects on Japanese quail (*Coturnix coturnix* japonica) on: behavioral patterns and histopathological findings. Alexandria Journal of Veterinary Sciences, 58, 91–101. https://doi.org/10.5455/ajvs302643231

Goren E, Jong WA, Doornenbal P and Laurense T, 1988. Therapeutic efficacy of doxycycline hyclate in experimental *Escherichia coli* infection in broilers. Veterinary Quarterly, 10, 48–52. https://doi.org/10.1080/01652176.1988.9694145

Gottlob RO, DeRouchey JM, Tokach MD, Goodband RD, Dritz SS, Nelssen JL, Hastad CW, Groesbeck CN and Lawrence KR, 2004. The effects of different feed-grade antibiotics on growth performance of weanling pigs in a research environment. In: Kansas State University Swine Day 2004. Report of Progress 940, Kansas State University, Kansas, pp. 32–35.

Gottlob RO, Tokach MD, Dritz SS, Goodband RD, DeRouchey JM, Nelssen JL, Hastad CW, Groesbeck CN, Neill CR, Schneider JD and Frantz NZ, 2005a. Comparison of water-based and in-feed antimicrobials for growth performance enhancement of weanling pigs. In: Kansas State University Swine Day. Report of Progress 964. Kansas State University, Kansas. pp. 38–43.

Gottlob RO, Tokach MD, Dritz SS, Goodband RD, DeRouchey JM, Nelssen JL, Hastad CW, Groesbeck CN, Neill CR, Schneider JD and Frantz NZ, 2005b. Effects of different dosages of water-based neomycin sulfate on growth performance of weanling pigs. In: Kansas State University Swine Day. Report of Progress 964. Kansas State University, Kansas. pp. 44–50.

Gottlob RO, Tokach MD, Dritz SS, Goodband RD, DeRouchey JM, Nelssen JL, Hastad CW, Groesbeck CN, Neill CR, Schneider JD and Frantz NZ, 2005c. Effects of intermittent usage of water-based neomycin sulfate on the growth performance of weanling pigs. In: Kansas State University Swine Day. Report of Progress 964. Kansas State University, Kansas. pp. 51–59.

Gottlob RO, Tokach MD, Dritz SS, Goodband RD, DeRouchey JM, Nelssen JL, Hastad CW, Groesbeck CN, Neill CR, Schneider JD and Frantz NZ, 2006a. Effects of dietary calcium formate and malic acid on nursery pig growth performance. In: Kansas State University Swine Day. Report of Progress 966. Kansas State University, Kansas. pp. 67–71.

Gottlob RO, Tokach MD, Dritz SS, Goodband RD, DeRouchey JM, Nelssen JL, Hastad CW, Groesbeck CN, Neill CR, Schneider JD and Frantz NZ, 2006b. Effects of water-soluble and in-feed organic acids on the growth performance of weanling pigs. In: Kansas State University Swine Day. Report of Progress 966. Kansas State University, Kansas. pp. 60–66.

Gottlob RO, Dritz SS, Tokach MD, Derouchev JM, Goodband RD, Nelssen JL, Hastad CW, Groesbeck CN and Neill CR, 2007. Effects of water-based antimicrobials on growth performance of weanling pigs. Journal of Swine Health and Production, 15, 198–205.

Greenfield J, Macdonald KR and Pope WH, 1973. Protection of Arizona disease infected turkey poults. Canadian Veterinary Journal, 14, 110–113.

Greer N, 2006. Tigecycline (Tygacil): The first in the glycylcycline class of antibiotics. Proceedings (Baylor University Medical Center), 19, 155–161. https://doi.org/10.1080/08998280.2006.11928154

Grossman TH, 2016. Tetracyclines antibiotics and resistance. Cold Spring Harbor Perspectives in Medicine, 6.

Gullberg E, Cao S, Berg OG, Ilback C, Sandegren L, Hughes D and Andersson DI, 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathogens, 7. https://doi.org/10.1371/journal.ppat.1002158

Guo S, Ma J, Xing Y, Xu Y, Jin X, Yan S and Shi B, 2020. *Artemisia annua* L. aqueous extract as an alternative to antibiotics improving growth performance and antioxidant function in broilers. Italian Journal of Animal Science, 19, 399–409. https://doi.org/10.1080/1828051X.2020.1745696

Gutierrez L, Vargas-Estrada D, Rosario C and Sumano H, 2012. Serum and tissue concentrations of doxycycline in broilers after the sub-cutaneous injection of a long-acting formulation. British Poultry Science, 53, 366–373. https://doi.org/10.1080/00071668.2012.701004

Hahn TW, Lohakare JD, Lee SL, Moon WK and Chae BJ, 2006. Effects of supplementation of β-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. Journal of Animal Science, 84, 1422–1428.

Hamid H, Zhao LH, Ma GY, Li WX, Shi HQ, Zhang JY, Ji C and Ma QG, 2019. Evaluation of the overall impact of antibiotics growth promoters on broiler health and productivity during the medication and withdrawal period. Poultry Science, 98, 3685–3694. https://doi.org/10.3382/ps/pey598

Han Y-K and Thacker PA, 2009. Performance, nutrient digestibility and nutrient balance in weaned pigs fed diets supplemented with antibiotics or zinc oxide. Journal of Animal and Veterinary Advances, 8, 868–875. https://doi.org/10.3923/javaa.2009.868.875

Han Y-K and Thacker PA, 2010. Effects of antibiotics, Zinc oxide or a rare earth mineral-yeast product on performance, nutrient digestibility and serum parameters in weanling pigs. Asian-Australasian Journal of Animal Sciences, 23, 1057–1065. https://doi.org/10.5713/ajas.2010.90569

Han X, Piao XS, Zhang HY, Li PF, Yi JQ, Zhang Q and Li P, 2012. *Forsythia suspensa* extract has the potential to substitute antibiotic in broiler chicken. Asian-Australasian Journal of Animal Sciences, 25, 569–576. https://doi.org/10.5713/ajas.2011.11425

Han J, Zhang F, Du LB, Han XH, Chen WF and Meng J, 2014. Effects of dietary biochar including vinegar liquid on growth performance, nutrient digestibility, blood characteristics and fecal noxious gas emission in weaned piglets. Journal of Animal and Veterinary Advances, 13, 1072–1079. https://doi.org/10.3923/javaa.2014.1072.1079

Han YS, Tang CH, Zhao QY, Zhan TF, Zhang K, Han YM and Zhang JM, 2018. Effects of dietary supplementation with combinations of organic and medium chain fatty acids as replacements for chlortetracycline on growth performance, serum immunity, and fecal microbiota of weaned piglets. Livestock Science, 216, 210–218. https://doi.org/10.1016/j.livsci.2018.08.013

Hansen MF, Petri LH and Ackert JE, 1954. Effects of aureomycin and vitamin B12 used separately as feed supplements on resistance of chickens to *Ascaridia galli* (Schrank). Experimental Parasitology, 3, 122–127. https://doi.org/10.1016/0014-4894(54)90002-8

Harper AF, Kornegay ET, Bryant KL and Thomas HR, 1983. Efficacy of virginiamycin and a commercially-available lactobacillus probiotic in swine diets. Animal Feed Science and Technology, 8, 69–76. https://doi.org/10.1016/0377-8401(83)90044-5

Hart WS, Heuzenroeder MW and Barton MD, 2006. A study of the transfer of tetracycline resistance genes between *Escherichia coli* in the intestinal tract of a mouse and a chicken model. Journal of Veterinary Medicine B, Infectious Diseases and Veterinary Public Health, 53, 333–340. https://doi.org/10.1111/j.1439-0450.2006.00967.x

Hathaway MR, Dayton WR, White ME, Henderson TL and Henningson TB, 1996. Serum Insulin-Like Growth Factor I (IGF-I) concentrations are increased in pigs fed antimicrobials. Journal of Animal Science, 74, 1541–1547. https://doi.org/10.2527/1996.7471541x

Hathaway MR, Dayton WR, White ME, Henderson TL, Young DA and Doan TN, 1999. Effect of feed intake on antimicrobially induced increases in porcine serum insulin-like growth factor I. Journal of Animal Science, 77, 3208–3214. https://doi.org/10.2527/1999.77123208x

Hathaway MR, Dayton WR, White ME and Pampusch MS, 2003. Effects of antimicrobials and weaning on porcine serum insulin-like growth factor binding protein levels. Journal of Animal Science, 81, 1456–1463. https://doi.org/10.2527/2003.8161456x

Hays VW, 1977. Effectiveness of feed additive usage of antibacterial agents in swine and poultry production. 110 pp. Available online: https://archive.org/details/effectivenessoff00hays

He T, Long S, Mahfuz S, Wu D, Wang X, Wei X and Piao X, 2019. Effects of probiotics as antibiotics substitutes on growth performance, serum biochemical parameters, intestinal morphology, and barrier function of broilers. Animals, 9. https://doi.org/10.3390/ani9110985

Hegazy AM, Tolba HMN, Abd EL-Samie LK, Abdelaziz AM and Ali AMA, 2018. Effect of the medicinal plant (*Azadirachta Indica*) on *Chlamydophila psittaci* infection in broiler chickens. Slovenian Veterinary Research, 55, 85–93. https://doi.org/10.26873/SVR-633-2018

Heinrichs AJ, Jones CM and Heinrichs BS, 2003. Effects of mannan oligosaccharide or antibiotics in neonatal diets on health and growth of dairy calves. Journal of Dairy Science, 86, 4064–4069. https://doi.org/10.3168/jds.S0022-0302(03)74018-1

Heinrichs AJ, Jones CM, Elizondo-Salazar JA and Terrill SJ, 2009. Effects of a prebiotic supplement on health of neonatal dairy calves.

Helm ET, Curry S, Trachsel JM, Schroyen M and Gabler NK, 2019. Evaluating nursery pig responses to in-feed sub-therapeutic antibiotics. PLoS ONE, 14. https://doi.org/10.1371/journal.pone.0216070

Henry PR, Ammerman CB, Campbell DR and Miles RD, 1987. Effect of antibiotics on tissue trace mineral concentration and intestinal tract weight of broiler chicks. Poultry Science, 66, 1014–1018. https://doi.org/10.3382/ps.0661014

Hersom M, Imler A, Thrift T, Yelich J and Arthington J, 2015. Comparison of feed additive technologies for preconditioning of weaned beef calves. Journal of Animal Science, 93, 3169–3178. https://doi.org/10.2527/jas.2014-8689

Hibbs JW and Conrad HR, 1958. High roughage system for raising calves based on the early development of rumen function. VIII. Effect of rumen inoculations and chlortetracycline on performance of calves fed high roughage pellets. Journal of Dairy Science, 41, 1230–1247. https://doi.org/10.3168/jds.S0022-0302(58)91079-8

Hildabrand BM, Neill CR, Burkey TE, Dritz SS, Johnson BJ and Minton JE, 2004. Growth performance of nursery pigs fed BIOSAF yeast alone or in combination with in-feed antimicrobial. In: Kansas State University Swine Day. Report of Progress 940. Kansas State University, Kansas. pp. 25–28.

Hill WJ, Gill DR and Ball RL, 1993. Effects of aureomycin, delivered through the drinking water, on shipping stressed stocker cattle. Animal Science Research Report, 304–307.

Holman DB and Chénier MR, 2013. Impact of subtherapeutic administration of tylosin and chlortetracycline on antimicrobial resistance in farrow-to-finish swine. FEMS Microbiology Ecology, 85, 1–13. https://doi.org/10.1111/1574-6941.12093

Holt JP, van Heugten E, Graves AK, See MT and Morrow WEM, 2011. Growth performance and antibiotic tolerance patterns of nursery and finishing pigs fed growth-promoting levels of antibiotics. Livestock Science, 136, 184–191. https://doi.org/10.1016/j.livsci.2010.09.007

Hong JW, Kim IH, Kwon OS, Min BJ, Lee WB and Shon KS, 2004. Influences of plant extract supplementation on performance and blood characteristics in weaned pigs. Asian-Australasian Journal of Animal Sciences, 17, 374–378. https://doi.org/10.5713/ajas.2004.374

Hong J-C, Steiner T, Aufy A and Lien T-F, 2012. Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. Livestock Science, 144, 253–262. https://doi.org/10.1016/j.livsci.2011.12.008

Hong Y, Cheng Y, Li Y, Li X, Zhou Z, Shi D, Li Z and Xiao Y, 2019. Preliminary study on the effect of *Bacillus amyloliquefaciens* TL on cecal bacterial community structure of broiler chickens. BioMed Research International, 2019. https://doi.org/10.1155/2019/5431354

Hossain Md and Yang C-J, 2014. Effect of fermented water plantain on growth performance, meat composition, oxidative stability, and fatty acid composition of broiler. Livestock Science, 162. https://doi.org/10.1016/j.livsci.2014.01.016

Hossain ME, Ko SY, Park KW, Firman JD and Yang CJ, 2012a. Evaluation of green tea by-product and green tea plus probiotics on the growth performance, meat quality and immunity of growing-finishing pigs. Animal Production Science, 52, 857–866.

Hossain ME, SeokYoung K and ChulJu Y, 2012b. Dietary supplementation of green tea by-products on growth performance, meat quality, blood parameters and immunity in finishing pigs. Journal of Medicinal Plants Research, 6, 2458–2467.

Hossain ME, Kim GM, Lee SK and Yang CJ, 2012c. Growth performance, meat yield, oxidative stability, and fatty acid composition of meat from broilers fed diets supplemented with a medicinal plant and probiotics. Asian-Australasian Journal of Animal Sciences, 25, 1159–1168. https://doi.org/10.5713/ajas.2012.12090

Hossain ME, GwiMan K, SangSoo S, Firman JD and ChulJu Y, 2012d. Evaluation of water plantain (*Alisma canaliculatum* A. Br. et Bouche) and mistletoe (*Viscum album L.*) effects on broiler growth performance, meat composition and serum biochemical parameters. Journal of Medicinal Plants Research, 6, 2160–2169.

Hossain ME, Ko SY, Kim GM, Firman JD and Yang CJ, 2012e. Evaluation of probiotic strains for development of fermented *Alisma canaliculatum* and their effects on broiler chickens. Poultry Science, 91, 3121–3131. https://doi.org/10.3382/ps.2012-02333

Hosseini SA and Meimandipour A, 2018. Feeding broilers with thyme essential oil loaded in chitosan nanoparticles: an efficient strategy for successful delivery. British Poultry Science, 59, 669–678. https://doi.org/10.1080/00071668.2018.1521511

Hu J and McDougald LR, 2002. Effect of anticoccidials and antibiotics on the control of blackhead disease in broiler breeder pullets. Journal of Applied Poultry Research, 11, 351–357. https://doi.org/10.1093/japr/11.4.351

Hu C, Xing W, Liu X, Zhang X, Li K, Liu J, Deng B, Deng J, Li Y and Tan C, 2019. Effects of dietary supplementation of probiotic *Enterococcus faecium* on growth performance and gut microbiota in weaned piglets. AMB Express, 9. https://doi.org/10.1186/s13568-019-0755-z

Huang CW, Lee TT, Shih YC and Yu B, 2012. Effects of dietary supplementation of Chinese medicinal herbs on polymorphonuclear neutrophil immune activity and small intestinal morphology in weanling pigs. Journal of Animal Physiology and Animal Nutrition, 96, 285–294. https://doi.org/10.1111/j.1439-0396.2011.01151.x

Huang P, Zhang Y, Xiao K, Jiang F, Wang H, Tang D, Liu D, Liu B, Liu Y, He X, Liu H, Liu X, Qing Z, Liu C, Huang J, Ren Y, Yun L, Yin L, Lin Q, Zeng C, Su X, Yuan J, Lin L, Hu N, Cao H, Huang S, Guo Y, Fan W and Zeng J, 2018. The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids. Microbiome, 6. https://doi.org/10.1186/s40168-018-0590-5

Humam AM, Loh TC, Foo HL, Samsudin AA, Mustapha NM, Zulkifli I and Izuddin WI, 2019. Effects of feeding different postbiotics produced by *Lactobacillus plantarum* on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. Animals, 9. https://doi.org/10.3390/ani9090644

Hustvedt SO, Storebakken T and Salte R, 1991. Does oral administration of oxolinic acid or oxytetracycline affect feed intake of rainbow trout? Aquaculture, 92, 109–113. https://doi.org/10.1016/0044-8486(91)90012-V

Isikwenu JO and Udomah JE, 2015. Phytotherapic potentials of *Xylopia aethiopica* dried fruits (grains of selim) as additive in broiler production. Journal of Agricultural Science and Technology A, 5, 122–129.

Islam KMS, Afrin S, Das PM, Hassan MM, Valks M, Klein U, Burch DGS and Kemppainen BW, 2008. Compatibility of a combination of tiamulin and chlortetracycline with salinomycin in feed during a pulsed medication program coadministration in broilers. Poultry Science, 87, 2528–2534. https://doi.org/10.3382/ps.2008-00219

Islam MJ, Rasul MG, Kashem MA, Hossain MM, Liza AA, Sayeed MA and Motaher Hossain M, 2015. Effect of oxytetracycline on Thai silver barb (*Barbonymus gonionotus*) and on it's culture environment. Journal of Fisheries and Aquatic Science, 10, 323–336. https://doi.org/10.3923/jfas.2015.323.336

Jiang S, Yang Z, Huang L, Yang W, Song D, Liu F, Ge J and Wang Y, 2019. Effect of Illicium verum or *Eucommia ulmoides* leaf extracts on the anti-stress ability, and mRNA and protein expression of Nrf2 and TNF-α in Duroc $\times$ Landrace $\times$ Yorkshire and Chinese native Licha-black nursery piglets. Journal of Animal Physiology and Animal Nutrition, 104, 1085–1095. https://doi.org/10.1111/jpn.13235

Johnson JS and Lay DC Jr, 2017. Evaluating the behavior, growth performance, immune parameters, and intestinal morphology of weaned piglets after simulated transport and heat stress when antibiotics are eliminated from the diet or replaced with L-glutamine. Journal of Animal Science, 95, 91–102. https://doi.org/10.2527/JAS.2016.1070

Johnson WP, Elliott RF and Shor AL, 1956. The effect of chlortetracycline on the incidence of enterotoxemia and weight gains in lambs maintained under commercial feed-lot conditions. Journal of Animal Science, 15, 781–787. https://doi.org/10.2527/jas1956.153781x

Jones JE, Derieux WT, Hughes BL and Dick JW, 1978. Effect of cholera preventative treatments on reproductive performance of turkey hens. Poultry Science, 57, 523–526. https://doi.org/10.3382/ps.0570523

Jukes TH, 1971. The present status and background of antibiotics in the feeding of domestic animals. Annals of the New York Academy of Sciences, 182, 362–379. https://doi.org/10.1111/j.1749-6632.1971.tb30672.x

Jung JY, Ahn Y, Khare S, Gokulan K, Pineiro SA and Cerniglia CE, 2018. An *in vitro* study to assess the impact of tetracycline on the human intestinal microbiome. Anaerobe, 49, 85–94. https://doi.org/10.1016/j.anaerobe.2017.12.011

Jutkina J, Rutgersson C, Flach CF and Joakim Larsson DG, 2016. An assay for determining minimal concentrations of antibiotics that drive horizontal transfer of resistance. Science of the Total Environment, 548–549, 131–138. https://doi.org/10.1016/j.scitotenv.2016.01.044

Kalavathy R, Abdullah N, Jalaludin S, Wong CMVL and Ho YW, 2008. Effect of *Lactobacillus* cultures and oxytetracycline on the growth performance and serum lipids of chickens. International Journal of Poultry Science, 7, 385–389. https://doi.org/10.3923/ijps.2008.385.389

Kareem KY, TeckChwen L, HooiLing F, Asmara SA, Akit H, Abdulla NR and MayFoong O, 2015. Carcasses, meat and bone quality of broiler chickens fed with postbiotic and prebiotic combinations. International Journal of Probiotics and Prebiotics, 10, 23–30.

Katya K, Park G, Bharadwaj AS, Browdy CL, Vazquez-Anon M and Bai SC, 2018. Organic acids blend as dietary antibiotic replacer in marine fish olive flounder, *Paralichthys olivaceus*. Aquaculture Research, 49, 2861–2868. https://doi.org/10.1111/are.13749

Ke YL, Jiao LF, Song ZH, Xiao K, Lai TM, Lu JJ and Hu CH, 2014. Effects of cetylpyridinium-montmorillonite, as alternative to antibiotic, on the growth performance, intestinal microflora and mucosal architecture of weaned pigs. Animal Feed Science and Technology, 198, 257–262. https://doi.org/10.1016/j.anifeedsci.2014.10.010

Keegan TP, DeRouchey JM, Nelssen JL, Tokach MD, Goodband RD and Dritz SS, 2003. Comparison of antibiotics on growth performance of weanling pigs in a commercial environment. In: Kansas State University Swine Day. Report of Progress 920. Kansas State University, Kansas. pp. 119–122.

Keegan TP, Dritz SS, Nelssen JL, DeRouchey JM, Tokach MD and Goodband RD, 2005. Effects of in-feed antimicrobial alternatives and antimicrobials on nursery pig performance and weight variation. Journal of Swine Health and Production, 13, 12–18.

Kehoe SI and Carlson DB, 2015. Influence of nonmedicated additives as alternatives to antibiotics on calf growth and health. Professional Animal Scientist, 31, 516–522. https://doi.org/10.15232/pas.2015-01416

Keijser BJF, Agamennone V, van den Broek TJ, Caspers M, van de Braak A, Bomers R, Havekes M, Schoen E, van Baak M, Mioch D, Bomers L and Montijn RC, 2019. Dose-dependent impact of oxytetracycline on the veal calf microbiome and resistome. BMC Genomics, 20, 65. https://doi.org/10.1186/s12864-018-5419-x

Khadem A, Soler L, Everaert N and Niewold TA, 2014. Growth promotion in broilers by both oxytetracycline and *Macleaya cordata* extract is based on their anti-inflammatory properties. British Journal of Nutrition, 112, 1110–1118. https://doi.org/10.1017/S0007114514001871

Kiarie E, Voth C, Wey D, Zhu C, Vingerhoeds P, Borucki S and Squires EJ, 2018. Comparative efficacy of antibiotic growth promoter and benzoic acid on growth performance, nutrient utilization, and indices of gut health in nursery pigs fed corn– soybean meal diet. Canadian Journal of Animal Science, 98, 868–874. https://doi.org/10.1139/cjas-2018-0056

Kijparkorn S, Jamikorn U, Wangsoonean S and Ittitanawong P, 2009. Antioxidant and acidifier properties of Roselle (*Hibicus sabdariffa* Linn.) calyx powder on lipid peroxidation, nutrient digestibility and growth performance in fattening pigs. Thai Journal of Veterinary Medicine, 39, 41–51.

Kilroy CR, Hall WF, Bane DP, Bevill RF and Koritz GD, 1990. Chlortetracycline in swine–bioavailability and pharmacokinetics in fasted and fed pigs. Journal of Veterinary Pharmacology and Therapeutics, 13, 49–58. https://doi.org/10.1111/j.1365-2885.1990.tb00747.x

Kim YJ and Choi IH, 2014. Comparison of the effects of supplemental Korean mistletoe (*Viscum album* var. coloratum) powder and antibiotic on growth performance, serum cholesterol profiles, and meat quality of broilers. Acta Agriculturae Scandinavica Section A-Animal Science, 64, 154–160. https://doi.org/10.1080/09064702.2014.929169

Kim LM, Gray JT, Harmon BG, Jones RD and Fedorka-Cray PJ, 2005. Susceptibility of *Escherichia coli* from growing piglets receiving antimicrobial feed additives. Foodborne Pathogens and Disease, 2, 304–316. https://doi.org/10.1089/fpd.2005.2.304

Kim SG, Han X-Y, Wei D and Xu Z-R, 2009. Influence of Cu2+ loaded silicate on the growth performance and microflora of crucian carp *Carassius auratus*. Diseases of Aquatic Organisms, 85, 239–243. https://doi.org/10.3354/dao02064

Kim K-W, Kim S-S, Khosravi S, Rahimnejad S and Lee K-J, 2014. Evaluation of sargassum fusiforme and *Ecklonia cava* as dietary additives for olive flounder (*Paralichthys olivaceus*). Turkish Journal of Fisheries and Aquatic Sciences, 14, 321–330. https://doi.org/10.4194/1303-2712-v14_2_03

King JOL, 1968. Feeding of oxytetracycline to growing and laying ducks. British Poultry Science, 9, 317. https://doi.org/10.1080/00071666808415727

Kitts SE, Harmon DL, Vanzant ES and McLeod KR, 2006. Effects of chlortetracycline (CTC) and Revalor-S on the growth performance and carcass quality traits of finishing beef steers. Journal of Animal and Veterinary Advances, 5, 70–76.

Kitts SE, Matthews JC, Schillo KK, Rumsey TS, Elsasser TH, Kahl S, Baldwin RL and McLeod KR, 2007. Effects of chlortetracycline and Synovex-S (R) on growth rate and on plasma growth hormone and thyroid hormone concentrations following administration of thyrotropin-releasing hormone and GH-releasing hormone in beef steers. Canadian Journal of Animal Science, 87, 327–341. https://doi.org/10.4141/A06-053

Kniffen TS, Bane DP, Hall WF, Koritz GD and Bevill RF, 1989. Bioavailability, pharmacokinetics, and plasma concentration of tetracycline hydrochloride fed to swine. American Journal of Veterinary Research, 50, 518–521.

Ko SY and Yang CJ, 2008. Effect of green tea probiotics on the growth performance, meat quality and immune response in finishing pigs. Asian-Australasian Journal of Animal Sciences, 21, 1339–1347. https://doi.org/10.5713/ajas.2008.70597

Ko SY, Bae IH, Yee ST, Lee SS, Uuganbayar D, Oh JI and Yang CJ, 2008. Comparison of the effect of green tea by-product and green tea probiotics on the growth performance, meat quality, and immune response of finishing pigs. Asian-Australasian Journal of Animal Sciences, 21, 1486–1494. https://doi.org/10.5713/ajas.2008.70604

Koh C-B, Romano N, Zahrah AS and Ng W-K, 2016. Effects of a dietary organic acids blend and oxytetracycline on the growth, nutrient utilization and total cultivable gut microbiota of the red hybrid tilapia, *Oreochromis* sp., and resistance to *Streptococcus agalactiae*. Aquaculture Research, 47, 357–369. https://doi.org/10.1111/are.12492

Kovacs M, Kosa E, Horn P, Szendro Z and Milisits G, 2009. Effect of a grain extract on certain digestive physiological indicators in early weaned rabbits. Acta Veterinaria Brno, 78, 379–386. https://doi.org/10.2754/avb200978030379

Kratzer FH, Vohra P, Ekperigin HE and Ritchie WL, 1994. Growth performance of starting turkey poults fed diets subjected to an anaerobic pasteurizing conditioning system. Animal Feed Science and Technology, 46, 67–73. https://doi.org/10.1016/0377-8401(94)90065-5

Kulshreshtha G, Rathgeber B, MacIsaac J, Boulianne M, Brigitte L, Stratton G, Thomas NA, Critchley AT, Hafting J and Prithiviraj B, 2017. Feed supplementation with red seaweeds, *Chondrus crispus* and *Sarcodiotheca gaudichaudii*, reduce *Salmonella* Enteritidis in laying hens. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.00567

Kyriakis SC, Bourtzi-Hatzopoulou E, Alexopoulos C, Kritas SK, Polyzopoulou Z, Lekkas S and Gardey L, 2002. Field evaluation of the effect of in-feed doxycycline for the control of ileitis in weaned piglets. Journal of Veterinary Medicine B, Infectious Diseases and Veterinary Public Health, 49, 317–321. https://doi.org/10.1046/j.1439-0450.2002.00574.x

Lang JM, Roy JHB, Shillam KWG and Ingram PL, 1959. The effect of giving stilboestrol and chlortetracycline to colostrum-fed calves. British Journal of Nutrition, 13, 463–467. https://doi.org/10.1079/BJN19590059

Langlois BE, Cromwell GL and Hays VW, 1978. Influence of type of antibiotic and length of antibiotic feeding period on performance and persistence of antibiotic resistant enteric bacteria in growing-finishing swine. Journal of Animal Science, 46, 1383–1396. https://doi.org/10.2527/jas1978.4651383x

Larsen C, Acha M and Ehrich M, 1988. Chlortetracycline and aflatoxin interaction in two lines of chicks. Poultry Science, 67, 1229–1232. https://doi.org/10.3382/ps.0671229

Larsen I, Hjulsager CK, Holm A, Olsen JE, Nielsen SS and Nielsen JP, 2016. A randomised clinical trial on the efficacy of oxytetracycline dose through water medication of nursery pigs on diarrhoea, faecal shedding of *Lawsonia intracellularis* and average daily weight gain. Preventive Veterinary Medicine, 123, 52–59. https://doi.org/10.1016/j.prevetmed.2015.12.004

Lawal MO, Aderolu AZ, Adewumi GA and Mudiaga A, 2019. Growth, nutrient utilization, haematology and biochemical parameters of African catfish (*Clarias gariepinus*, Burchell, 1822) fed with varying levels of *Bacillus subtilis*. Agrosearch, 19, 13–27.

Lee SJ, Shin NH, Ok JU, Jung HS, Chu GM, Kim JD, Kim IH and Lee SS, 2009. Effects of dietary synbiotics from anaerobic microflora on growth performance, noxious gas emission and fecal pathogenic bacteria population in weaning pigs. Asian-Australasian Journal of Animal Sciences, 22, 1202–1208. https://doi.org/10.5713/ajas.2009.90045

Lee ON, Lyu SR, Wang RC, Weng CF and Chen BJ, 2011a. Exhibit differential functions of various antibiotic growth promoters in broiler growth, immune response and gastrointestinal physiology. International Journal of Poultry Science, 10, 216–220. https://doi.org/10.3923/ijps.2011.216.220

Lee CY, JungWon L, YoungHyun K, SunYoung K, ManJong P, TaeGu K, JiHoon L, Young H, KyuSik J and InSurk J, 2011b. Intestinal growth and development of weanling pigs in response to dietary supplementation of antibiotics, phytogenic products and brewer's yeast plus *Bacillus* spores. Journal of Animal Science and Technology, 53, 227–235. https://doi.org/10.5187/JAST.2011.53.3.227

Lee YK, Katya K, Yun HH, Yoon MY, Park JK, Sung JS, Shin HS and Bai SC, 2016. Evaluation of dietary yellow loess as an antibiotic replacer on growth, immune responses, serological characteristics and disease resistance in rainbow trout, *Oncorhynchus mykiss*. Aquaculture Nutrition, 22, 1018–1025. https://doi.org/10.1111/anu.12348

Lee S, Katya K, Hamidoghli A, Hong J, Kim D-J and Bai SC, 2018a. Synergistic effects of dietary supplementation of *Bacillus subtilis* WB60 and mannanoligosaccharide (MOS) on growth performance, immunity and disease resistance in Japanese eel, *Anguilla japonica*. Fish and Shellfish Immunology, 83, 283–291. https://doi.org/10.1016/j.fsi.2018.09.031

Lee SH, Lee YK, Katya K, Park JK and Bai SC, 2018b. Natural dietary additive yellow loess as potential antibiotic replacer in Japanese eel, *Anguilla japonica*: effects on growth, immune responses, serological characteristics and disease resistance against *Edwardsiella tarda*. Aquaculture Nutrition, 24, 1034–1040. https://doi.org/10.1111/anu.12641

Lema M and Nahashon S, 2006. Effect of antibiotic-supplemented feed on fecal enterohemorrhagic *Escherichia coli* O157:H7 population in lambs. Small Ruminant Research, 63, 256–261. https://doi.org/10.1016/j.smallrumres.2005.02.026

LeMieux FM, Southern LL and Bidner TD, 2003. Effect of mannan oligosaccharides on growth performance of weanling pigs.

Lerner RG, Rapaport SI and Spitzer JM, 1968. Effects of thorotrast upon the reactivity and intravascular disappearance rate of fibrinogen in the rabbit. Proceedings of the Society for Experimental Biology and Medicine, 127, 904–909. https://doi.org/10.3181/00379727-127-32832

Li XJ, Piao XS, Kim SW, Liu P, Wang L, Shen YB, Jung SC and Lee HS, 2007. Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. Poultry Science, 86, 1107–1114. https://doi.org/10.1093/ps/86.6.1107

Li Z, Yi G, Yin J, Sun P, Li D and Knight C, 2008. Effects of organic acids on growth performance, gastrointestinal pH, intestinal microbial populations and immune responses of weaned pigs. Asian-Australasian Journal of Animal Sciences, 21, 252–261. https://doi.org/10.5713/ajas.2008.70089

Li LL, Yin FG, Zhang B, Peng HZ, Li FN, Zhu NS, Hou DX, Yin YL, Luo JJ, Tang ZR and Liu G, 2011. Dietary supplementation with *Atractylodes Macrophala* Koidz polysaccharides ameliorates metabolic status and improve immune function in early-weaned pigs. Livestock Science, 142, 33–41. https://doi.org/10.1016/j.livsci.2011.06.013

Li P, Piao X, Ru Y, Han X, Xue L and Zhang H, 2012. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Australasian Journal of Animal Sciences, 25, 1617–1626. https://doi.org/10.5713/ajas.2012.12292

Li B, Zhang J-Q, Han X-G, Wang Z-L, Xu Y-Y and Miao J-F, 2018. *Macleaya cordata* helps improve the growth-promoting effect of chlortetracycline on broiler chickens. Journal of Zhejiang University (Science B), 19, 776–784. https://doi.org/10.1631/jzus.B1700435

Li J, Cheng Y, Chen Y, Qu H, Zhao Y, Wen C and Zhou Y, 2019a. Dietary chitooligosaccharide inclusion as an alternative to antibiotics improves intestinal morphology, barrier function, antioxidant capacity, and immunity of broilers at early age. Animals, 9. https://doi.org/10.3390/ani9080493

Li JH, Yousif MH, Li ZQ, Wu ZH, Li SL, Yang HJ, Wang YJ and Cao ZJ, 2019b. Effects of antibiotic residues in milk on growth, ruminal fermentation, and microbial community of preweaning dairy calves. Journal of Dairy Science, 102, 2298–2307. https://doi.org/10.3168/jds.2018-15506

Li D, Zhang K, Pan Z, Yu M, Lu Y, Wang G, Wu J, Zhang J and Du W, 2020. Antibiotics promote abdominal fat accumulation in broilers. Animal Science Journal, 91. https://doi.org/10.1111/asj.13326

Liao XD, Ma G, Cai J, Fu Y, Yan XY, Wei XB and Zhang RJ, 2015. Effects of *Clostridium butyricum* on growth performance, antioxidation, and immune function of broilers. Poultry Science, 94, 662–667. https://doi.org/10.3382/ps/pev038

Licht TR, Struve C, Christensen BB, Poulsen RL, Molin S and Krogfelt KA, 2003. Evidence of increased spread and establishment of plasmid RP4 in the intestine under sub-inhibitory tetracycline concentrations. FEMS Microbiology Ecology, 44, 217–223. https://doi.org/10.1016/s0168-6496(03)00016-3

Lien TF, Horng YM and Wu CP, 2007. Feasibility of replacing antibiotic feed promoters with the Chinese traditional herbal medicine Bazhen in weaned piglets. Livestock Science, 107, 97–102. https://doi.org/10.1016/j.livsci.2006.09.008

Limbu SM, Zhou L, Sun S-X, Zhang M-L and Du Z-Y, 2018. Chronic exposure to low environmental concentrations and legal aquaculture doses of antibiotics cause systemic adverse effects in Nile tilapia and provoke differential human health risk. Environment International, 115, 205–219. https://doi.org/10.1016/j.envint.2018.03.034

Limbu SM, Ma Q, Zhang M-L and Du Z-Y, 2019. High fat diet worsens the adverse effects of antibiotic on intestinal health in juvenile Nile tilapia (*Oreochromis niloticus*). Science of the Total Environment, 680, 169–180. https://doi.org/10.1016/j.scitotenv.2019.05.067

Limbu SM, Zhang H, Luo Y, Chen L-Q, Zhang M and Du Z-Y, 2020. High carbohydrate diet partially protects Nile tilapia (*Oreochromis niloticus*) from oxytetracycline-induced side effects. Environmental Pollution, 256. https://doi.org/10.1016/j.envpol.2019.113508

Linares JF, Gustafsson I, Baquero F and Martinez JL, 2006. Antibiotics as intermicrobial signaling agents instead of weapons. Proceedings of the National Academy of Sciences, 103, 19484.https://doi.org/10.1073/pnas.0608949103

Liu P, Piao XS, Kim SW, Wang L, Shen YB, Lee HS and Li SY, 2008. Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of *Escherichia coli* and *Lactobacillus* in weaning pigs. Journal of Animal Science, 86, 2609–2618. https://doi.org/10.2527/jas.2007-0668

Loh TC, Thanh NT, Foo HL, Hair-Bejo M and Azhar BK, 2010. Feeding of different levels of metabolite combinations produced by *Lactobacillus plantarum* on growth performance, fecal microflora, volatile fatty acids and *villi* height in broilers. Animal Science Journal, 81, 205–214. https://doi.org/10.1111/j.1740-0929.2009.00701.x

Loh TC, Thu TV, Foo HL and Bejo MH, 2013. Effects of different levels of metabolite combination produced by *Lactobacillus plantarum* on growth performance, diarrhoea, gut environment and digestibility of postweaning piglets. Journal of Applied Animal Research, 41, 200–207. https://doi.org/10.1080/09712119.2012.741046

Long S, Liu L, Liu S, Mahfuz S and Piao X, 2019. Effects of forsythia suspense extract as an antibiotics substitute on growth performance, nutrient digestibility, serum antioxidant capacity, fecal *Escherichia coli* concentration and intestinal morphology of weaned piglets. Animals, 9. https://doi.org/10.3390/ani9100729

Lundström SV, Östman M, Bengtsson-Palme J, Rutgersson C, Thoudal M, Sircar T, Blanck H, Eriksson KM, Tysklind M, Flach CF and Larsson DGJ, 2016. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. Science of the Total Environment, 553, 587–595. https://doi.org/10.1016/j.scitotenv.2016.02.103

Luthman J and Jacobsson SO, 1983. The availability of tetracyclines in calves. Nordisk Veterinaermedicin, 35, 292–299.

Ma XK, Shang QH, Wang QQ, Hu JX and Piao XS, 2019. Comparative effects of enzymolytic soybean meal and antibiotics in diets on growth performance, antioxidant capacity, immunity, and intestinal barrier function in weaned pigs. Animal Feed Science and Technology, 248, 47–58. https://doi.org/10.1016/j.anifeedsci.2018.12.003

Mader TL and Brumm MC, 1987. Effect of feeding Sarsaponin in cattle and swine diets. Journal of Animal Science, 65, 9–15. https://doi.org/10.2527/jas1987.6519

Mahfuz S, He T, Liu S, Wu D, Long S and Piao X, 2019. Dietary inclusion of mushroom (*Flammulina velutipes*) stem waste on growth performance, antibody response, immune status, and serum cholesterol in broiler chickens. Animals, 9. https://doi.org/10.3390/ani9090692

Mahmoud UT, Abdel-Mohsein HS, Mahmoud MAM, Amen OA, Hassan RIM, Abd-El-Malek AM, Rageb SMM, Waly HSA, Othman AA and Osman MA, 2020. Effect of zinc oxide nanoparticles on broilers' performance and health status. Tropical Animal Health and Production. https://doi.org/10.1007/s11250-020-02229-2

Manafi M, Hedayati M and Mirzaie S, 2018. Probiotic Bacillus species and *Saccharomyces boulardii* improve performance, gut histology and immunity in broiler chickens. South African Journal of Animal Science, 48, 379–389. https://doi.org/10.4314/sajas.v48i2.19

Mane PM, 2010. Clinical efficacy of different intrauterine preparations in repeat breeder bovines. Research Journal of Animal Husbandry and Dairy Science, 1, 77–79.

Maneewan C, Yamauchi K, Thirabunyanon M, Siri S, Mekbungwan A and Thongwittaya N, 2011. Development of *Bacillus subtilis* MP and effective utilization on productivity and microorganisms in feces of suckling piglets. International Journal of Applied Research in Veterinary Medicine, 9, 382–387.

Marking LL, Howe GE and Crowther JR, 1988. Toxicity of erythromycin, oxytetracycline, and tetracycline administered to lake trout in water baths, by injection, or by feeding. Progressive Fish-Culturist, 50, 197–201. https://doi.org/10.1577/1548-8640(1988)050<0197:TOEOAT>2.3.CO;2

Martin SW, 1985. A mail survey of the efficacy of prophylactic medication in feed and/or water of feedlot calves. Canadian Journal of Comparative Medicine, 49, 15–20.

Maurin M and Raoult D, 2001. Use of aminoglycosides in treatment of infections due to intracellular bacteria. Antimicrobial Agents and Chemotherapy, 45, 2977–2986. https://doi.org/10.1128/AAC.45.11.2977-2986.2001

Maxwell CV, Combs GE, Knabe DA, Kornegay ET and Noland PR, 1994. Effect of dietary chlortetracycline during breeding and(or) farrowing and lactation on reproductive performance of sows: a cooperative study. https://doi.org/10.2527/1994.72123169x

May KD, Wells JE, Maxwell CV and Oliver WT, 2012. Granulated lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of 10-day-old pigs. Journal of Animal Science, 90, 1118-U1169. https://doi.org/10.2527/jas.2011-4297

Mazón-Suástegui JM, García-Bernal M, Saucedo PE, Campa-Córdova Á and Abasolo-Pacheco F, 2016. Homeopathy outperforms antibiotics treatment in juvenile scallop *Argopecten ventricosus*: effects on growth, survival, and immune response. Homeopathy, 106, 18–26. https://doi.org/10.1016/j.homp.2016.12.002

McOrist S, Shearn MFH and Morgan J, 1999. Control of porcine proliferative enteropathy by oral administration of chlortetracycline. Veterinary Record, 144, 48–49. https://doi.org/10.1136/vr.144.2.48

Meijer LA, Ceyssens KGF, de Grève BIJAC and de Bruijn W, 1993. Pharmacokinetics and bioavailability of doxycycline hyclate after oral administration in calves. Veterinary Quarterly, 15, 1–5. https://doi.org/10.1080/01652176.1993.9694358

Messersmith RE, Johnson DD, Elliott RF and Drain JJ, 1966. Value of chlortetracycline in breeding rations for sows. Journal of Animal Science, 25, 752–755. https://doi.org/10.2527/jas1966.253752x

Migliore L, Rotini A and Thaller MC, 2013. Low doses of tetracycline trigger the *E. coli* growth: a case of hormetic response. Dose Response, 11, 550–557. https://doi.org/10.2203/dose-response.13-002.Migliore

Mir Z, 1989. Monensin, chlortetracycline and tylosin effects on performance and digestion in lambs fed a ground alfalfa diet. Canadian Journal of Animal Science, 69, 505–508. https://doi.org/10.4141/cjas89-059

Mosleh N, Shomali T, Namazi F, Marzban M, Mohammadi M and Boroojeni AM, 2016. Comparative evaluation of therapeutic efficacy of sulfadiazine-trimethoprim, oxytetracycline, enrofloxacin and florfenicol on *Staphylococcus aureus*-induced arthritis in broilers. British Poultry Science, 57, 179–184. https://doi.org/10.1080/00071668.2016.1148263

Motl MA, Fritts CA and Waldroup PW, 2005. Effects of intestinal modification by antibiotics and antibacterials on utilization of methionine sources by broiler chickens. Journal of Applied Poultry Research, 14, 167–173. https://doi.org/10.1093/japr/14.1.167

Murdock FR, Hodgson AS and Blosser TH, 1961. Milk replacers for dairy calves. I. a comparison of an all milk by-product replacer and limited whole milk with and without chlortetracycline. Journal of Dairy Science, 44, 1711–1716. https://doi.org/10.3168/jds.S0022-0302(61)89944-X

Myers DJ and Speer VC, 1973. Effects of an antibiotic and flushing on performance of sows with short farrowing intervals. Journal of Animal Science, 36, 1125–1128. https://doi.org/10.2527/jas1973.3661125x

Natsir MH, Hartutik Sjofjan O, Widodo E and Widyastuti ES, 2017. Use of acidifiers and herb-Acidifier combinations with encapsulated and non-encapsulated intestinal microflora, intestinal histological and serum characteristics in Broiler. In: Hamid NA, Shrestha BG and Mustafa I (eds.). pp. 020012–1–02012-7.

NCR-89 Committee on confinement management of swine, 1984. Effect of space allowance and antibiotic feeding on performance of nursery pigs. Journal of Animal Science, 58, 801–804. https://doi.org/10.2527/jas1984.584801x

Neill CR, Nelssen JL, Tokach MD, Goodband RD, DeRouchey JM, Dritz SS, Groesbeck CN, Lawrence KR, Hastad CW, Gottlob RO and Hildabrand BM, 2004. Evaluating oregano oil as a growth enhancer in nursery pig diets. In: Kansas State University Swine Day 2004. Report of Progress 940, Kansas State University, Kansas, pp. 29–31.

Neill CR, Nelssen JL, Tokach MD, Goodband RD and DeRouchey JM, 2005. Effects of increasing oregano oil on nursery pig performance. In: Kansas State University Swine Day. Report of Progress 964. Kansas State University, Kansas. pp. 64–67.

Neill CR, Nelssen JL, Tokach MD, Goodband RD, DeRouchey JM, Dritz SS, Groesbeck CN and Brown KR, 2006. Effects of oregano oil on growth performance of nursery pigs. Journal of Swine Health and Production, 14, 312–316.

Nelson ML and Levy SB, 2011. The history of the tetracyclines. Annals of the New York Academy of Sciences, 1241, 17–32. https://doi.org/10.1111/j.1749-6632.2011.06354.x

Neuvonen PJ, Gothoni G, Hackman R and af Björksten K, 1970. Interference of iron with the absorption of tetracylines in man. British Medical Journal, 4, 532. https://doi.org/10.1136/bmj.4.5734.532

Neveling DP, van Emmenes L, Ahire JJ, Pieterse E, Smith C and Dicks LMT, 2020. Effect of a multi-species probiotic on the colonisation of salmonella in broilers. Probiotics and Antimicrobial Proteins. https://doi.org/10.1007/s12602-019-09593-y

Nielsen P and Gyrd-Hansen N, 1996. Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs. Journal of Veterinary Pharmacology and Therapeutics, 19, 305–311. https://doi.org/10.1111/j.1365-2885.1996.tb00054.x

Nitikanchana S, Dritz SS, Tokach MD, DeRouchey JM, Goodband RD and Nelssen JL, 2012. Effect of dietary addition of Denagard (Tiamulin) and CTC (Chlortetracycline) on pig performance immediately after placement in the finishing barn. In: Kansas State University Swine Day 2012. Report of progress 1074, Kansas State University, Kansas, pp. 343–347.

NRC (National Research Council), 1980. National Research Council (US) Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feed. The effects on human health of subtherapeutic use of antimicrobials in animal feeds. National Academy of Sciences, Washington, DC. https://doi.org/10.17226/21

Nyachoti CM, Kiarie E, Bhandari SK, Zhang G and Krause DO, 2012. Weaned pig responses to *Escherichia coli* K88 oral challenge when receiving a lysozyme supplement. Journal of Animal Science, 90, 252–260. https://doi.org/10.2527/jas.2010-3596

Oe O and Arakawa A, 1975. Effect of feed additive antibiotics on chickens infected with *Eimeria tenella*. Poultry Science, 54, 1008–1018. https://doi.org/10.3382/ps.0541008

Oguntona T. 1988a. Effects of dietary levels of oxytetracycline on the growth and organ weights of guinea fowl (*Numida meleagris*). The Journal of Agricultural Science, 111, 217–220. https://doi.org/10.1017/S0021859600083167

Oguntona T, 1988b. Studies on the response of guinea fowls (*Numida Meleagris*) to antibiotics. British Poultry Science, 29, 683–687. https://doi.org/10.1080/00071668808417095

Ogunwole OA, Abu OA and Adepoju IA, 2011. Performance and carcass characteristics of broiler finishers fed acidifier based diets. Pakistan Journal of Nutrition, 10, 631–636. https://doi.org/10.3923/pjn.2011.631.636

Ohe O and Arakawa A, 1976. Effect of dietary antibiotics on chickens infected with *Eimeria tenella*. Poultry Science, 55, 660–665. https://doi.org/10.3382/ps.0550660

Okerman L, Devriese LA, Gevaert D, Uyttebroek E and Haesebrouck F, 1990. *In vivo* activity of orally administered antibiotics and chemotherapeutics against acute septicaemic pasteurellosis in rabbits. Laboratory Animals, 24, 341–344. https://doi.org/10.1258/002367790780865994

Oko OOK, Ozung PO and Abang FB, 2018. Influence of ethanolic extract of *Aspilia africana* leaf on the performance and egg qualities of Japanese quails. Global Journal of Pure and Applied Sciences, 24, 135–140.

Oliver WT, Wells JE and Maxwell CV, 2014. Lysozyme as an alternative to antibiotics improves performance in nursery pigs during an indirect immune challenge. Journal of Animal Science, 92, 4927–4934. https://doi.org/10.2527/jas.2014-8033

Ologhobo AD, Akangbe EI, Adejumo IO and Adeleye O, 2014. Effect of *Moringa oleifera* leaf meal as replacement for oxytetracycline on carcass characteristics of the diets of broiler chickens. Annual Research and Review in Biology, 4, 423–431. https://doi.org/10.9734/ARRB/2014/6017

Olson LD, 1977a. Comparison of low-level Rofenaid, low-level chlortetracycline, and vaccination with commercial bacterin for preventing pulmonary form of fowl cholera in turkeys. Avian diseases, 21, 160–166. https://doi.org/10.2307/1589336

Olson LD, 1977b. Evaluation of Aureomycin for prevention of arthritic, pulmonary and cranial forms of fowl cholera in turkeys. Poultry Science, 56, 1102–1106. https://doi.org/10.3382/ps.0561102

Olson NO and Sahu SP, 1976. Efficacy of chlortetracycline against *Mycoplasma synoviae* isolated in two periods. Avian Diseases, 20, 221–229. https://doi.org/10.2307/1589259

Olusola SE, Ajiwoju IJ and Emikpe BO, 2020. Efficacy of tamarind *Tamarindus indica* leaves and *Mango mangifera* indica leaves as feed additives on growth, blood status and resistance to *Aeromonas hydrophila* in Juvenile African Catfish *Clarias gariepinus*. Ribarstvo, Croatian Journal of Fisheries, 78, 11–20. https://doi.org/10.2478/cjf-2020-0002

Onifade AA, 1997. Growth performance, carcass characteristics, organs measurement and haematology of broiler chickens fed a high fibre diet supplemented with antibiotics or dried yeast. Nahrung - Food, 41, 370–374. https://doi.org/10.1002/food.19970410612

Onifade AA and Babatunde GM, 1997. Comparative response of broiler chicks to a high fibre diet supplemented with four antibiotics. Animal Feed Science and Technology, 64, 337–342. https://doi.org/10.1016/S0377-8401(96)01043-7

Oso AO, Idowu OMO, Haastrup AS, Ajibade AJ, Olowonefa KO, Aluko AO, Ogunade IM, Osho SO and Bamgbose AM, 2013. Growth performance, apparent nutrient digestibility, caecal fermentation, ileal morphology and caecal microflora of growing rabbits fed diets containing probiotics and prebiotics. Livestock Science, 157, 184–190. https://doi.org/10.1016/j.livsci.2013.06.017

Oso AO, Suganthi RU, Reddy GBM, Malik PK, Thirumalaisamy G, Awachat VB, Selvaraju S, Arangasamy A and Bhatta R, 2019. Effect of dietary supplementation with phytogenic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. Poultry Science, 98, 4755–4766. https://doi.org/10.3382/ps/pez191

Papaioannou DS, Kyriakis SC, Papasteriadis A, Roumbies N, Yannakopoulos A and Alexopoulos C, 2002. A field study on the effect of in-feed inclusion of a natural zeolite (clinoptilolite) on health status and performance of sows/gilts and their litters. Research in Veterinary Science, 72, 51–59. https://doi.org/10.1053/rvsc.2001.0521

Park Y, Lee S, Hong J, Kim D, Moniruzzaman M and Bai SC, 2016a. Use of probiotics to enhance growth, stimulate immunity and confer disease resistance to *Aeromonas salmonicida* in rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research, 48, 2672–2682. https://doi.org/10.1111/are.13099

Park Y, Moniruzzaman M, Lee S, Hong J, Won S, Lee JM, Yun H, Kim K-W, Ko D and Bai SC, 2016b. Comparison of the effects of dietary single and multi-probiotics on growth, non-specific immune responses and disease resistance in starry flounder, *Platichthys stellatus*. Fish and Shellfish Immunology, 59, 351–357. https://doi.org/10.1016/j.fsi.2016.11.006

Patel KP and Baker DH, 1996. Supplemental iron, copper, zinc, ascorbate, caffeine and chlortetracycline do not affect riboflavin utilization in the chick. Nutrition Research, 16, 1943–1952. https://doi.org/10.1016/S0271-5317(96)00217-5

Patterson R, Heo JM, Wickramasuriya SS, Yi YJ and Nyachoti CM, 2019. Dietary nucleotide rich yeast extract mitigated symptoms of colibacillosis in weaned pigs challenged with an enterotoxigenic strain of *Escherichia coli*. Animal Feed Science and Technology, 254. https://doi.org/10.1016/j.anifeedsci.2019.114204

Pavlova I, Lukanov H, Ivanov V, Petrova Y and Genchev A, 2018. Simultaneous administration of silymarin and doxycycline in Japanese quails suggests probable herb-drug interaction. Bulgarian Journal of Agricultural Science, 24, 126–131.

Peeters LE, Daeseleire E, Devreese M, Rasschaert G, Smet A, Dewulf J, Heyndrickx M, Imberechts H, Haesebrouck F, Butaye P and Croubels S, 2016. Residues of chlortetracycline, doxycycline and sulfadiazine-trimethoprim in intestinal content and feces of pigs due to cross-contamination of feed. BMC Veterinary Research, 12, 209. https://doi.org/10.1186/s12917-016-0803-8

Penttilä O, Hurme H and Neuvonen PJ, 1975. Effect of zinc sulphate on the absorption of tetracycline and doxycycline in man. European Journal of Clinical Pharmacology, 9, 131–134. https://doi.org/10.1007/BF00614009

Percy DH and Black WD, 1988. Pharmacokinetics of tetracycline in the domestic rabbit following intravenous or oral administration. Canadian Journal of Veterinary Research, 52, 5–11.

Pérez VG, Waguespack AM, Bidner TD, Southern LL, Fakler TM, Ward TL, Steidinger M and Pettigrew JE, 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiota of pigs after weaning.

Perrin-Guyomard A, Cottin S, Corpet DE, Boisseau J and Poul JM, 2001. Evaluation of residual and therapeutic doses of tetracycline in the human-flora-associated (HFA) mice model. Regulatory Toxicology and Pharmacology, 34, 125–136. https://doi.org/10.1006/rtph.2001.1495

Perry TW, Riley JG, Mohler MT and Pope RV, 1986. Use of chlortetracycline for treatment of new feedlot cattle. Journal of Animal Science, 62, 1215–1219. https://doi.org/10.2527/jas1986.6251215x

Peterson RA, Carpenter GH, Jones WT and Kula JA, 1991. Research note: effect of tetracycline hydrochloride and oxytetracycline hydrochloride given via drinking water on early mortality of broiler chicks from twenty-eight-week-old dams. Poultry Science, 70, 1040–1042. https://doi.org/10.3382/ps.0701040

Phelps PV, Gildersleeve RP and Edens FW, 1987. Effect of prefeeding on physiological parameters associated with turkey poult mortality. Poultry Science, 66, 1882–1884. https://doi.org/10.3382/ps.0661882

Piva A, Grilli E, Fabbri L, Pizzamiglio V and Campani I, 2007. Free versus microencapsulated organic acids in medicated or not medicated diet for piglets. Livestock Science, 108, 214–217. https://doi.org/10.1016/j.livsci.2007.01.065

Pollet RA, Glatz CE and Dyer DC, 1985. The pharmacokinetics of chlortetracycline orally administered to turkeys: influence of citric acid and *Pasteurella multocida* infection. Journal of Pharmacokinetics and Biopharmaceutics, 13, 243–264. https://doi.org/10.1007/bf01065655

Powley JS, Cheeke PR, England DC, Davidson TP and Kennick WH, 1981. Performance of growing-finishing swine fed high levels of alfalfa meal: effects of alfalfa level, dietary additives and antibiotics. Journal of Animal Science, 53, 308–316. https://doi.org/10.2527/jas1981.532308x

Proudfoot FG, Hulan HW and Jackson ED, 1988. The response of male broiler chicks to the consumption of low levels of chlortetracycline as a growth promoter. Canadian Journal of Animal Science, 68, 1285–1290. https://doi.org/10.4141/cjas88-144

Puls CL, Allee GL, Hammer JM and Carr SN, 2019a. Effects of different antibiotic feeding programs on morbidity and mortality and growth performance of nursery pigs housed in a wean-to-finish facility. Translational Animal Science, 3, 124–133. https://doi.org/10.1093/tas/txy096

Puls CL, Hammer JM, Eggers K, Graham A, Knopf B, Greiner L and Carr SN, 2019b. Effects of two feeding periods of tiamulin fed in combination with chlortetracycline for control and treatment of swine respiratory and enteric disease and subsequent growth performance of growing-finishing pigs. Translational Animal Science, 3, 113–122. https://doi.org/10.1093/tas/txy097

Purushothaman MR, Chandrasekaran D and Janani SR, 2014. Fenugreek residue as nutraceutical and antibacterial agent in broiler ration. Indian Journal of Animal Sciences, 84, 1295–1299.

Purwanti S, Agustina L, Jamilah X, Syamsu JA and Putra RD, 2019. Histology of the liver and small intestine broiler using phytobiotic in the ration infected *Salmonella pullorum*. In. 1st International Conference of Animal Science and Technology (ICAST) 2018, pp.

Qu H, Cheng Y, Chen Y, Li J, Zhao Y and Zhou Y, 2019. Effects of dietary zeolite supplementation as an antibiotic alternative on growth performance, intestinal integrity, and cecal antibiotic resistance genes abundances of broilers. Animals, 9. https://doi.org/10.3390/ani9110909

Quigley JD III and Drew MD, 2000. Effects of oral antibiotics or bovine plasma on survival, health and growth in dairy calves challenged with *Escherichia coli*. Food and Agricultural Immunology, 12, 311–318. https://doi.org/10.1080/09540100020008173

Quigley JD III, Drewry JJ, Murray LM and Ivey SJ, 1997. Body weight gain, feed efficiency, and fecal scores of dairy calves in response to galactosyl-lactose or antibiotics in milk replacers. Journal of Dairy Science, 80, 1751–1754. https://doi.org/10.3168/jds.S0022-0302(97)76108-3

Radecki SV, Juhl MR and Miller ER, 1988. Fumaric and citric acids as feed additives in starter pig diets: effect on performance and nutrient balance. Journal of Animal Science, 66, 2598–2605. https://doi.org/10.2527/jas1988.66102598x

Rae DO, Ramsay KH and Morrison RL, 2002. Effect of chlortetracycline in a trace mineral salt mix on fertility traits in beef cattle females in Florida. Journal of Animal Science, 80, 880–885. https://doi.org/10.2527/2002.804880x

Ramezanzadeh S, Kenari AA and Esmaeili M, 2020. Immunohematological parameters of rainbow trout (*Oncorhynchus mykiss*) fed supplemented diet with different forms of barberry root (*Berberis vulgaris*). Comparative Clinical Pathology, 29, 177–187.

Ran T, Gomaa WMS, Shen YZ, Saleem AM, Yang WZ and McAllister TA, 2019. Use of naturally sourced feed additives (*Lactobacillus* fermentation products and enzymes) in growing and finishing steers: effects on performance, carcass characteristics and blood metabolites. Animal Feed Science and Technology, 254. https://doi.org/10.1016/j.anifeedsci.2019.05.013

Reda RM, Mahmoud R, Selim KM and El-Araby IE, 2016. Effects of dietary acidifiers on growth, hematology, immune response and disease resistance of Nile tilapia, *Oreochromis niloticus*. Fish and Shellfish Immunology, 50, 255–262. https://doi.org/10.1016/j.fsi.2016.01.040

Redden RR, Kott RW, Boles JA, Layton AW and Hatfield PG, 2010. Effects of late gestation supplementation of rumen undegradable protein, vitamin E, zinc, and chlortetracycline to ewes on indices of immune transfer and productivity. Journal of Animal Science, 88, 1125–1134. https://doi.org/10.2527/jas.2009-2442

Reid ED, Erickson PS, Hodgdon S, Lennon E and Tsang PCW, 2014. Chlortetracycline supplementation of yearling dairy heifers. Journal of Animal Science, 84, 2406–2409. https://doi.org/10.2527/jas.2005-761

Rhee C, Kim H, Aalfin Emmanuel S, Kim H-G, Won S, Bae J, Bai SC and Koh S-C, 2018. Microbial community analysis of an eco-friendly recirculating aquaculture system for olive flounder (*Paralichthys olivaceus*) using complex microbial probiotics. Korean Journal of Microbiology, 54, 369–378. https://doi.org/10.7845/kjm.2018.8085

Rhee C, Kim H, Emmanuel SA, Kim H-G, Won S, Bae J, Bai SC and Koh S-C, 2020. Probiotic effects of mixture of *Groenewaldozyma salmanticensis* and *Gluconacetobacter liquefaciens* on growth and immune responses in *Paralichthys olivaceus*. Letters in Applied Microbiology, 70, 431–439. https://doi.org/10.1111/lam.13282

Ribeiro de Lima F, Stahly TS and Cromwell GL, 1981. Effects of copper, with and without ferrous sulfide, and antibiotics on the performance of pigs. Journal of Animal Science, 52, 241–247. https://doi.org/10.2527/jas1981.522241x

Riond JL and Riviere JE, 1990. Pharmacokinetics and metabolic inertness of doxycycline in young pigs. American Journal of Veterinary Research, 51, 1271–1275.

Riond JL, Tyczkowska K and Riviere JE, 1989. Pharmacokinetics and metabolic inertness of doxycycline in calves with mature or immature rumen function. American Journal of Veterinary Research, 50, 1329–1333.

Riviere JE and Papich MG, 2017. Veterinary Pharmacology and Therapeutics, 10th Edition. Wiley-Blackwell. 1252 pp.

Robbins RC, Artuso-Ponte VC, Moeser AJ, Morgan Morrow WE, Spears JW and Gebreyes WA, 2013. Effects of quaternary benzo(c)phenanthridine alkaloids on growth performance, shedding of organisms, and gastrointestinal tract integrity in pigs inoculated with multidrug-resistant *Salmonella* spp. American Journal of Veterinary Research, 74, 1530–1535. https://doi.org/10.2460/ajvr.74.12.1530

Roberts MC and Schwarz S, 2016. Tetracycline and phenicol resistance genes and mechanisms: importance for agriculture, the environment, and humans. Journal of Environmental Quality, 45, 576–592. https://doi.org/10.2134/jeq2015.04.0207

Rogstad A, Hormazabal V, Ellingsen OG and Rasmussen KE, 1991. Pharmacokinetic study of oxytetracycline in fish. I. Absorption, distribution and accumulation in rainbow trout in freshwater. Aquaculture, 96, 219–226. https://doi.org/10.1016/0044-8486(91)90151-V

Rollins LD, Gaines SA, Pocurull DW, Mercer HD and Frobish LT, 1976. Persistence of transferable drug resistance in the lactose fermenting enteric flora of swine following antimicrobial feeding. Canadian Journal of Comparative Medicine : Revue Canadienne de Médécine Comparée, 40, 175–183.

Roose-Amsaleg C, Yan C, Hoang AM and Laverman AM, 2013. Chronic exposure of river sediments - environmentally relevant levels of tetracycline affects bacterial communities but not denitrification rates. Ecotoxicology, 22, 1467–1478. https://doi.org/10.1007/s10646-013-1133-2

Rossi F, Morlacchini M, Gatti P, Soldi S, Callegari ML and Piva G, 2008. Effects of a gluco-oligosaccharide supplement on the morphological characteristics of the gastro-intestinal tract and growth performance in weaned piglets. Italian Journal of Animal Science, 7, 185–198. https://doi.org/10.4081/ijas.2008.185

Roura E, Homedes J and Klasing KC, 1992. Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. The Journal of Nutrition, 122, 2383–2390. https://doi.org/10.1093/jn/122.12.2383

Roy S, Ghosh RC, Misra SK and Chauhan HVS, 1991. Use of tetracycline sorbate for the treatment of *Aspergillus fumigatus* infection in broiler chicks. The British Veterinary Journal, 147, 549–555. https://doi.org/10.1016/0007-1935(91)90025-I

Rueff L, Mellencamp MA and Pantoja LG, 2019. Performance of immunologically castrated pigs at a commercial demonstration farm over 3.5 years. Journal of Swine Health and Production, 27, 322–328.

Rumsey TS, Bitman J, Wrenn TR, Baldwin KA, Tao H and Thompson MJ, 1982. Performance, ruminal fermentation and blood constituents of lambs fed N, N-dimethyldodecanamine and chlortetracycline. Journal of Animal Science, 54, 1040–1050. https://doi.org/10.2527/jas1982.5451040x

Rumsey TS, McLeod K, Elsasser TH, Kahl S and Baldwin RL, 1999. Effects of oral chlortetracycline and dietary protein level on plasma concentrations of growth hormone and thyroid hormones in beef steers before and after challenge with a combination of thyrotropin-releasing hormone and growth hormone-releasing hormone. Journal of Animal Science, 77, 2079–2087. https://doi.org/10.2527/1999.7782079x

Rumsey TS, McLeod K, Elsasser TH, Kahl S and Baldwin RL, 2000. Performance and carcass merit of growing beef steers with chlortetracycline-modified sensitivity to pituitary releasing hormones and fed two dietary protein levels. Journal of Animal Science, 78, 2765–2770. https://doi.org/10.2527/2000.78112765x

Rusoff LL, Cummings AH, Stone EJ and Johnston JE, 1959. Effect of high-level administration of chlortetracycline at birth on the health and growth of young dairy calves. Journal of Dairy Science, 42, 856–862. https://doi.org/10.3168/jds.S0022-0302(59)90663-0

Sacristán RDP, Rodríguez AL, Sierens A, Vranckx K, Boyen F, Dereu A, Haesebrouck F and Maes DGD, 2012. Efficacy of in-feed medication with chlortetracycline in a farrow-to-finish herd against a clinical outbreak of respiratory disease in fattening pigs. Veterinary Record, 171, 645. https://doi.org/10.1136/vr.100976

Saha AK and Ray AK, 1998. Cellulase activity in rohu fingerlings. Aquaculture International, 6, 281–291. https://doi.org/10.1023/A:1009210929594

Salaheen S, Kim S-W, Haley BJ, van Kessel JAS and Biswas D, 2017. Alternative growth promoters modulate broiler gut microbiome and enhance body weight gain. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.02088

Saleh AA, Ebeid TA and Abudabos AM, 2018. Effect of dietary phytogenics (herbal mixture) supplementation on growth performance, nutrient utilization, antioxidative properties, and immune response in broilers. Environmental Science and Pollution Research, 25, 14606–14613. https://doi.org/10.1007/s11356-018-1685-z

Salichs M, Sabaté D and Homedes J, 2013. Efficacy of ketoprofen administered in drinking water at a low dose for the treatment of porcine respiratory disease complex. Journal of Animal Science, 91, 4469–4475. https://doi.org/10.2527/jas.2012-6165

Saloma AE, Creger CR and Couch JR, 1970. The intermittent feeding of anti-bacterial and/or antifungal agents to laying hens. British Poultry Science, 11, 281–289. https://doi.org/10.1080/00071667008415818

Samanta AK, Jayaram C, Jayapal N, Sondhi N, Kolte AP, Senani S, Sridhar M and Dhali A, 2015. Assessment of fecal microflora changes in pigs supplemented with herbal residue and prebiotic. PLoS ONE, 10. https://doi.org/10.1371/journal.pone.0132961

Sanchez-Martínez JG, Pérez-Castañeda R, Rábago-Castro JL, Aguirre-Guzmán G and Vázquez-Sauceda ML, 2008. A preliminary study on the effects on growth, condition, and feeding indexes in channel catfish, *Ictalurus punctatus*, after the prophylactic use of potassium permanganate and oxytetracycline. Journal of the World Aquaculture Society, 39, 664–670. https://doi.org/10.1111/j.1749-7345.2008.00195.x

Sandhu TS and Dean WF, 1980. Effect of chemotherapeutic agents on *Pasteurella anatipestifer* infection in White Pekin ducklings. Poultry Science, 59, 1027–1030. https://doi.org/10.3382/ps.0591027

Santos MDF, Vermeersch H, Remon JP, Schelkens M, De Backer P, Van Bree HJ, Ducatelle R and Haesebrouck F, 1996. Pharmacokinetics and bioavailability of doxycycline in turkeys. Journal of Veterinary Pharmacology and Therapeutics, 19, 274–280. https://doi.org/10.1111/j.1365-2885.1996.tb00049.x

Santos MDF, Vermeersch H, Remon JP, Schelkens M, Backer P, Ducatelle R and Haesebrouck F, 1997. Administration of doxycycline hydrochloride via drinking water to turkeys under laboratory and field conditions. Poultry Science, 76, 1342–1348. https://doi.org/10.1093/ps/76.10.1342

Sarker MSK, Kim GM and Yang CJ, 2010a. Effect of green tea and biotite on performance, meat quality and organ development in ross broiler. Egyptian Poultry Science Journal, 30, 77–88.

Sarker MSK, Ko S-Y, Kim G-M and Yang C-J, 2010b. Effects of *Camellia sinensis* and mixed probiotics on the growth performance and body composition in broiler. Journal of Medicinal Plants Research, 4, 546–550.

Sarker SK, Park S-R, Kim G-M and Yang C-J, 2010c. Hamcho (*Salicornia herbacea*) with probiotics as alternative to antibiotic for broiler production. Journal of Medicinal Plants Research, 4, 415–420.

Sarker MSK, Yim KJ, Ko SY, Uuganbayar D, Kim GM, Bae IH, Oh JI, Yee ST and Yang CJ, 2010d. Green tea level on growth performance and meat quality in finishing pigs. Pakistan Journal of Nutrition, 9, 10–14. https://doi.org/10.3923/pjn.2010.10.14

Sbiraki AP, Saoulidis K, Kyriakis SC, Saratsis P, Alexopoulos C and Fthenakis GC, 2003. Effects of chlortetracycline administration on the health status and performance of sows: results of a field trial. Journal of Swine Health and Production, 11, 117–126.

Schach von Wittenau M and Twomey TM, 1971. The disposition of doxycyline by man and dog. Chemotherapy, 16, 217–228. https://doi.org/10.1159/000220730

Schifferli D, Galeazzi RL, Nicolet J and Wanner M, 1982. Pharmacokinetics of oxytetracycline and therapeutic implications in veal calves. Journal of Veterinary Pharmacology and Therapeutics, 5, 247–257. https://doi.org/10.1111/j.1365-2885.1982.tb00440.x

Scornec H, Bellanger X, Guilloteau H, Groshenry G and Merlin C, 2017. Inducibility of Tn*916* conjugative transfer in *Enterococcus faecalis* by subinhibitory concentrations of ribosome-targeting antibiotics. Journal of Antimicrobial Chemotherapy, 72, 2722–2728. https://doi.org/10.1093/jac/dkx202

Serafin JA, 1982. Reduced mortality among young endangered masked bobwhite quail fed oxytetracycline-supplemented diets. Avian Diseases, 26, 422–425. https://doi.org/10.2307/1590116

Shaddad SAI, Wasfi IA, Maglad MA and Adam SEI, 1985a. The effect of oxytetracycline on growth and lipid metabolism in poultry. Comparative Biochemistry and Physiology Part C, 80, 375–380. https://doi.org/10.1016/0742-8413(85)90071-4

Shaddad SAI, Wasfi IA, Yassein OE, Ali AE, Maglad MA and Adam SEI, 1985b. The effect of oxytetracycline on the fat content and fatty acid composition of the egg yolk. Comparative Biochemistry and Physiology Part C, 81, 223–226. https://doi.org/10.1016/0742-8413(85)90119-7

Shalaei M, Hosseini SM and Zergani E, 2014. Effect of different supplements on eggshell quality, some characteristics of gastrointestinal tract and performance of laying hens. Veterinary Research Forum, 5, 277–286.

Shelton NW, Dritz SS, Tokach MD, Goodband RD, Nelssen JL, DeRouchey JW and Murray LW, 2009a. Effects of experimental design and its role in interpretation of results. In: Kansas State University Swine Day. Report of Progress 1020. Kansas State University, Kansas. pp. 96–105.

Shelton NW, Jacob ME, Tokach MD, Nelssen JL, Goodband RD, Dritz SS, DeRouchey JM, Amachawadi RG and Nagaraja TG, 2009b. Effects of copper sulfate, zinc oxide, and neoterramycin on weanling pig growth and antibiotic resistance rate for fecal Escherichia coli. In: Kansas State University Swine Day. Report of Progress 1020. Kansas State University, Kansas. pp. 73–79.

Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I and Zhen YG, 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. Journal of Animal Science, 87, 2614–2624. https://doi.org/10.2527/jas.2008-1512

Shi BL, Li DF, Piao XS and Yan SM, 2005. Effects of chitosan on growth performance and energy and protein utilisation in broiler chickens. British Poultry Science, 46, 516–519. https://doi.org/10.1080/00071660500190785

Shields D, Blome R, Wood D and Sowinski J, 2010. Effects of neomycin and oxytetracycline (N/T) fed at treatment rate for 14 days in calf milk replacer (CMR) on calf performance and health. Journal of Dairy Science, 93, 24.

Shokaiyan M, Ashayerizadeh O, Shargh SM and Dastar B, 2019. Algal crude fucoidan alone or with *Bacillus subtilis* DSM 17299 in broiler chickens diet: growth performance, carcass characteristics, blood metabolites, and morphology of intestine. Poultry Science Journal, 7, 87–94. https://doi.org/10.22069/psj.2019.16314.1411

Shon KS, Hong JW, Kwon OS, Min BJ, Lee WB, Kim IH, Park YH and Lee IS, 2005. Effects of *Lactobacillus reuteri*-based *direct-fed microbial supplementation for growing-finishing pigs. Asian-Australasian Journal of Animal Sciences, 18, 370–374. https://doi.org/10.5713/ajas.2005.370*

Shor AL, Johnson WP and Abbey A, 1959. Effects of various amounts of chlortetracycline in the rations of lactating dairy cattle. Journal of Dairy Science, 42, 1203–1208. https://doi.org/10.3168/jds.S0022-0302(59)90713-1

Shrimpton DH, Barnes EM and Miller WS, 1958. The control of spoilage of uneviscerated poultry carcasses by treatment with antibiotics before slaughter. Journal of the Science of Food and Agriculture, 9, 353–360. https://doi.org/10.1002/jsfa.2740090606

Sinclair RW, Robinson FE and Hardin RT, 1990. The effects of parent age and posthatch treatment on broiler performance. Poultry Science, 69, 526–534. https://doi.org/10.3382/ps.0690526

Singh A, Din ZZ, Maurer AJ and Sunde ML, 1985. Effects of sodium diacetate on the growth, feed efficiency, and intestinal microflora of broilers. Poultry Science, 64, 844–851. https://doi.org/10.3382/ps.0640844

Singh J, Sethi APS, Sikka SS, Chatli MK and Mehta N, 2014a. Effect of sun dried whole leaf *Aloe vera* powder on growth, carcass characteristics and meat quality of commercial broilers. Indian Journal of Poultry Science, 49, 21–24.

Singh J, Sethi APS, Sikka SS, Chatli MK and Kumar P, 2014b. Effect of cinnamon (*Cinnamomum cassia*) powder as a phytobiotic growth promoter in commercial broiler chickens. Animal Nutrition and Feed Technology, 14, 471–479. https://doi.org/10.5958/0974-181X.2014.01349.3

Singh J, Sethi APS, Sikka SS, Chatli MK and Kumar P, 2015. Effect of sun dried whole bulb garlic powder on growth, carcass characteristics and meat quality of commercial broilers. Indian Journal of Animal Sciences, 85, 67–71.

Singh J, Sharma M, Mehta N, Singh ND, Kaur P, Sethi APS and Sikka SS, 2018. Influence of supplementation of black pepper powder through feed in broiler chickens on their growth performance, blood profile, meat sensory qualities and duodenum morphology. Indian Journal of Animal Sciences, 88, 215–221.

Singh J, Kaur P, Sharma M, Mehta N, Singh ND, Sethi APS and Sikka SS, 2019. Effect of combination of garlic powder with black pepper, cinnamon and aloe vera powder on the growth performance, blood profile, and meat sensory qualities of broiler chickens. Indian Journal of Animal Sciences, 89, 1370–1376.

Skinner LD, Levesque CL, Wey D, Rudar M, Zhu J, Hooda S and Lange CFM, 2014. Impact of nursery feeding program on subsequent growth performance, carcass quality, meat quality, and physical and chemical body composition of growing-finishing pigs. Journal of Animal Science, 92, 1044–1054. https://doi.org/10.2527/jas.2013-6743

Slyamova A, Sarsembayeva N, Valdovska A, Micinski J, Ussenbayev A, Paritova A and Mankibayev A, 2016. Effects of antibiotic growth promoters on biochemical and haematological parameters of broiler chickens' blood. Latvia University of Agriculture. pp. 131–136.

Smith WC, Adam JL and Tonks HM, 1964. Effects of oxytetracycline and oleandomycin. Separately and together in pig diets. Animal Production, 6, 363–368. https://doi.org/10.1017/S0003356100022169

Sokoudjou JB, Fodouop SPC, Djoueudam FG, Kodjio N, Kana JR, Fowa AB, Kamsu GT and Gatsing D, 2019. Antisalmonellal and antioxidant potential of hydroethanolic extract of *Canarium schweinfurthii* Engl. (Burseraceae) in *Salmonella enterica* serovar Typhimurium-infected chicks. Asian Pacific Journal of Tropical Biomedicine, 9, 474–483. https://doi.org/10.4103/2221-1691.270980

Soler L, Miller I, Hummel K, Razzazi-Fazeli E, Jessen F, Escribano D and Niewold T, 2016. Growth promotion in pigs by oxytetracycline coincides with down regulation of serum inflammatory parameters and of hibernation-associated protein HP-27. Electrophoresis, 37, 1277–1286. https://doi.org/10.1002/elps.201500529

Song J, Li YL and Hu CH, 2013. Effects of copper-exchanged montmorillonite, as alternative to antibiotic, on diarrhea, intestinal permeability and proinflammatory cytokine of weanling pigs. Applied Clay Science, 77–78, 52–55. https://doi.org/10.1016/j.clay.2013.01.016

Sotak KM, Tokach MD, Hammer M, Jacela JY, Dritz SS, Mechler D, Goodband RD, DeRouchey JM and Nelssen JL, 2010. A comparison of Denagard, Denagard/CTC and Pulmotil on nursery pig growth performance and economic return. In: Kansas State University Swine Day. Report of Progress 1038. Kansas State University, Kansas. pp. 72–78.

Souza KA, Cooke RF, Schubach KM, Brandaõ AP, Schumaher TF, Prado IN, Marques RS and Bohnert DW, 2018. Performance, health and physiological responses of newly weaned feedlot cattle supplemented with feed-grade antibiotics or alternative feed ingredients. Animal, 12, 2521–2528. https://doi.org/10.1017/S1751731118000551

Stahly TS, Cromwell GL and Monegue HJ, 1980. Effects of the dietary inclusion of copper and(or) antibiotics on the performance of weanling pigs. Journal of Animal Science, 51, 1347–1351. https://doi.org/10.2527/jas1981.5161347x

Stanford K, Gibb DJ, Schwartzkopf-Genswein KS, van Herk F and McAllister TA, 2015. Feeding subtherapeutic antimicrobials to low-risk cattle does not confer consistent performance benefits. Canadian Journal of Animal Science, 95, 589–597. https://doi.org/10.4141/cjas-2015-008

Steidinger MU, Tokach MD, Dau D, Dritz SS, DeRouchey JM, Goodband RD and Nelssen JL, 2008. Influence of antimicrobial sequence in the nursery on pig performance and economic return. In: Kansas State University Swine Day. Report of Progress 1001. Kansas State University, Kansas. pp. 74–81.

Steidinger MU, Tokach MD, Dau D, Dritz SS, DeRouchey JM, Goodband RD and Nelssen JL, 2009. Comparison of different antimicrobial sequences on nursery pig performance and economic return. In: Kansas State University Swine Day. Report of Progress 1020. Kansas State University, Kansas. pp. 122–131.

Stipkovits L, Salyi G, Glavits R and Burch DGS, 1999. Testing the compatibility of a combination of tiamulin/chlortetracycline 1:3 premix (Tetramutin-Novartis) given in feed at different levels with salinomycin in chickens. Avian Pathology, 28, 579–586. https://doi.org/10.1080/03079459994371

Stipkovits L, Miller D, Glavits R, Fodor L and Burch D, 2001. Treatment of pigs experimentally infected with *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae* with various antibiotics. Canadian Journal of Veterinary Research, 65, 213–222.

Stutz MW and Lawton GC, 1984. Effects of diet and antimicrobials on growth, feed efficiency, intestinal *Clostridium perfringens*, and ileal weight of broiler chicks. Poultry Science, 63, 2036–2042. https://doi.org/10.3382/ps.0632036

Subagja J, Slembrouck J, Hung LT and Legendre M, 1999. Larval rearing of an Asian catfish *Pangasius hypophthalmus* (*Siluroidei, Pangasiidae*): analysis of precocious mortality and proposition of appropriate treatments. Aquatic Living Resources, 12, 37–44. https://doi.org/10.1016/S0990-7440(99)80013-8

Suchy P, Strakova E, Kummer V, Herzig I, Pisarikova V, Blechova R and Maskova J, 2008. Hepatoprotective effects of milk thistle (*Silybum marianum*) seed cakes during the chicken broiler fattening. Acta Veterinaria Brno, 77, 31–38. https://doi.org/10.2754/avb200877010031

Sukandhiya K, Mani K, Moorthy M, Purushothaman MR and Rajendran K, 2016. Influence of dietary supplementation of sodium diformate on the production performance of broilers in environmentally controlled housing system. Indian Veterinary Journal, 93, 32–33.

Sulabo RC, Jacela JY, DeRouchey JM, Tokach MD, Neher F, Goodband RD, Dritz SS and Nelssen JL, 2007. Effects of phytobiotics (Biomin® P.E.P.) on nursery pig performance. In: Kansas State University Swine Day 2007. Report of Progress 985, Kansas State University, Kansas, pp. 94–98.

Swanson EW, 1963. Effects of chlortetracycline in calf starter and M ilk. Journal of Dairy Science, 46, 955–958. https://doi.org/10.3168/jds.S0022-0302(63)89185-7

Szasz JI, McMurphy CP, Bryant TC, Luque J, Barcelo C, Sepulveda G, Blood KS, Bernhard BC and Hughes HD, 2019. Influence of therapeutic use of feedgrade tetracyclines in combination with tulathromycin metaphylaxis on animal health and performance of Holstein steer calves. Translational Animal Science, 3, 185–194. https://doi.org/10.1093/tas/txy135

Tang X, Fatufe AA, Yin Y, Tang Z, Wang S, Liu Z, Xinwu X and Li T-J, 2012. Dietary supplementation with recombinant lactoferrampin-lactoferricin improves growth performance and affects serum parameters in piglets. Journal of Animal and Veterinary Advances, 11, 2548–2555. https://doi.org/10.3923/javaa.2012.2548.2555

Tang ZG, Wen C, Wang LC, Wang T and Zhou YM, 2014. Effects of zinc-bearing clinoptilolite on growth performance, cecal microflora and intestinal mucosal function of broiler chickens. Animal Feed Science and Technology, 189, 98–106. https://doi.org/10.1016/j.anifeedsci.2013.12.014

Teague HS, Grifo AP Jr and Rutledge EA, 1966. Response of growing-finishing swine to different levels and methods of feeding chlortetracycline. Journal of Animal Science, 25, 693–700. https://doi.org/10.2527/jas1966.253693x

Ternus GS, Vetter RL and Danley MM, 1971. Feeder lamb response to chlortetracycline-sulfamethazine supplementation. Journal of Animal Science, 33, 878–880. https://doi.org/10.2527/jas1971.334878x

Thaler RC, Nelssen JL, Anderson GA, Blecha F, Chitko CG, Chapes SK and Clough ER, 1989. Evaluation of a biological response modifier: effects on starter pig performance. Journal of Animal Science, 67, 2341–2346. https://doi.org/10.2527/jas1989.6792341x

Thanh NT, Loh TC, Foo HL, Hair-Bejo M and Azhar BK, 2009. Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microbial population, small intestine *villus* height and faecal volatile fatty acids in broilers. British Poultry Science, 50, 298–306. https://doi.org/10.1080/00071660902873947

Thomson DU, Swingle RS, Branine M, Bartle SJ and Yates DA, 2014. Effects of timing of chlortetracycline in combination with decoquinate on growth performance, health, and carcass characteristics of feeder steers. Bovine Practitioner, 48, 120–128.

Thongsong B, Kalandakanond-Thongsong S and Chavananikul V, 2008. Effects of the addition of probiotic containing both bacteria and yeast or an antibiotic on performance parameters, mortality rate and antibiotic residue in broilers. Thai Journal of Veterinary Medicine, 38, 17–26.

Thu TV, Loh TC, Foo HL, Yaakub H and Bejo MH, 2011. Effects of liquid metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faeces characteristics, intestinal morphology and diarrhoea incidence in postweaning piglets. Tropical Animal Health and Production, 43, 69–75. https://doi.org/10.1007/s11250-010-9655-6

Toften H and Jobling M, 1997. Feed intake and growth of Arctic charr, *Salvelinus alpinus* (L.), fed diets supplemented with oxytetracycline and squid extract. Aquaculture Nutrition, 3, 255–259. https://doi.org/10.1046/j.1365-2095.1997.00098.x

Touchburn SP and Nestor KE, 1971. Effect of dietary neomycin-terramycin on reproductive performance in turkeys. Poultry Science, 50, 151–155. https://doi.org/10.3382/ps.0500151

Trushenski JT, Aardsma MP, Barry KJ, Bowker JD, Jackson CJ, Jakaitis M, McClure RL and Rombenso AN, 2018. Oxytetracycline does not cause growth promotion in finfish. Journal of Animal Science, 96, 1667–1677. https://doi.org/10.1093/jas/sky120

Unno T, Kim J, Guevarra RB and Nguyen SG, 2015. Effects of antibiotic growth promoter and characterization of ecological succession in swine gut microbiota. Journal of Microbiology and Biotechnology, 25, 431–438. https://doi.org/10.4014/jmb.1408.08063

Ürüşan H and Bölükbaşı ŞC, 2017. Effects of dietary supplementation levels of turmeric powder (*Curcuma longa*) on performance, carcass characteristics and gut microflora in broiler chickens. JAPS, Journal of Animal and Plant Sciences, 27, 732–736.

Uuganbayar D, Bae IH, Choi KS, Shin IS, Firman JD and Yang CJ, 2005. Effects of green tea powder on laying performance and egg quality in laying hens. Asian-Australasian Journal of Animal Sciences, 18, 1769–1774. https://doi.org/10.5713/ajas.2005.1769

Vandersall JH, Hibbs JW and Conrad HR, 1957. I131 Uptake in calves fed chlortetracycline. Journal of Dairy Science, 40, 1365. https://doi.org/10.3168/jds.S0022-0302(57)94637-4

Vandonkergoed J, 1992. Metaanalysis of field trials of antimicrobial mass medication for prophylaxis of bovine respiratory-disease in feedlot cattle. Canadian Veterinary Journal, 33, 786–795.

Verbrugghe E, Van Parys A, Haesendonck R, Leyman B, Boyen F, Haesebrouck F and Pasmans F, 2016. Subtherapeutic tetracycline concentrations aggravate *Salmonella Typhimurium* infection by increasing bacterial virulence. Journal of Antimicrobial Chemotherapy, 71, 2158–2166. https://doi.org/10.1093/jac/dkw152

Vernon J, Mercer EA and Rosen GD, 1962. The effects on production efficiency of oleandomycin and oxytetracycline fed at low levels in the diet of heavy pigs. Animal Production, 4, 279–287. https://doi.org/10.1017/S0003356100034292

Veum TL, Lauxen R and Yen J-T, 1980. Efficacy of feed additives in enhancing performance of growing pigs. Animal Production, 30, 95–103. https://doi.org/10.1017/S0003356100023849

Waldroup PW, Owen JA, Blackman JR, Short JR, Ramsey BE, Slagter PJ and Johnson ZB, 1981. Comparison of low dietary calcium and sodium sulfate for the potentiation of tetracycline antibiotics in broiler diets. Avian Diseases, 25, 857–865. https://doi.org/10.2307/1590060

Walker-Love J, Laird R, Thomson JM and Gray KW, 1959. Ultra-violet irradiation of fattening pigs. Animal Production, 1, 21–29. https://doi.org/10.1017/S0003356100033146

Walsh MC, Sholly DM, Hinson RB, Saddoris KL, Sutton AL, Radcliffe JS, Odgaard R, Murphy J and Richert BT, 2007. Effects of water and diet acidification with and without antibiotics on weanling pig growth and microbial shedding. Journal of Animal Science, 85, 1799–1808. https://doi.org/10.2527/jas.2006-049

Wang Y, Shan T, Xu Z, Liu J and Feng J, 2006. Effect of lactoferrin on the growth performance, intestinal morphology, and expression of PR-39 and protegrin-1 genes in weaned piglets. Journal of Animal Science, 84, 2636–2641. https://doi.org/10.2527/jas.2005-544

Wang M-Q, Du Y-J, Wang C, Tao W-J, He Y-D and Li H, 2012. Effects of copper-loaded chitosan nanoparticles on intestinal microflora and morphology in weaned piglets. Biological Trace Element Research, 149, 184–189. https://doi.org/10.1007/s12011-012-9410-0

Wang Y, Wu Y, Wang B, Cao X, Fu A, Li Y and Li W, 2017. Effects of probiotic *Bacillus* as a substitute for antibiotics on antioxidant capacity and intestinal autophagy of piglets. AMB Express, 7. https://doi.org/10.1186/s13568-017-0353-x

Wang S, Yao B, Gao H, Zang J, Tao S, Zhang S, Huang S, He B and Wang J, 2019. Combined supplementation of *Lactobacillus fermentum* and *Pediococcus acidilactici* promoted growth performance, alleviated inflammation, and modulated intestinal microbiota in weaned pigs. BMC Veterinary Research, 15. https://doi.org/10.1186/s12917-019-1991-9

Wanner M, Walker W, Sutter HM, Riond JL and Broz J, 1991. Influence of dietary citric acid and calcium on the bioavailability of orally administered chlortetracycline in piglets. Zentralbl Veterinarmed A, 38, 755–762. https://doi.org/10.1111/j.1439-0442.1991.tb01075.x

Weber NR, Pedersen KS, Hansen CF, Denwood M, Hjulsager CK and Nielsen JP, 2017. Batch medication of intestinal infections in nursery pigs. A randomised clinical trial on the efficacy of treatment strategy, type of antibiotic and bacterial load on average daily weight gain. Preventive Veterinary Medicine, 137, 69–76. https://doi.org/10.1016/j.prevetmed.2016.12.018

Wenner BA, Zerby HN, Boler DD, Gebreyes WA and Moeller SJ, 2013. Effect of mannan oligosaccharides (Bio-Mos) and outdoor access housing on pig growth, feed efficiency and carcass composition. Journal of Animal Science, 91, 4936–4944. https://doi.org/10.2527/jas.2013-6582

Whittle G, Shoemaker NB and Salyers AA, 2002. Characterization of genes involved in modulation of conjugal transfer of the *Bacteroides* conjugative transposon CTnDOT. Journal of Bacteriology, 184, 3839. https://doi.org/10.1128/JB.184.14.3839-3847.2002

Widodo N, Wihandoyo Zuprizal and Dono ND, 2018. The effect of dietary Binahong [*Anredera cordifolia* (Ten.) Steenis] leaf meal supplementation on total ileal bacteria and jejunal histomorphology in broiler chickens. International Journal of Poultry Science, 17, 473–478. https://doi.org/10.3923/ijps.2018.473.478

Wieser MF, Preston TR, Macdearmid A and Rowland AC, 1966. Intensive beef production. 8. The effect of chlortetracycline on growth, feed utilisation and incidence of liver abscesses in barley beef cattle. Animal Production, 8, 411–423. https://doi.org/10.1017/S0003356100038095

Williams BJ, 1985. The effects of neomycin and oxytetracycline alone or combined upon the incidence of salmonellosis in broiler chickens. Poultry Science, 64, 1455–1457. https://doi.org/10.3382/ps.0641455

Williams HE, Tokach MD, Dritz SS, Woodworth JC, DeRouchey JM, Amachawadi RG, Nagaraja TG and Goodband RD, 2017. 172 Effects of feeding probiotic or chlortetracycline or a combination on nursery pig growth performance. Journal of Animal Science, 95, 81–82. https://doi.org/10.2527/asasmw.2017.12.172

Williams HE, Tokach MD, Dritz SS, Woodworth JC, DeRouchey JM, Nagaraja TG, Goodband RD, Pluske JR, Chitakasempornkul K, Bello NM and Amachawadi RG, 2018. Effects of chlortetracycline alone or in combination with direct fed microbials on nursery pig growth performance and antimicrobial resistance of fecal *Escherichia coli*. Journal of Animal Science, 96, 5166–5178. https://doi.org/10.1093/jas/sky370

Wilson DN, 2009. The A-Z of bacterial translation inhibitors. Critical Reviews in Biochemistry and Molecular Biology, 44, 393–433. https://doi.org/10.3109/10409230903307311

Winther L, Honoré Hansen S, Baptiste KE and Friis C, 2011. Antimicrobial disposition in pulmonary epithelial lining fluid of horses, part II Doxycycline. Journal of Veterinary Pharmacology Theory, 34, 285–289. https://doi.org/10.1111/j.1365-2885.2010.01229.x

Won S, Moniruzzaman M, Lee S, Hong J, Park J-K, Kim S and Bai SC, 2017. Evaluation of dietary natural mineral materials as an antibiotic replacer on growth performance, non-specific immune responses and disease resistance in rainbow trout, *Oncorhynchus mykiss*. Aquaculture Research, 48, 4735–4747. https://doi.org/10.1111/are.13295

Won S, Hamidoghli A, Choi W, Park Y, Jang WJ, Kong I-S and Bai SC, 2020. Effects of *Bacillus subtilis* wb60 and *Lactococcus lactis* on growth, immune responses, histology and gene expression in nile tilapia, *Oreochromis niloticus*. Microorganisms, 8. https://doi.org/10.3390/microorganisms8010067

Woods GT, Jensen AH, Gossling J, Rhoades HE and Nickelson WF, 1972. The effect of medicated feed on the nasal microflora and weight gain of pigs. Canadian Journal of Comparative Medicine: Revue Canadienne De Medecine Comparee, 36, 49–54.

Xiao D, Tang Z, Yin Y, Zhang B, Hu X, Feng Z and Wang J, 2013. Effects of dietary administering chitosan on growth performance, jejunal morphology, jejunal mucosal sIgA, occluding, claudin-1 and TLR4 expression in weaned piglets challenged by enterotoxigenic *Escherichia coli*. International Immunopharmacology, 17, 670–676. https://doi.org/10.1016/j.intimp.2013.07.023

Xu N, Fu Y, Cheng B, Liu Y, Yang Q, Dong J, Yang Y, Zhou S, Song Y and Ai X, 2020. The pharmacokinetics of doxycycline in channel catfish (*Ictalurus punctatus*) following intravenous and oral administrations. Frontiers Veterinary Science, 7. https://doi.org/10.3389/fvets.2020.577234

Yakubchak OM, Zabarna IV, Taran TV, Prosaniy SB and Holovko NP, 2018. Indicators of broiler chickens' slaughter after Pharmazin (R) and Tilotsiklinvet (R). Ukrainian Journal of Ecology, 8, 649–653. https://doi.org/10.15421/2018_262

Yan R, Hui A, Kang Y, Zhou Y and Wang A, 2019. Effects of palygorskite composites on growth performance and antioxidant status in broiler chickens. Poultry Science, 98, 2781–2789. https://doi.org/10.3382/ps/pez070

Yang CJ, Yang IY, Oh DH, Bae IH, Cho SG, Kong IG, Uuganbayar D, Nou IS and Choi KS, 2003. Effect of green tea by-product on performance and body composition in broiler chicks. Asian-Australasian Journal of Animal Sciences, 16, 867–872. https://doi.org/10.5713/ajas.2003.867

Yang F, Li ZL, Shan Q and Zeng ZL, 2014. Pharmacokinetics of doxycycline in tilapia (*Oreochromis aureus* × *Oreochromis niloticus*) after intravenous and oral administration. Journal of Veterinary Pharmacology and Therapeutics, 37, 388–393. https://doi.org/10.1111/jvp.12095

Yang W, Moore IF, Koteva KP, Bareich DC, Hughes DW and Wright GD, 2004. TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. J Biol Chem, 279, 52346–52352. https://doi.org/10.1074/jbc.M409573200

Yeh HS, Weng BC and Lien TF, 2011. Effects of Chinese traditional herbal medicine complex supplementation on the growth performance, immunity and serum traits of pigs. Animal Science Journal, 82, 747–752. https://doi.org/10.1111/j.1740-0929.2011.00897.x

Yi H, Wang L, Xiong Y, Wen X, Wang Z, Yang X, Gao K and Jiang Z, 2018. Effects of *Lactobacillus reuteri* LR1 on the growth performance, intestinal morphology, and intestinal barrier function in weaned pigs. Journal of Animal Science, 96, 2342–2351. https://doi.org/10.1093/jas/sky129

Yin QQ, Chang J, Zuo RY, Chen LY, Chen QX, Wei XY, Guan QF, Sun JW, Zheng QH, Yang X and Ren GZ, 2010. Effect of the transformed *Lactobacillus* with phytase gene on pig production performance, nutrient digestibility, gut microbes and serum biochemical indexes. Asian-Australasian Journal of Animal Sciences, 23, 246–252. https://doi.org/10.5713/ajas.2010.90372

Young LG, Forshaw RP and Smith GC, 1973. Simplified diets based on barley for reproducing swine. Journal of Animal Science, 37, 898–905. https://doi.org/10.2527/jas1973.374898x

Yousif MH, Li J-H, Li Z-Q, Alugongo GM, Ji S-K, Li Y-X, Wang Y-J, Li S-L and Cao Z-J, 2018. Low concentration of antibiotics modulates gut microbiota at different levels in pre-weaning dairy calves. Microorganisms, 6, 118. https://doi.org/10.3390/microorganisms6040118

Yu M, Zhang C, Yang Y, Mu C, Su Y, Yu K and Zhu W, 2017. Long-term effects of early antibiotic intervention on blood parameters, apparent nutrient digestibility, and fecal microbial fermentation profile in pigs with different dietary protein levels. Journal of Animal Science and Biotechnology, 8. https://doi.org/10.1186/s40104-017-0192-2

Yuangklang C, Wensing T, Broek LVD, Jittakhot S and Beynen AC, 2005. Effect of oxytetracycline supplementation on nutrient digestibility in veal calves. Animal Feed Science and Technology, 118, 161–166. https://doi.org/10.1016/j.anifeedsci.2004.08.015

Zamora GM, Meléndez LAD, Hume ME and Vázquez RS, 2017. Performance, blood parameters, and carcass yield of broiler chickens supplemented with Mexican oregano oil. Revista Brasileira de Zootecnia, 46, 515–520. https://doi.org/10.1590/S1806-92902017000600006

Zeineldin M, Megahed A, Burton B, Blair B, Aldridge B and Lowe JF, 2019. Effect of single dose of antimicrobial administration at birth on fecal microbiota development and prevalence of antimicrobial resistance genes in piglets. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.01414

Zhang L, Li J, Yun TT, Li AK, Qi WT, Liang XX, Wang YW and Liu S, 2015. Evaluation of pilot-scale microencapsulation of probiotics and product effect on broilers. Journal of Animal Science, 93, 4796–4807. https://doi.org/10.2527/jas.2015-9243

Zhang JY, Baek DH and Kim IH, 2019. Effect of dietary supplemental medium chain fatty acids instead of antibiotics on the growth performance, digestibility and blood profiles in growing pigs. Journal of Animal Physiology and Animal Nutrition, 103, 1946–1951. https://doi.org/10.1111/jpn.13175

Zhang W-X, Zhang Y, Zhang X-W, Deng Z-X, Liu J-X, He M-L and Wang H-F, 2020. Effects of dietary supplementation with combination of tributyrin and essential oil on gut health and microbiota of weaned piglets. Animals, 10. https://doi.org/10.3390/ani10020180

Zhao X, Li L, Luo Q, Ye M, Luo G and Kuang Z, 2015. Effects of mulberry (*Morus alba* L.) leaf polysaccharides on growth performance, diarrhea, blood parameters, and gut microbiota of early-weanling pigs. Livestock Science, 177, 88–94. https://doi.org/10.1016/j.livsci.2015.03.001

Zhou X, Jin E, Li S, Wang C, Qiao E and Wu G, 2015. Effects of dietary supplementation of probiotics (*Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus natto*) on broiler muscle development and meat quality. Turkish Journal of Veterinary and Animal Sciences, 39, 203–210. https://doi.org/10.3906/vet-1406-67

Zhu C, Lv H, Chen Z, Wang L, Wu X, Zhang W, Liang R and Jiang Z, 2017. Dietary zinc oxide modulates antioxidant capacity, small intestine development, and jejunal gene expression in weaned piglets. Biological Trace Element Research, 175, 331–338. https://doi.org/10.1007/s12011-016-0767-3

Zinkl JG, Hurt JJ, Hyland JM, Dey N, Studnicka D and King DD, 1977. Treatment of captive giant Canada geese affected by avian cholera. Journal of Wildlife Diseases, 13, 294–296. https://doi.org/10.7589/0090-3558-13.3.294

Zinn RA, 1986. Short-term effect of antibiotic feeding on site and extent of digestion of growing and finishing diets in feedlot cattle. Journal of Animal Science, 63, 2013–2017. https://doi.org/10.2527/jas1986.6362013x

Zinn RA, 1993. Influence of oral antibiotics on digestive function in Holstein steers fed a 71% concentrate diet. Journal of Animal Science, 71, 213–217. https://doi.org/10.2527/1993.711213x

Ziółkowski H, Madej-Śmiechowska H, Grabowski T, Jaroszewski JJ and Maślanka T, 2019. Hard water may increase the inhibitory effect of feed on the oral bioavailability of oxytetracycline in broiler chickens. Polish Journal of Veterinary Sciences, 22, 251–258. https://doi.org/10.24425/pjvs2019.127093

Zulkifli I, Abdullah N, Azrin MN and Ho YW, 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. British Poultry Science, 41, 593–597. https://doi.org/10.1080/713654979

Abbreviations

ADF	acid-detergent fibre
ADG	average daily gain
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AQ	assessment question
AST	aspartate aminotransferase
BCS	body condition score
BW/bw	body weight
CF	crude fat
Con A	concanavalin A
CP	crude protein
CVMP	EMA Committee for Medicinal Products for Veterinary Use
DAO	oxidase
DM	dry matter
DMI	dry matter intake
EMA	European Medicines Agency
EMEA	see EMA
EUCAST	database for a certain antimicrobial
EUCAST	European Committee on Antimicrobial Susceptibility testing
F:G	feed conversion ratio or feed to gain ratio
FA	fatty acids
FARSC	Feed Antimicrobial Resistance Selection Concentration
FE	feed efficiency
F	fraction of the antimicrobial that is absorbed from the digestive tract to the blood
FI	feed intake
FM	fresh matter
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
GE	gross energy
GH	growth hormone
GHRH	growth hormone-releasing hormone
GOT	glutamic oxaloacetic transaminase
GPT	glutamic pyruvic transaminase
GSH-Px	glutathione peroxidase
HDL	high-density lipoprotein
I	fraction of the antimicrobial present in the digestive tracts that would be inactive on the microbiota
LDL	low-density lipoprotein
LPS	lipopolysaccharide
MDA	malondialdehyde
MIC	minimum inhibitory concentration
MIC_{lowest}	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)
MICres	minimum inhibitory concentration of the resistant strain
MICsusc	minimum inhibitory concentration of the susceptible strain
MICtest	minimum inhibitory concentration of the susceptible isolate used in the competition experiments to calculate the MSC
MPO	myeloperoxidase
MRL	maximum residues limit
MSC	selective concentration
MT	muscular thickness
NBT	respiratory burst activity
NDF	neutral-detergent fibre
Nrf2	nuclear factor erythroid 2-related factor 2

OCLN	occludin
OM	organic matter
PCV	packed cell volume
PER	protein efficiency ratio
PK	pharmacokinetic(s)
PMSC	predicted MSC
PUFA	polyunsaturated fatty acids
PUFA/SFA	PUFA to saturated fatty acid ratio
RBC	red blood cells
SCFA	short-chain fatty acid
SGR	specific growth rate
SOD	superoxide dismutase
SR	survival rate
T3	triiodothyronine
T4	thyroxine
T-AOC	total antioxidant capacity
TBARS	thiobarbituric acid-reactive substances
TBA	thiobarbituric acid value
TLR	toll-like receptor
ToRs	terms of reference
TP	total protein
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
T-SOD	superoxide dismutase
VFA	volatile fatty acids
VL	villi length
VSI	viscero-somatic index
wt	wild type
ZO-1	zonulin-1

Appendix A – Table of uncertainties

Uncertainty analysis specific for tetracycline. chlortetracycline, oxytetracycline and doxycycline

A.1. Uncertainties associated with the FARSC calculation

Table A.1: Potential sources of uncertainty identified in the estimation of the maximum concentrations of tetracyclines in non-target feed that would not select for antimicrobial resistance in the rumen or large intestines and assessment of the impact that these uncertainties could have on the conclusion

Source or location of the uncertainty	Nature or cause of uncertainty as described by the experts	Impact of the uncertainty on the determination of the Feed Antimicrobial Resistance Selective Concentration (FARSC)
Estimation of PMSC data		
	Limited MSC data from competition experiments. MSC data is available for *S. enterica*.	This limitation was overcome by the PMSC approach. Nevertheless, this could lead to an overestimation of FARSC if a bacteria with a lower MIC is described.
	Data extrapolation from tetracycline to the other tetracyclines was performed. It is a reasonable assumption to consider that MSCs are similar if the different antimicrobials within a class share similar MICs, mechanism of action and resistance.	This could lead to an overestimation or underestimation of FARSC if new data pointing for differences will be available.
	Impact of bacterial community complexity on the MSCs values. It is a reasonable assumption to consider that MSCs are similar if the different antimicrobials within a class share similar mechanism of action and resistance.	If this assumption is not correct, the PMSC, and accordingly the FARSC, could either be over or underestimated, depending on the specific species and the targeted community.
Antimicrobial pharmacokinetic and degradation data		
	The percentage of inactive tetracycline was extracted from human data and applied to other species.	The percentage of inactive drug can be higher or lower depending on the digestive content leading to potential over or underestimation of FARSC. So, other simulations were made with other values for binding to determine the range of FARSC that could be obtained.
	With the exception of one old study data that suggests that inactivation in the distal part of the intestines could be high, the percentage of inactive doxycycline was not found. Simulations were performed considering absence (0) and $I = 0.7$.	The assumption used for inactivation determinations might lead to underestimation or overestimation of FARSC if inactivation would occur at different levels than the ones considered.

Source or location of the uncertainty	Nature or cause of uncertainty as described by the experts	Impact of the uncertainty on the determination of the Feed Antimicrobial Resistance Selective Concentration (FARSC)
	The average values for bioavailability were extracted from literature for each species.	The complete range of possible individual values for bioavailability was not explored even if additional simulations were performed. These values could be higher or lower and thus, the FARSC could be over or underestimated.
	For doxycycline, the intestinal elimination of the parent drug was described as very low by EMEA, but high in two old publications. The selected value was derived from EMEA position. Since the EMEA reported that doxycycline was mainly excreted in faeces, mostly in a microbiologically inactive form, the *GE* was set to 0.	There is potentially an intestinal elimination of the parent drug. However, the influence on FARSC would be limited due to low bioavailability for most species. The FARSC could be overestimated.

DOX: doxycycline; EMEA: currently, European Medicines Agency (EMA); FARSC: Feed Antimicrobial Resistance Selection Concentration; *I*: inactive fraction, fraction of antimicrobial that would not have any activity on bacteria; *GE*: Gastrointestinal elimination, fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream; MSC: minimal selective concentration; PMSC: predicted minimal selective concentration.

A.2. Uncertainties associated with the growth promotion assessment

Table A.2: Potential sources of uncertainty identified in the levels of tetracycline 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 A.3: Potential sources of uncertainty identified in the levels of chlortetracycline 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), although meta-analysis was not applicable to the studies retrieved, evidence synthesis was done, since: • 19 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 on 21 studies, 19 showing positive effect and 2 showing no effects; • 11 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 19 studies, 11 showing positive effect and 7 showing no effects and 1 showing negative effects; • 17 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 27 studies, 17 showing positive effect and 10 showing no effects. For cattle for fattening, calves, lambs for fattening, sows and laying hens and fish the low number of studies retrieved prevented evidence synthesis.	The extent of the underestimation or overestimation on the levels which shown growth-promoting effect is modulated by the consistency of the results.

Table A.4: Potential sources of uncertainty identified in the levels of oxytetracycline 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), although meta-analysis was not applicable to the studies retrieved, evidence synthesis was done, since: • 15 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 26 studies, 15 showing positive effect and 11 showing no effects; • 9 studies showing consistent (positive) results in a comparable range of concentrations were available in fish. The uncertainty resulting in the process of evidence synthesis was based on 11 studies, 9 showing positive effect and 2 showing no effects. For pigs for fattening, weaned piglets, growing Guinea fowls, Japanese quail, the low number of studies retrieved prevented evidence synthesis.	The extent of the underestimation or overestimation on the levels which shown growth-promoting effect is modulated by the consistency of the results.

Appendix B – List of excluded publications and their shortcomings

B.1. Tetracycline

The publications excluded from the assessment of the effects of tetracycline on growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue) are summarised in Table B.1.

Table B.1: Publications not relevant for the assessment of the effects of tetracycline 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)**
Abdul-Aziz and Weber (1999)	Poultry	X			X		X	X		
Abu-Ruwaida et al. (1995)	Poultry	X				X			X	
Berge et al. (2005)	Ruminants	X					X		X	
Berge et al. (2009)	Ruminants	X			X		X			
Dabrowski and Poczyczyński (1988)	Fish	X			X				X	
Das (2004)	Ruminants			X				X		
Ekperigin et al. (1983)	Poultry	X					X	X		
Fox (1980)	Various		X		X				X	
Gazdzinski and Julian (1992)	Poultry				X		X			
Goren et al. (1988)	Poultry	X			X	X				
Hays (1977)	Pigs, Poultry	X	X		X				X	
Jukes (1971)	Various							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)
Lema and Nahashon (2006)	Ruminants				X					
Lerner et al. (1968)	Rabbit							X		
Li et al. (2019b)	Ruminants	X						X		
Mane (2010)	Ruminants			X	X			X		
Marking et al. (1988)	Fish				X				X	
Martin (1985)	Ruminants				X			X	X	
Natsir et al. (2017)	Poultry								X	
NRC (1980)								X	X	
Okerman et al. (1990)	Rabbit	X			X	X	X			X[1]
Peterson et al. (1991)	Poultry				X		X			
Purwanti et al. (2019)	Poultry	X				X		X		
Roy et al. (1991)	Poultry				X					
Roura et al. (1992)	Poultry	X					X			
Saha and Ray (1998)	Fish				X			X		
Samanta et al. (2015)	Pigs							X		
Shrimpton et al. (1958)	Poultry							X		X[2]

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)
Slyamova et al. (2016)	Poultry							X	X	
Widodo et al. (2018)	Poultry							X		
Yousif et al. (2018	Ruminants	X						X		
Zinkl et al. (1977)	Poultry	X			X			X		

(1): Small number of animals per group.
(2): Antimicrobial was administered 24 h before death as a mean to reduce carcass spoilage.

B.2. Chlortetracycline

The publications excluded from the assessment of the effects of chlortetracycline on growth/production yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue) are summarised in Table B.2.

Table B.2: Publications not relevant for the assessment of the effects of chlortetracycline on growth promotion/production 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)
Alexopoulos et al. (2003)	Pigs					X	X			
Aluko et al. (2017)	Pigs					X	X			
Bains (1974)	Poultry	X			X		X			X(1)

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)
Banerjee et al. (2018)	Poultry	X						X		
Betancourt et al. (2014)	Poultry						X			
Bhandari et al. (2008)	Pigs	X				X	X			
Braude and Johnson (1953)	Pigs									X(2)
Bridge et al. (1982)	Pigs	X								
Burch et al. (1986)	Pigs	X					X		X	
Burnell et al. (1988)	Pigs	X								X(1)
Çelýk et al. (2003)	Poultry									X(3)
Cernicchiaro et al. (2016)	Ruminants	X								X(4)
Che et al. (2017)	Pigs	X				X				
Clawson et al. (1955)	Pigs								X	
Colby et al. (1950)	Ruminants								X	
Connor and Neill (1971)	Poultry									X(1)
Del Castillo et al. (2002)	Pigs			X	X				X	X(5)
Dritz et al. (2002)	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)
Duff et al. (2000)	Ruminants				X					X[(6)]
Duttlinger et al. (2019)	Pigs	X								
Eckerman et al. (2011)	Ruminants	X								
Edmonds et al. (1985)	Pigs	X							X	
Feldpausch et al. (2014a)	Pigs									X[(7)]
Feldpausch et al. (2014b)	Pigs									X[(7)]
Fomenky et al. (2017)	Ruminants	X								
Foreman et al. (1961)	Ruminants								X	
Freeman et al. (1975)	Poultry									X[(8)]
Furr et al. (1968)	Ruminants								X	
Furusawa (2001)	Poultry								X	X[(9)]
Gadberry et al. (2014)	Ruminants								X	
Gallo and Berg (1995)	Ruminants	X								
Gebru et al. (2010)	Pigs					X				
George et al. (1977)	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)**
Gibb et al. (2006)	Ruminants				X					X[1]
Gibb et al. (2008)	Ruminants	X								X[1]
Gottlob et al. (2004)	Pigs	X								
Hahn et al. (2006)	Pigs	X								
Hamid et al. (2019)	Poultry	X								
Han and Thacker (2009)	Pigs	X								
Han and Thacker (2010)	Pigs	X								
Hansen et al. (1954)	Poultry					X				
Harper et al. (1983)	Pigs	X			X					
Hathaway et al. (1996)	Pigs	X								
Hathaway et al. (1999)	Pigs	X							X	
Hathaway et al. (2003)	Pigs	X								
Hersom et al. (2015)	Ruminants								X	
Hill et al. (1993)	Ruminants				X				X	
Holt et al. (2011)	Pigs									X[1]

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)
Hu and McDougald (2002)	Poultry				X	X				
Hu et al. (2019)	Pigs								X	
Huang et al. (2012)	Pigs	X								
Islam et al. (2008)	Poultry	X								X[(1)]
Johnson and Lay (2017)	Pigs	X								
Johnson et al. (1956)	Ruminants				X		X			
Jones et al. (1978)	Poultry									X[(10)]
Keegan et al. (2003)	Pigs	X								
Keegan et al. (2005)	Pigs	X								X[(11)]
Kiarie et al. (2018)	Pigs	X								
Kim et al. (2005)	Pigs						X		X	
Ko et al. (2008)	Pigs									X[(12)]
Kratzer et al. (1994)	Poultry	X								
Kulshreshtha et al. (2017)	Poultry					X			X	
Lang et al. (1959)	Ruminants				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)
Larsen et al. (1988)	Poultry									X(13)
Lee et al. (2011b)	Pigs	X								
Li et al. (2008)	Pigs	X								
Li et al. (2011)	Pigs	X								
Li et al. (2012)	Pigs	X								
Li et al. (2018)	Poultry									X(13)
Lien et al. (2007)	Pigs	X								
Maneewan et al. (2011)	Pigs						X			
McOrist et al. (1999)	Pigs					X				
Motl et al. (2005)	Poultry	X								
NCR-89 (1984)	Pigs	X			X					
Nyachoti et al. (2012)	Pigs	X			X	X			X	
Oe and Arakawa (1975)	Poultry	X				X				
Ohe and Arakawa (1976)	Poultry					X				
Oliver et al. (2014)	Pigs	X								
Olson (1977a)	Poultry				X	X				
Olson (1977b)	Poultry				X	X				
Olson and Sahu (1976)	Poultry								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)
Oso et al. (2019)	Poultry						X			
Patel and Baker (1996)	Poultry	X								
Patterson et al. (2019)	Pigs	X								
Perry et al. (1986)	Ruminants				X		X			
Phelps et al. (1987)	Poultry	X								
Piva et al. (2007)	Pigs	X							X	X(1)
Powley et al. (1981)	Pigs	X								
Puls et al. (2019a)	Pigs	X								
Puls et al. (2019b)	Pigs	X								
Radecki et al. (1988)	Pigs	X								
Rae et al. (2002)	Ruminants								X	
Ran et al. (2019)	Ruminants	X								
Redden et al. (2010)	Ruminants	X								X(14)
Robbins et al. (2013)	Pigs					X				
Rollins et al. (1976)	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)
Rossi et al. (2008)	Pigs	X								
Rueff et al. (2019)	Pigs	X			X					X(1)
Rumsey et al. (1999)	Ruminants							X		
Rusoff et al. (1959)	Ruminants				X				X	
Sacristán et al. (2012)	Pigs				X		X			X(1)
Saloma et al. (1970)	Poultry									X(15)
Sandhu and Dean (1980)	Poultry					X		X		
Shelton et al. (2009a)	Pigs	X								
Shon et al. (2005)	Pigs								X	
Shor et al. (1959)	Ruminants								X	X(16)
Shrimpton et al. (1958)	Poultry							X		X(17)
Singh et al. (1985)	Poultry								X	
Skinner et al. (2014)	Pigs				X		X		X	
Sotak et al. (2010)	Pigs	X					X			
Souza et al. (2018)	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)**
Steidinger et al. (2008)	Pigs	X								
Steidinger et al. (2009)	Pigs	X								
Stipkovits et al. (1999)	Poultry	X			X			X		
Stipkovits et al. (2001)	Pigs	X				X				
Suchy et al. (2008)	Poultry									X(18),(19)
Swanson (1963)	Ruminants						X		X	
Szasz et al. (2019)	Ruminants				X		X			X(1)
Tang et al. (2012)	Pigs					X				
Thaler et al. (1989)	Pigs	X								X(1)
Thongsong et al. (2008)	Poultry								X	
Thomson et al. (2014)	Ruminants	X								X(1),(20)
Unno et al. (2015)	Pigs	X						X		X(21)
Vandersall et al. (1957;	Ruminants							X	X	X(2)
Veum et al. (1980)	Pigs	X								
Waldroup et al. (1981)	Poultry									X(1)

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)
Walsh et al. (2007)	Pigs	X								
Wang et al. (2006)	Pigs	X								
Wang et al. (2017)	Pigs									X(1)
Wieser et al. (1966)	Ruminants								X	X(22)
Williams et al. (2017)	Pigs							X		X(18)
Woods et al. (1972)	Pigs	X	X							X(21)
Xiao et al. (2013)	Pigs				X	X				
Yan et al. (2019)	Poultry									X(1)
Yang et al. (2003)	Poultry						X			
Yeh et al. (2011)	Pigs	X								
Yi et al. (2018)	Pigs	X								
Yin et al. (2010)	Pigs									X(13)
Young et al. (1973)	Pigs	X								
Zhang et al. (2019)	Pigs	X								
Zhou et al. (2015)	Poultry	X								
Zhu et al. (2017)	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)
Zinn (1986)	Ruminants	X			X					X(13)
Zinn (1993)	Ruminants	X			X					

(1): Absence of a negative control group without antimicrobial.
(2): Low number of animals in the experiment.
(3): Very high levels of aflatoxins in feed.
(4): The study is a meta-analysis.
(5): The focus of the study was the pharmacokinetics of chlortetracycline and oxytetracycline.
(6): The design of this study was not appropriate to test performance/yield.
(7): This study is a conference paper and it is the same as Feldpausch et al. (2018), Experiment 2, and therefore, it should not be considered twice.
(8): Animals challenged with adrenocorticotrophic hormone three times weekly.
(9): Designed to study the transfer of antimicrobials to eggs.
(10): The study aimed to test a cholera vaccine and the chlortetracycline was given before the experiment started.
(11): The relevant trial, Experiment 3, is the same as study of Keegan et al. (2003a).
(12): The study appears to contain the same animals (and some results) as in Hossain et al. (2012a).
(13): No replication.
(14): Diets of the experiment not comparable.
(15): Study based on the intermittent (i.e. not continuative over the whole experimental period) use of the antimicrobial.
(16): Old paper (1959) aimed at defining the dietary level of antimicrobial that produces residues in milk. Very short duration (14 days). Many confounding factors: error in dosage during the trial, sick animals, negative effect of hot season.
(17): The study deals with the prevention of carcass spoilage.
(18): It is the abstract of the study of Williams et al. (2018).
(19): Chlortetracycline was administered only during the last 8 days of trial (total duration 52 days), at a high dosage (2 g/10 kg BW) and with the aim of producing liver toxicity.
(20): Animals treated with an estrogen-trenbolone.
(21): Insufficient replication.
(22): All animals implanted with hexoestrol.

B.3. Oxytetracycline

The publications excluded from the assessment of the effects of oxytetracycline on growth/production yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue) are summarised in Table B.3.

Table B.3: Publications not relevant for the assessment of the effects of oxytetracycline on growth promotion/production 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)
Abdelhamid et al. (2009)	Fish									X(1)
al-Ankari and Homeida (1996)	Poultry							X		
Alonge et al. (2017b)	Poultry				X			X		
Azam and Narayan (2013)	Crustaceans			X						
Battaglene et al. (2006)	Fish	X								
Bray et al. (2006)	Crustaceans				X					
Bergstrom et al. (2007a)	Pigs	X								
Bergstrom et al. (2007b)	Pigs	X								
Bergstrom et al. (2007c)	Pigs	X								
Bónai et al. (2008)	Rabbit	X								
Bónai et al. (2010)	Rabbit	X								
Bovera et al. (2010)	Rabbit	X					X			X(2)
Chansiripornchai (2009)	Poultry				X	X				
Cunha et al. (2017)	Rabbit	X					X		X	X(2)
Cusack (2004)	Ruminants				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)
Dean et al. (1973)	Poultry				X	X	X		X	
Del Castillo et al. (2002)	Pigs			X	X				X	X(3)
Dennis et al. (2019)	Ruminants	X								
Dobsikova et al. (2013)	Fish									
Donovan et al. (2002)	Ruminants	X								
Dritz et al. (1993)	Pigs	X							X	X(2)
Dritz et al. (2002)	Pigs	X								
El-Deek et al. (2012a)	Poultry								X	X(4)
El-Deek et al. (2012b)	Poultry								X	X(4)
El-Tahawy and El-Shafey (2015)	Fish									
Fonseca et al. (2005)	Rabbit									X(2)
Franti et al. (1971)	Poultry							X	X	
Frantz et al. (2004)	Pigs	X								
Furusawa (2001)	Poultry								X	X(5)
Gaikowski et al. (2003)	Fish					X				
Geidam et al. (2015)	Poultry					X	X			
Glisson et al. (2004)	Poultry				X	X	X			
Gottlob et al. (2004)	Pigs	X							X	
Gottlob et al. (2005a)	Pigs								X	
Gottlob et al. (2005b)	Pigs	X							X	
Gottlob et al. (2005c)	Pigs								X	
Gottlob et al. (2006a)	Pigs	X							X	
Gottlob et al. (2006b)	Pigs	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)
Gottlob et al. (2007)	Pigs								X	
Greenfield et al. (1973)	Poultry	X			X	X				
Hegazy et al. (2018)	Poultry				X	X				
Heinrichs et al. (2003)	Ruminants	X								
Heinrichs et al. (2009)	Ruminants	X								
Hildabrand et al. (2004)	Pigs	X								
Hong et al. (2004)	Pigs	X							X	X[(2),(6)]
Humam et al. (2019)	Poultry				X	X				
Hustvedt et al. (1991)	Fish				X					X[(2),(7)]
Islam et al. (2015)	Fish							X		
Kareem et al. (2015)	Poultry	X								
Katya et al. (2018)	Fish					X				
Keegan et al. (2003)	Pigs	X								
Keegan et al. (2005)	Pigs	X								
Kehoe and Carlson (2015)	Ruminants	X								
Kim et al. (2014)	Fish				X	X				
King (1968)	Poultry							X		X[(8)]
Koh et al. (2016)	Fish					X				
Kovacs et al. (2009)	Rabbit	X								
Larsen et al. (2016)	Pigs				X		X			
Lee et al. (2009)	Pigs	X								
Lee et al. (2011b)	Pigs	X								
Lee et al. (2016)	Fish					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)
Lee et al. (2018a)	Fish					X				
Lee et al. (2018b)	Fish					X				
LeMieux et al. (2003)	Pigs	X								
Lien et al. (2007)	Pigs	X					X			
Limbu et al. (2018)	Fish				X				X	X(9)
Limbu et al. (2019)	Fish				X					
Limbu et al. (2020)	Fish				X					
Loh et al. (2010)	Poultry	X								
May et al. (2012)	Pigs	X								
Mazón-Suástegui et al. (2016)	Other aquatic animals			X		X				
Mosleh et al. (2016)	Poultry				X	X			X	X(10)
Neill et al. (2004)	Pigs	X								
Neill et al. (2005)	Pigs	X								
Neill et al. (2006)	Pigs	X								
Neveling et al. (2020)	Poultry				X	X				
Ogunwole et al. (2011)	Poultry									X(11)
Ologhobo et al. (2014)	Poultry									X(2)
Onifade (1997)	Poultry	X								
Onifade and Babatunde (1997)	Poultry	X								
Oso et al. (2013)	Rabbit				X					
Pérez et al. (2011)	Pigs	X								X(2)
Peterson et al. (1991)	Poultry				X		X			
Puls et al. (2019a)	Pigs	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)
Purushothaman et al. (2014)	Poultry	X								X[(2)]
Quigley et al. (1997)	Ruminants	X			X	X				
Quigley and Drew (2000)	Ruminants	X								
Ramezanzadeh et al. (2020)	Fish				X				X	
Rhee et al. (2018)	Fish								X	
Salaheen et al. (2017)	Poultry	X								
Serafin (1982)	Poultry								X	
Shaddad et al. (1985a,b)	Poultry								X	X[(12)]
Shelton et al. (2009b)	Pigs	X								
Shields et al. (2010)	Ruminants	X			X					
Shrimpton et al. (1958)	Poultry									X[(9)]
Sinclair et al. (1990)	Poultry	X								
Smith et al. (1964)	Pigs								X	X[(2)]
Soler et al. (2016)	Pigs							X		X[(10)(13)]
Sokoudjou et al. (2019)	Poultry				X	X				
Steidinger et al. (2008)	Pigs	X								
Steidinger et al. (2009)	Pigs	X								
Subagja et al. (1999)	Fish			X						
Sukandhiya et al. (2016)	Poultry								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)
Sulabo et al. (2007)	Pigs	X								
Szasz et al. (2019)	Ruminants	X			X		X			X(2)
Thanh et al. (2009)	Poultry	X							X	
Toften and Jobling (1997)	Fish				X					
Touchburn and Nestor (1971)	Poultry	X						X	X	
Vandonkergoed (1992)	Ruminants									X(14)
Vernon et al. (1962)	Pigs	X							X	
Veum et al. (1980)	Pigs	X								
Waldroup et al. (1981)	Poultry	X			X					X(2)
Walker-Love et al. (1959)	Pigs						X			X(15)
Williams (1985)	Poultry				X	X				
Yeh et al. (2011)	Pigs	X								
Yu et al. (2017)	Pigs	X								
Zeineldin et al. (2019)	Pigs			X						X(16)
Zhang et al. (2020	Pigs	X								

(1): The antimicrobial was not administered to fish but used for an *in vitro* study.
(2): No untreated control group.
(3): Short-term study in pigs on the tolerance and palatability of high doses of oxytetracycline. The pharmacokinetics of chlortetracycline and oxytetracycline was studied.
(4): Low number of animals per replicate.
(5): Designed to study the transfer of antimicrobials to eggs.
(6): Antimicrobial diet vs diets with SDEP (spray-dried egg protein). Low number of animals (4 pens of 3 pigs/treatment).
(7): Short-term (6-h) study on the tolerance of therapeutic use in trout.
(8): Old outdated study (1968). The design does not allow to examine the results of the effects of oxytetracycline alone.
(9): Antimicrobial was administered 24 h before death as a mean to reduce carcass spoilage.
(10): Low number of animals.

(11): Inadequate study design, including the selection of animals for carcass evaluation.
(12): No replicates.
(13): The study focused on blood parameters and proteome.
(14): The article is a meta-analysis.
(15): Animals were also exposed to UV irradiation.
(16): Single-dose administration at birth.

B.4. Doxycycline

The publications excluded from the assessment of the effects of doxycycline on growth promotion/increase yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue) are summarised in Table B.4.

Table B.4: Publications not relevant for the assessment of the effects of doxycycline on growth promotion/increase 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)**
Bosi et al. (2011)	Pigs								X	
Bousquet et al. (1998)	Pigs				X		X			
Conejos et al. (2012)	Pigs							X[1]		X[2]
George et al. (1977)	Poultry				X	X				
Goma et al. (2018)	Poultry									X[3][4]
Goren et al. (1988)	Poultry	X			X	X				
Gutierrez et al. (2012)	Poultry									X[5]
Isikwenu and Udomah (2015)	Poultry	X								X[6]

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)**
Kyriakis et al. (2002)	Pigs				X		X			
Pavlova et al. (2018)	Poultry				X				X	X(3)
Saleh et al. (2018)	Poultry	X								
Salichs et al. (2013)	Pigs				X		X			X(6)
Santos et al. (1997)	Poultry									X(6)
Weber et al. (2017)	Pigs									X(6)
Yakubchak et al. (2018)	Poultry	X								X(6)

(1): Only digestibility and intestinal morphology/bacteria were reported.
(2): Small number of animals per treatment.
(3): No replicates.
(4): The study investigated doxycycline-overdose induced toxicity.
(5): Single administration. The study investigated pharmacokinetics after different administration routes (water bolus vs parenteral).
(6): No untreated control group.