Poultry production methods and use of coccidiostats with special reference to coccidial and bacterial resistance and possible future alternatives

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Advisory memorandum from DCA – Danish Centre for Food and Agriculture

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Data sheet

Title:	Poultry production methods and use of coccidiostats with special reference to coccidial and bacterial resistance and possible future alternatives
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Commissioned by:	Government Agency
Date for request/submission:	24-04-2020, revised 08-07-2020/ 04.06.2021
File no.:	2020-0101360
Funding:	This memorandum has been prepared as part of the "Framework Agreement on the Provision of research-based Policy Support" between the Danish Ministry of Food, Agriculture and Fisheries (MFVM) and Aarhus University (AU) according to ID H3-11. "Performance Agreement Animal Production 2020-2023".
Eksternal contributions:	The review was conducted by Copenhagen University
To be cited as:	Engberg, RM. 2021. Poultry production methods and use of coccidiostats with special reference to coccidial and bacterial resistance and possible future alternatives. 16 pages. Advisory memorandum from DCA – Danish Centre for Food and Agriculture, Aarhus University, submitted: 04.06.2021.
Policy support from DCA:	Read more here https://dca.au.dk/raadgivning/

Background and task

Conventional poultry production including the production of broilers is characterised by high intensity, where birds selected for high body weight gain, high feed efficiency and a high breast meat yield are raised at a high stocking rate. These conditions require adequate biosecurity and disease control in order to avoid disease outbreaks and economic losses. The control of infections with *Eimeria spp.* (coccidiosis) and related intestinal bacterial infection is crucial in order to maintain gut health, bird welfare and consequently production results.

In conventional broiler production, coccidiosis control is provided by coccidiostatica which are drugs that are added to poultry feed to minimize disease and production losses related to the infection with coccidia *(Eimeria* spp.). Currently, these substances are considered feed additives and are therefore regulated by Regulation (EC) No 1831/2003 on additives for use in animal nutrition. Article 11 of this regulation lays down that the Commission shall submit a report to the European Parliament and the Council regarding the use of coccidiostats and histomonostats as feed additives with a view to a decision on the phasing out of the use of these substances as feed additives by 31 December 2012. The report of the commission (Commission of the European Communities, 2008) concluded that the use of coccidiostats as a preventive measure for the control of coccidiosis in modern poultry production is essential, that alternatives currently do not offer the same advantages as the use of coccidiostats as feed additives and that the regulatory framework (Regulation (EC) No 1831/2003) can be considered as working properly.

However, in a recent position paper, the Federation of Veterinarians of Europe (2016), recommends that coccidiostats should be under veterinary prescription following clinical examination and diagnosis. This would allow for better surveillance and the veterinarian to diagnose and choose the best strategy to extend the useful life of coccidiostats, such as through 'shuttle use' or 'rotational use' or the use of vaccines. Additionally it would allow more frequent reporting of any adverse reactions seen, including lack of efficiency, ensure withdrawal periods are respected and could allow monitoring through the the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) monitoring system.

The aim of the present note is, to describe current practises in poultry production with respect to management and feeding and the use of coccidiostats addressing specifically the risk of coccidial and bacterial resistance to coccidiostats with antimicrobial effect (ionophore coccidiostats). Finally, available alternatives to the use of ionophore coccidiostats are described.

Coccidia

Coccidia are microscopic, spore-forming, single-celled eukaryote parasites of the subclass Coccidiasina. Unless otherwise noted in this note, the term "coccidia" is used to describe coccidia of the genus Eimeria which can infect poultry. Coccidia are obligate intracellular parasites with a quite complex lifecycle (Figure 1) involving asexual and sexual formation within the host intestinal cells. It is the intracellular stages that may lead to an intestinal injury, which may exceed beyond immunization of the infected chicken at high infection levels. The product of the sexual formation is the oocyst which is shed with the faeces and sporulates (sporogony) outside the host in the litter to become infective. *Eimeria* spp. have a narrow host spectrum, meaning that coccidial species that can infect chickens can not infect turkeys, and vise versa.

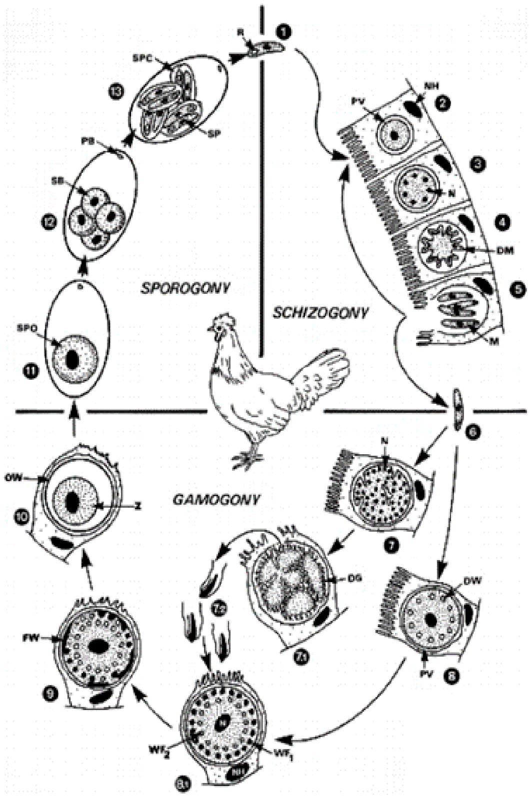

Figure 1. *Life cycle of* Eimeria *spp. in chickens*
1 After oral uptake of sporulated oocysts the sporozoites hatch in the small intestine from the sporocysts. *2–6* After penetration, multinucleate schizonts are formed (**3**) inside a parasitophorous vacuole (*PV*). The schizonts produce motile merozoites (*DM, M*), which may initiate another generation of schizonts in other intestinal cells (**2–5**) or become gamonts of different sex (**7, 8**). *7* Formation of multinucleate microgamonts, which develop many flagellated microgametes (**7.1-7.2**). *8* Formation of uninucleate macrogamonts, which grow to be macrogametes (**8.1**) that are characterized by the occurrence of two types of wall-forming bodies (*WF_1, WF_2*). *9* After fertilization the young zygote forms the oocyst wall by consecutive fusion of both types of wall-forming bodies (*FW*). *10* Unsporulated oocysts are set free via faeces. *11–13* Sporulation (outside the host) is temperature dependent and leads to formation of four sporocysts, each containing two sporozoites (*SP*), which are released when the oocyst is ingested by the next host. Adopted from Peek (2010).

Coccidiostats and their mode of action

Coccidiostats are drugs added to poultry feed that serve to retard the life cycle or reduce the population of pathogenic coccidia to the point that disease is minimized and the host develops immunity. According to the register of approved feed additives (Table 1), the following 11 types of coccidiostats are currently approved within the EU (European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 Annex I: List of additives). These are: Narasin, lasalocid sodium, monensin sodium, salinomycin sodium, maduramycin ammonium, semduamicin sodium, robenidine hydrochloride, diclazuril, decoquinate, halofuginon and nicarbacin. Basically three categories of anticoccidial products are currently used in poultry production (Peek and Landman, 2011).

a. *Synthetic compounds*. These compounds are produced by chemical synthesis and are often referred to as 'chemicals'. Synthetic drugs usually have a specific mode of action against the parasite metabolism. There are products affecting cofactor synthesis, e.g. the folate pathway, or affecting the mitochondrial function of the parasite. However, the precise mode of action of some synthetic coccidiostats, e.g. diclazuril, halofuginone, nicarbaczin and robenidine, is still not yet completely clarified (Noack et al., 2019).

b. *Polyether antibiotics or ionophores*. These products are produced by the fermentation of Streptomyces spp. or Actinomadura spp. and destroy coccidia by interfering with the balance of important ions like sodium and potassium. Polyether antibiotics influence the transport of mono- or divalent cations (Na^{+}, K^{+}, Ca^{++}) across cell membranes inducing osmotic damage. The following groups of ionophores exist: Monovalent ionophores (monensin, narasin and salinomycin). Monovalent glycosidic ionophores (maduramicin and semduramycin). Divalent ionophores (lasalocid).

c. *Mixed products.* These products consist of two components, e.g. a synthetic compound and ionophore (nicarbazin/narasin; nicarbacin/monensin sodium).

It is important to note that in contrast to synthetic coccidiostats, most ionophores inhibit growth of Gram-positive bacteria in the digestive tract of poultry (Table1).

Table 1. Active substances of coocidiostats currently registered as feed additives within the EU (European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003) and their antibacterial activity (adopted from VKM report, 2015)

Type	Active substance	Antibacterial activity
Ionophore coccidiostats	Monensin sodium	Mainly active against Gram-positive bacteria
	Salinomycin sodium	Active against Gram-positive bacteria
	Maduramycin ammonium	Active against Gram-positive bacteria
	Semiduramycin sodium	Has limited antibacterial activity
	Lasalocid sodium	Active against Gram-positive bacteria
	Narasin	Mainly active against Gram-positive bacteria
Synthetic coccidiostats	Nicarbazin	No known antibacterial activity
	Diclazuril	No substantial antibacterial activity
	Robenidine hydrochloride	No known antibacterial activity
	Halofuginon hydrobromide	No known antibacterial activity
	Decoquinate	No substantial antimicrobial activity

Poultry production practices in Denmark

As the turkey production with only 20 producers in 2019 in Denmark (Danmarks statistik) is very small, in the following major attention is focused on broiler production.

In Danmark, the genetical line Ross 308 (Aviagen) has been preferentially used in conventional broiler production. However, slower growing broilers lines, i.e. Ranger Gold (Aviagen) and Hubbard/Cobb have recently been introduced. Figures adopted from Danmarks Statistik and Statistics of the Danish poultry branch (Fjerkræbranchens årstatistik) reveal that during the period from 2008 to 2019, over 100 million broilers are slaughtered in Denmark annually (Figure 1). The highest number of broilers slaughtered during this period was in 2010 with approximately 208 million birds. In the period from 2010 to 2019, a decrease in the number of slaughtered broilers was evident with the lowest numbers (approx. 95 mio broilers) slaughtered in 2015, where conventional broiler production faced disease challenges resulting in increased mortality, increased carcass condemnations at slaughter and increased use of antibiotics for therapy, which otherwise is very low in Danish broiler production. (Det Danske Fjerkræråd, Årsberetning 2015). After 2015, the number of slaughtered broilers increased again by 8.86% (Fjerkræbranchens Årsstatistik 2020).

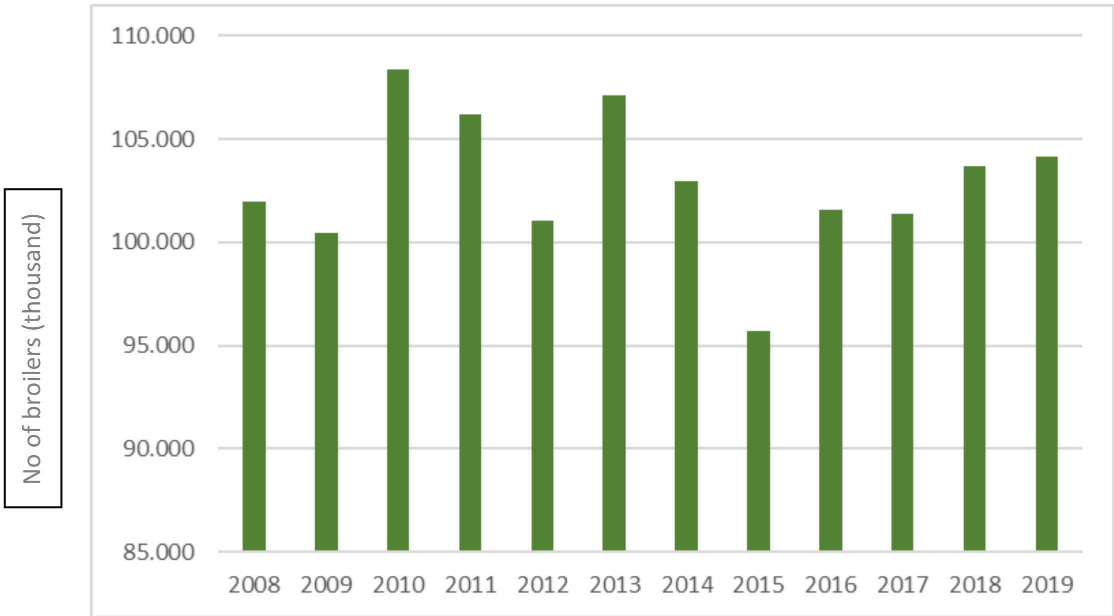

Figure 1. Total number of slaughtered broilers in Denmark (thousand) (Danmarks statistik)

The weight of produced broilers reflects the number of slaughtered broilers (Figure 2). However, the body weight at slaughter (Figure 3) has increased quite considerably from 2016 to 2020 from an average of 2131g to 2348g, which corresponds to a body weight increase of 217g or 10% which is remarkable.

It is obvious that the feed conversion ratio (kg feed/kg broiler) has improved significantly during recent years (Figure 4). From 2014 to 2020, feed conversion ratio decreased from 1.6 kg feed/kg chicken to 1.5 kg/kg chicken. Both the increase in body weight gain and the improvement of feed conversion ratio are a result of intense breeding. The number of chickens placed /m^2 has decreased through the years (Figure 5), which is in accordance with the increased target weight of the broilers. In Danmark, a stocking density of up to 40 kg/ m^2 is permitted provided that bird welfare is not negatively affected (control at the slaughter house or at the production site) and mortality is low (Lov om hold af slagtekyllinger, 2002).

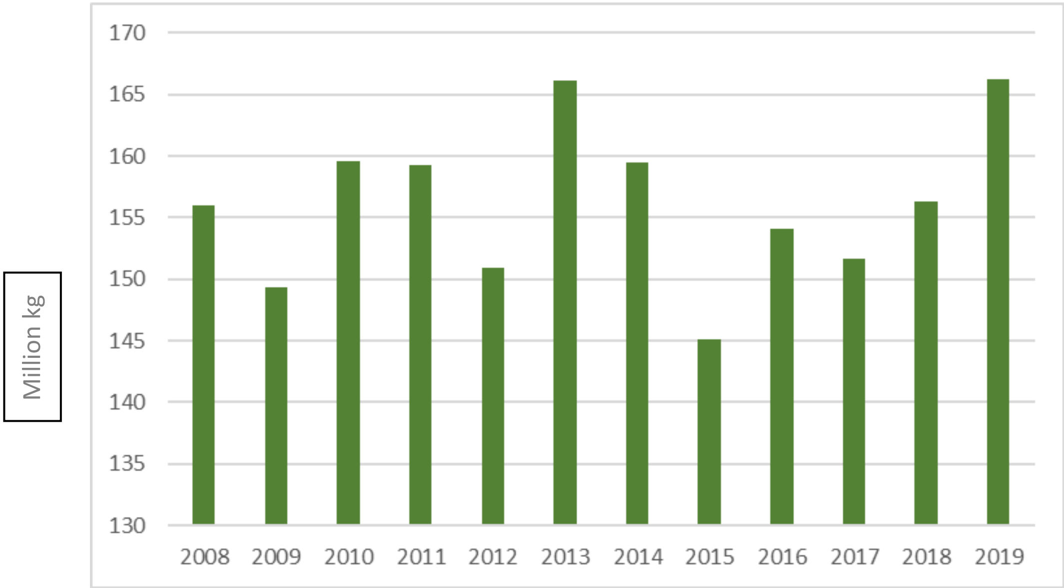

Figure 2. Production of broiler meat in Denmark (mio. kg) (Danmarks statistik)

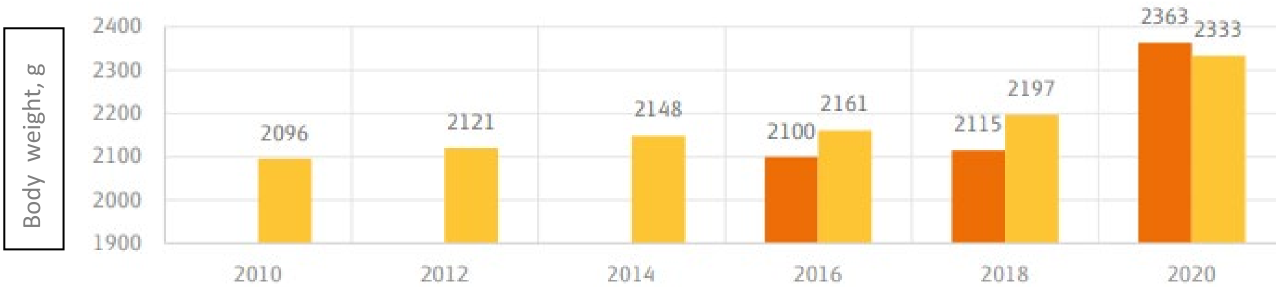

Figure 3. Body weight (g) of broilers at slaughter (Fjerkræbranchens Årsstatistik 2020)
From 2016 values are shown separately for broiler houses up to 7 years old (orange colums) and broiler houses being at least 8 years old (yellow colums).

Generally, broiler production is a very intensive production form, where birds reach their slaughter weight in just 35 days. From the figures it appears that selection for increased body weight and improved feed conversion ratio has been very successful. However, the achievement of these breeding goals do come with health risks, in particular negative consequences for intestinal health. Accoring to Ducatelle *et al.,* 2018, the intensive selection for higher daily weight gain and lower feed conversion ratio has generated breeds that are characterized by an extremely high feed intake. Excessive amounts of feed may put considerable stress on the digestive system. Passed a certain threshold, even in the absence of any specifc pathogens, this may damage the health status of the GI tract, leading to partial loss of function. Although conventional broilers are exposed to light programes which provide dark periods allowing the birds to rest thus controlling feed intake, the digestive tract is still exposed to large amounts of feed.

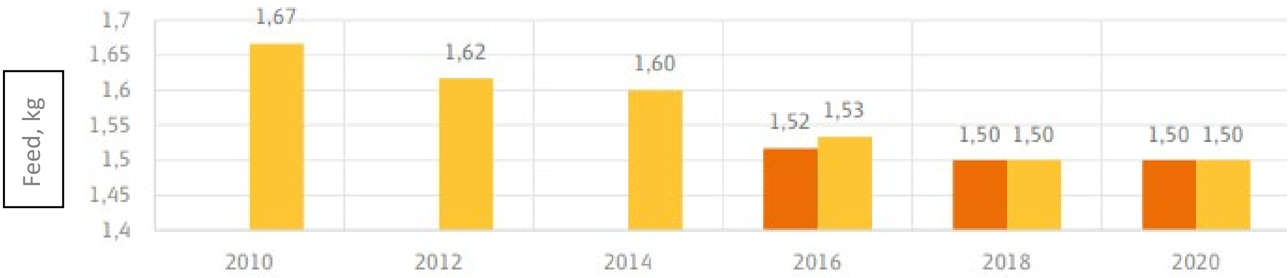

Figure 4. Feed gain ratio, kg feed /kg broiler (Fjerkræbranchens Årsstatistik 2020)
From 2016 values are shown separately for broiler houses up to 7 years old (orange colums) and broiler houses being at least 8 years old (yellow colums).

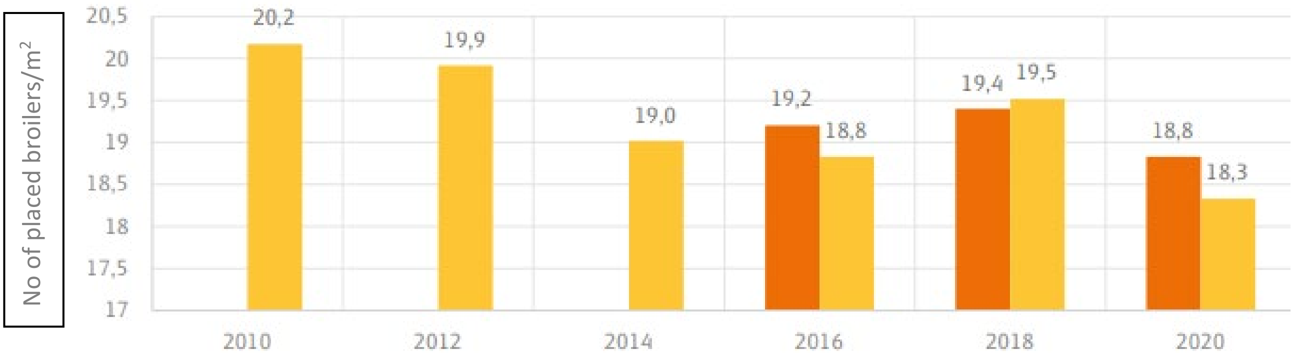

Figure 5. Number of broilers placed /square meter (Fjerkræbranchens Årsstatistik 2020)
From 2016 values are shown separately for broiler houses up to 7 years old (orange colums) and broiler houses being at least 8 years old (yellow colums).

Another factor contributing to the pressure on the intestinal tract is the feed management and the physical form of the feed. In order to achieve an average weight of 2.3 kg in just 35 days, it is important that the birds are offered a feed balancend with respect to protein, amino acids and energy content. Conventional broilers are fed *ad libitum* with pelleted diets to avoid sorting of feed particles and to support efficient nutrient uptake. However, pelleted diets have the disadvantage that they are dissolved rapidly, pass the proventriculus and gizzard ard arrive quickly in the upper small intestine. These means that the function of the gizzard in regulating the filling of the small intestine is circumvented (Engberg *et al*., 2004; Bjerrum *et al*., 2005) and a bulk of nutrients arrives in the intestine to be used as nutrients by the host, but also as substrate for both commensals and pathogenic bacteria, which then will increase in number.

Gut health has become an increasingly important issue.The control of infections with *Eimeria spp.* (coccidiosis) and related bacterial diseases is crucial in order to maintain gut health, bird welfare and consequently production results under these intensive production conditions. For this reason, the largest chicken abattoir in Denmark (Danpo) has decided to phase out the very fast growing chicken by the end of 2021 aiming at the production of slower growing birds, i.e. Ranger Gold.

Consumption of coccidiostats in Denmark

As shown above, conventional broiler production in Europe and Denmark is characterized by highly intensive production conditions with regard to stocking density, growth rate and feeding management which require adequate biosecurity and disease control in order to avoid disease outbreaks and economic losses. In the control of coccidiosis, ionophore coccidiostats are the most extensively used drugs in poultry production within the EU (Noak et al., 2019) In accordance with this, a glance at the historical and current consumption of coccidiostats in Denmark (Vet-STAT) clearly reveals that from 2008 to 2020, ionophores are by far the preferred coccidiostats in poultry production (Table 2). Taking only the currently approved coccidiostats into account, ionophores present approximately 98% of the applied coccidiostats. The most extensively used ionophore coccidiostat in Denmark through all the years has been salinomycin sodium (60.3%), followed by the combination of narasin/nicarbacin (ionophore and synthetic coccidiostat) (27.7%), then narasin (6.3%), lasalocide sodium (3.4%) , and monensin sodium (2.2). Monovalent glycosidic ionophores (maduramicin and semduramycin) are used to a very low extent (0.02%). Syntethic coccidiostats (diclazuril, halofuginon nicarbazin and decoquinate) alone are generally very rarely used and present only 0.12 % of the applied coccidiostats. The pattern of coccidiostat consumption has not really changed between 2008 and 2020. It seems that salinomycin and narasin/nicarbacin substitute each other, which means, in years where salinomycin use is lower, the consumption of nicarbacin/narasin increases correspondingly. However, it has to be noted that the general coccidiostat use has increased considerably from 17,733 kg in 2008 to 26,335 kg in 2020, which corresponds to an increase by 48.5%. The reason for this increase is not immediately clear. When comparing for instance the years 2013 and 2019, it shows that in 2013, 107 million broilers were slaughtered corresponding to 166 mio kg and a coccidiostat consumption of 22,368 kg. In 2019, 104 million broilers were slaughtered corresponding to 166 million kg and a total coccidiostat consumption of 27,441 kg. In this connection, it has to be noted that turkeys are extremely sensitive to salinomycin and narasin (Markiewicz et al. 2014) which can cause severe intoxications with high flock mortality. Therefore, in Denmark coccidiosis control in turkey production, is managed by dietary supplementation of either monensin sodium or lasalocid (Andres Katholm, DLG, personal communication). Another category of birds that consume coccidiostats are gallinaceus game birds reared for hunting, e. g. pheasants, which are supplemented with lasalocid to control coccidiosis (Andres Katholm, DLG, personal communication). This means that salinomycin, narasin/nicarbazine and narasin are exclusively applied in broiler production.

Table 2. Total consumption of coccidiostats (kg) in Danish poultry production (adopted from Vet-Stat)

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total
E757 monensin sodium	489	640	622	683	503	327	347	441	576	546	487	564	439	6.664
E714 monensin sodium				98	86									183
E26 salinomycin sodium	4													4
E766 salinomycin sodium	14.978	16.405	16.293	12.428	12.280	11.429	11.466	11.864	12.371	13.527	14.875	16.756	18.864	183.536
51766 salinomycin													721	721
E770 maduramacin ammonium							26	6		4	3	1	3	43
E773 semiduramicin sodium							1					2		4
51763 lasalocid sodium													130	130
E763 lasalosid sodium	1.177	1.178	539	497	1.060	546	584	771	670	670	814	867	877	10.251
E765 narasin	490	617	872	1.853		490	1.569	1.718	1.684	2.103	3.212	2.698	1.809	19.114
E772/51772 narasin/nicarbazin	593	348	2.994	8.538	8.798	9.574	9.341	10.981	9.632	6.932	6.879	6.549	3.485	84.645
E768 nicarbazin		3	3	8		1	2	8	4		10	2	4	45
E771 diclazuril		1	7	1						1			1	11
E764 halofuginon			1					0			1	1	.	3
E25 halofuginon	1							5	6	1				14
E756 decoquinate										2	304		2	309
Consumption total	17.733	19.190	21.331	24.105	22.726	22.368	23.338	25.794	24.943	23.786	26.586	27.441	26.335	305.675

Table 3 shows a rough index calculation between total consumption of coccidiostats (kg) according to the information of Vet-STAT (Table 2) and number of slaughtered broilers (million) from 2008 to 2019 (Danmarks statistik). This calculation reveals that the total consumption of coccidiostats has increased significantly, while the number of slaughtered birds has been quite stable. As salinomycin and narasin/nicarbacin by far make up the largest part of coccidiostats consumed in Denmark, it can be deduced that the use of ionophores, has increased considerably during the past 11 years.

Table 3. Calculation of index between consumption (kg) of coccidiostats (Table 2, VetStat) and number (millions) of slaughtered broilers (Danmarks statistik)

	2008	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**
Index coccidiostats	100	108.2	120.3	135.9	128.2	126.1	131.6	145.5	140.7	134.1	149.9	154.7
No. slaughtered broilers	102,0	100,5	108,4	106,2	101,1	107,1	102,9	95,7	101,6	101,4	103,7	104,2
Index slaughtered broilers	100	98.5	106.3	104.2	99.1	105.0	101.0	93.8	99.6	99.4	101.7	102.1

Why are ionophores the preferred coccidiostats?

A reason why ionophores or combinations of ionophores and synthetic coccidiostats are so extensively used in the field can be explained by the combined anticoccidal and antibacterial properties (Table 1) targeting primarily Gram-positive bacteria. Broiler production is challenged by problems related to gut health that most commonly occur in the production period from day 21 to day 28. *Eimeria* infection is considered a predisposing factor for necrotic enteritis, a severe intestinal infection caused by *Clostridium perfringens* Type A, an anaerobic Gram-positive bacterium producing potent toxins (alpha toxin and net B toxin). These toxins lead to necrosis of the mucosal membrane in the small intestine, resulting in severe lesions, which in turn reduces nutrient absorption causing significant weight loss. Another condition that can be observed at the same broiler age is the so-called dysbacteriosis, a not very well-defined bacterial infection of the small intestine (Teirlynck *et al*., 2011). In relation to these intestinal disorders, ionophore coccidiostats replace the formerly used antibiotic growth promoters. Although their mode of action is different, ionophores like antibiotic growth promoters target Gram-positive bacteria and improve wheight gain in poultry. The improved weight gain that is achieved by ionophore coccidiostats with antibacterial effect is not only due to their growth inhibiting effect of intestinal pathogens like *Clostridium perfringens*. Also growth of potentially beneficial commensal bacteria i.e, lactobacilli that colonize the small intestine of broilers in high numbers is reduced (Engberg *et al*., 2000), which balances nutrient consumption in favour of the host. Further, it has ben observed that the use of ionophores (salinomycin) in combination with an antibiotic growth promoter reduces bacterial bile salt de-conjugation. Bile salts in their conjugated form, (conjugated with taurine or glycine) play an important role in the emulsification of fat in the small intestine. A number of different Gram-positive commensal bacteria, i.e. enterococci, lactobacilli and *Clostridium perfringens* can de-conjugate bile salts (Knarreborg et al., 2002). When the number of these bacteria decreases, their activity will consequently be reduced, which in turn leads to an increased fat absorption (Knarreborg *et al*., 2004) and contributes to the observed increase in weight gain.

A further advantage of ionophore coccidiostats is that some of these compounds, e.g. salinomycin significantly reduce water intake, which has a positive influence on litter quality improving bird welfare as related to the occurrence of foot pad dermatitis. (Engberg et al., 2000). The preferential use of ionophore coccidiostats in the field clearly reflects the unique properties of ionophores in the control of gut health both with respect to parasitic and bacterial intestinal infections. Although substantial research efforts have been made during the last two decades to identify alternatives to ionophores, currently no other single feed additives or combinations of different feed additives are available that provide the same advantages (Granstad *et al*., 2020).

Resistance of *Eimeria spp*. against coccidiostats

The World Health Organization (WHO) defines drug resistance in antimalarial chemotherapy, which can also be applied to coccidiology, as 'the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject' (WHO, 1965). Generally, drug resistance in coccidia can be complete, in which case increasing doses up the maximum tolerated by the host is ineffective (i.e. diclazuril and nicarbazin). In contrast, relative resistance to anticoccidial drugs is characterized by the fact that increasing doses tolerated by the host still will show efficacy (i.e. ionophores) (Peek and Landman, 2011).

Resistance of *Eimeria* spp. against coccidiostats is a common phenomenon (Chapman et al., 1984). Drugs with a similar mode of action, such as ionophores, are likely to induce similar mechanism of resistance in *Emeria spp*., a phenomenon called cross-resistance. Cross-resistance between different monovalent ionophores has been reported (Chapman and Shirley, 1989; Marien et al., 2007; Chapman et al., 2010). However, in relation to the development of coccidial resistance in *Eimeria* spp., it has been shown that resistance against ionophores generally occurs much slower than that against synthetic coccidiostats (Chapman, 1984). Further, it has been suggested that ionophores prevent the clinical effects of coccidiosis while allowing birds to acquire natural immunity to *Eimeria* infections due to a low grade infection with coccidia (Chapman, 1999).

In order to avoid resistance, the use of so-called "shuttle programs" (shift from one coccidiostat to another within the same rotation), the use of combinations of ionophore and synthetic coccidiostat, or a so called "switch" (the change of coccidiostat from one rotation to the next rotation) are commonly applied in practice (DANMAP, 2015). However, looking at the consumption of different coccidiostats in Danmark (Table 3), it is obvious that the use of in particular salinomycin and the combination of narasin/nicarbacin by far outcompetes the use of other coccidiostats. It is suspected that the use of shuttle and switch programs may not be so common, and the same ionophores are continuously used over many rotations. A similar situation has been reported from Norway, where narasin has been almost exclusively used in broiler production for decades (VKM, 2015) before it was withdrawn from broiler production. In this relation, it should be noted, that the slower development of resistance is a another advantage of ionophore coccidiostats compared to synthetic coccidiostats and may further explain why ionophores are used so extensively in the field.

Currently, in Denmark, no systematic monitoring of resistance to coccidiostats is conducted in the field. This is due to the complex life cycle of of *Eimeria spp*. coccidia, which makes the assessment of their resistance quite laborious. Due to the lack of appropriate *in vitro* assays, resistance assessment currently requires *in vivo* passage/infection in the host/target animal. In practice, this means that the decision to shift to another coccidiostat is made empirically, and is based on observations such as gut health problems, poor feed conversion ratio, decreased flock uniformity and increased carcass condemnations at slaughter.

Resistance of bacteria to coccidiostats

The use of ionophore coccidiostats has generally been considered safe with regard to antibiotic resistance of bacteria, seen in light that these substances are currently not applied in human medicin for antibiotic therapy. Recent research indicates that ionophores may become a role in human cancer therapy (Naujokat and Steinhart, 2012).

As mentioned above, most ionophore coccidiostats inhibit growth of primarily Gram-positive bacteria in the digestive tract of poultry (Table 1). The continuous use of the same ionophore in the control of coccidiosis does therefore not only result in resistance development of *Eimeria* spp. but may also induce resistance in certain intestinal bacteria to these compounds.

The Panel on Animal Feed of the Norwegian Scientific Committee for Food Safety (VKM, 2015) concluded that enterococci (*Enterococcucus faecium* and *Enterococcus faecalis*) isolated from poultry fed with narasin, monensin and salinomycin may become resistant to these drugs.

Recent evidence suggests that ionophore use in some cases may co-select for resistance to vancomycin (Wong, 2019). Vancomycin is considered a critical antibiotic used in human medicine (WHO, 2019). In Denmark, resistance to vancomycin in isolates of *Enterococcucus faecium* and *Enterococcus faecalis* has increased through recent years (DANMAP, 2019). Further, blood infections in humans related to these types of enterococci appear likewise to be an increasing problem in Denmark in the period from 2012-2020, (Statens Serum Institut, Denmark, Statistik). However, whether or not the extensive use of ionophores contributes to bacterial antimicrobial resistance problems is not very well established. Nielsen *et al*. (2019) demonstrated a significant decrease in vancomycin-resistant intestinal bacteria in Swedish broilers which coincidences with an increased use of narasin. This finding does not really support the hypothesis of an association between selective pressure caused by ionophores and persistence of antimicrobial resistance vancomycin. However, Wong (2019) suggested that there might be an urgent need to systematically investigate the contribution of ionophores to the burdon of antimicrobial resistance. In line with this, the Federation of Veterinarians of Europe (2016), advises the inclusion of coccidiostats in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) ESVAC monitoring system.

It is concluded that on basis of the currently available literature, the question whether or not the extensive use of ionophores in coccidiosis control and prevention of related intestinal health problems contributes to the development of antimicrobial resistance can not be answered unequivocally.

Alternatives to ionophore coccidiostats

In relation to the control of coccidiosis, a number of commercially available attenuated (weakened) and non-attenuated vaccines exists. These vaccines are based on live sporulated oocysts of different *Eimeria* spp. Attenuated vaccines contain oocysts which are manipulated in a way that they are either less virulent, or that they have a shortenened life cycle, which ultimatievely results in a reduced number of oocysts produced after vaccination/infection (Peek and Landman, 2011). These vaccines can be applied to day-old chicks via spraying or via the drinking water. The alternating application of vaccines and coccidiostats in different rotations has been suggested to reduce the development of resistance against the coccidiostat (Peek and Landman, 2011; Kadykalo et al., 2018).

The production of these vaccines involves infection of live chickens for the propagation of the parasite. In this relation, it should be mentioned that these vaccines are specific to *Eimeria* spp. occurring in chickens and can therefore not be used in turkeys in the control of coccidiosis. Another drawback of these vaccines are their costs, which are significantly higher compared to the costs of ionophores. Further, dosage errors may result in insufficient immune response when the dose is too low, or in a significant damage of the intestinal mucosa or coccidiosis when too high doses are administered. The development of new and effective vaccine candidates are needed. Progress has been made towards identification of immunoprotective antigens that can be used in recombinant or vectored vaccines (Blake et al., 2017). According to Blake (2021), one major constraint in the development of such antigen-specific vaccines is an appropriate and effective delivery system. Several possible vectors for oral administration, including Bacillus, Salmonella, transgenic *Eimeria* and yeasts such as *Saccharomyces cerevisiae*, are currently in development and could be appropriate.

Currently in Denmark attenuated live vaccines are routinely applied to a large extent in pullets raised either for egg production or for parent flocks for the production of hatching eggs for broiler production. Further, organic broilers are vaccinated against coccidiosis.

However, as described above, it is not only coccidiosis but also bacterial pathogens, e.g. *Clostridium perfringens* and dysbiosis that have to be addressed in broiler production. Therefore, the use of vaccines solves the problem with coccidiosis but does not solve intestinal disorders related to bacteria. Currently, only Norway has experience in broiler production without ionophores, which upon consumer demand, were gradually phased out from 2015 to 2016 on a voluntary basis. During recent years different feed additives have been developed and are commercially available for broiler production. Among these products are prebiotics, probiotics, organic acids and phytogenics. Grandstad et al. (2020) investigated different commercially available feed additives with respect to their effect on production results and gut health in broilers challenged with a 10-fold overdose of an attenuated live vaccine against coccidiosis. The strength of this study is that it was carried out under identical experimental conditions for all the investigated products, which reduces the bias found in literature considering the effect of different alternative feed additives. The authors found that some products providing probiotics, prebiotics and organic acids improved performance results under these conditions. Some phytogenic additives improved feed conversion and showed growth inhibiting effect on *Clostridium perfringens*. However, none of the investigated products could compete with the ionophore narasin, which was used as positive control in the experiment.

In order to control coccidiosis and bacteria related intestinal problems in broiler production, a combination of different actions have to be taken, where biosecurity, disinfection strategy and the length of the period between rotations where the broiler houses are empty play an important role to decrease the infection pressure. As mentioned above, the use of highly efficient broiler breeds with exessive appetite, high stocking density and the current feeding management are very likely to contribute to intestinal problems in broiler production. Therefore, if ionophores should be phased out from broiler feed, it is very likely that we have to reconsider our management strategies including the choice of the breed.

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