Lohmann Information

Drinking water vaccination against *Salmonella* - a proven concept also in day-old chicks

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1 Introduction

Practical sanitation measures alone are usually not enough to protect poultry flocks against debilitating and costly diseases, especially if holdings are located in regions with high poultry densities (SCHOLTYSSEK, 1987). It has been found that prevention of infectious diseases in poultry is to a large extent a matter of good hygiene in conjunction with a customised vaccination programme.

The administration of poultry vaccines with the drinking water is a long-established and widely used form of vaccination. This method has several advantages: as well as being a relatively simple and quick way of administering the vaccine to a large number of animals it is completely stress-free for the chickens and cost-efficient for the farmer. Although "straightforward" at first glance, this route of administration requires great care in order to obtain satisfactory results.

In case of an inappropriate use of a live vaccine the uniformity of the immune response as measured by antibody titres for instance is less homogeneous after mass vaccination with the drinking water than after a carefully implemented individual vaccination of all birds by injection or eyedrop. This is partly due to variations in the water intake of individual birds but, more importantly, the potentially adverse effect of the water on the vaccine itself has to be considered. It is therefore essential to minimise the disadvantages of this form of vaccination in order to reap its undisputed benefits.

Drinking water vaccination is the preferred route of immunisation against *Salmonella* in particular because it mimics the natural route of the infection and thus provides rapid and powerful protection against these pathogens.

2 Drinking water vaccination - general considerations

As mentioned above, the drinking water is a convenient vehicle for delivering live vaccines to a large number of birds in a short time. This method is the preferred route for vaccines whose primary target organ is the digestive tract and its immune system (IBD, *Salmonella*). Due to an anatomical feature in the mouth of poultry, the choanal slit, the method is also suitable to some extent for vaccines that act mainly in the respiratory tract because the vaccine comes into direct contact with the mucous membranes of the upper airways.

The same fundamental considerations apply to both applications:

- All birds must have adequate access to drinkers during the limited time of the vaccination (drinking space).
- The amount of water provided must be sufficient for all birds (water requirement).
- The water must be of good quality and meet the needs of the chickens and of the vaccine (water quality).
- Vaccines must be mixed just prior to the vaccination (vaccine quality).
- The vaccine solution must not come into contact with disinfectants or other "harmful" chemicals (vaccine handling).

3 The vaccine

Salmonella live vaccines by LAH are intended for vaccination of chickens from day-old. The manufacturer recommends three vaccinations for laying hens, the first at day-old, followed by the second vaccination at six to eight weeks of age and the final vaccination at 16 to 18 weeks, at least three weeks before point of lay.

The vaccines induce an active immunity against *Salmonella* enteritidis and *Salmonella* typhimurium to the laying hens. This immunity results in reduced colonisation, invasion and excretion of *Salmonella* enteritidis and *Salmonella* typhimurium field strains, thus diminishing the potential risk to the consumer of becoming infected with the organisms via the food chain.

3.1 Calculating the required vaccine dose

The manufacturer's directions on vaccine vials usually contain information about the number of doses they contain. The rule is 1 dose per bird, regardless of age or type of production.

The recommended vaccine doses should never be reduced. 1000 doses must be used for 1000 birds. When in doubt, always round up (for instance 2750 birds should be vaccinated with 3000 doses if the smallest vial contains 1000 doses).

It is also essential to mix the correct volume per house, i.e. for three houses each holding 6000 birds prepare three lots of 6000 vaccine doses, not one lot of 18000 doses which is then distributed among the three houses. Dividing the vaccine solution between houses in this way leads to unequal amounts of antigen in the water and therefore to variable response rates.

3.2 Mixing the vaccine

Live vaccines have a limited shelf life. The storage life indicated on the label is only guaranteed if the vaccine is kept refrigerated in the absence of sunlight and the vacuum in the bottle is intact. As soon as the vaccine bottle is removed from the refrigerator and opened under water, the titre begins to decline. If the vaccine is to be given in the drinking water it must be dispersed in the water straight after mixing. The vaccine should be consumed within two hours after dilution.

Salmonella live vaccines are designed to have a reduced survival ability in the environment (which is absolutely essential to ensure user safety). The use of live vaccines therefore requires that certain precautions must be observed to prevent the vaccine from being damaged or destroyed even before it reaches the birds.

Live vaccines should always be mixed in a clean room, on a clean work surface and with clean equipment ("clean"

in this context also means “free of disinfectant residues”). Care must also be taken to ensure that the mixed vaccine solution does not come into direct contact with sunlight as UV light impairs the storage stability of live vaccines.

The following items of equipment are needed:

- Disposable gloves,
- Clean plastic buckets for mixing the vaccine solution,
- A plastic or stainless steel mixer (egg whisk),
- If the vaccine is added via the supply tank, a paddle for thorough mixing of water, milk and vaccine solutions,
- Skim milk powder (99 % fat-free, food quality) or skimmed milk (max. 0.5 % fat).

It is advisable that any equipment used for mixing the vaccines should be used only for this purpose, clearly labelled and stored separately. The addition of skimmed milk or skim milk powder to the drinking water improves the stability of vaccines in the drinking water and is therefore generally recommended in all drinking water vaccinations. It has been found that 2 g skim milk powder is equivalent to a use rate of 2 litres of skimmed milk per 1000 litres of drinking water, even if the dose rates when calculated on a dry matter basis differ by a factor of 10.

Recommendations for the addition of skimmed milk and skim milk powder to the drinking water:

- Skim milk powder 99 % fat-free (2 to 4 g/litre) or skimmed milk 0.1 % fat (2 to 4 ml/litre),
- Stir into cold water,
- Wait for 10 min,
- Then add the vaccine.

The vaccine solution is prepared as follows:

- Before starting, wash hands thoroughly with soap and water (no disinfectant!). The wearing of disposable gloves is highly recommended.
- In a clean bucket combine 5 to 10 litres of water with 2 to 4 grams of skim milk powder per litre, mix thoroughly and then leave to stand for 10 minutes.
- The required vials of vaccine are later dissolved in this water and the vaccine is thoroughly mixed in (the vials have to be opened under water to ensure that the vaccine pellet dissolves rapidly).
- If supply tanks are used as water reservoir the water in these tanks must also be mixed with a sufficient amount of skim milk powder (2 to 4 g skim milk powder/l or 2.0 to 4.0 l skimmed milk per 1000 l water).
- Finally, pour the vaccine solution into the supply tank or into the total volume of drinking water required.
- If a dose metering device is used, which continuously delivers a small amount of the vaccine solution to the drinking water, the situation is different. In this case the amount of skim milk powder or skimmed milk is calculated not with reference to the volume of vaccine solution but based on the total volume of drinking water to be administered. For example, if 5 litres of the vaccine solution is added to the drinking water at a rate of 5 %, i.e. mixed into 100 litres of drinking water, the vaccine solution should contain not 5 x 2 (to 4) grams of skim milk powder but 100 x 2 (to 4) grams.
- Empty vaccine vials must be properly disposed of (disinfection or autoclaving).

The equipment should be thoroughly cleaned after use, first with cold and then with hot water (>70 °C), dried and stored in a dry, clean and dust-free place until it is used again.

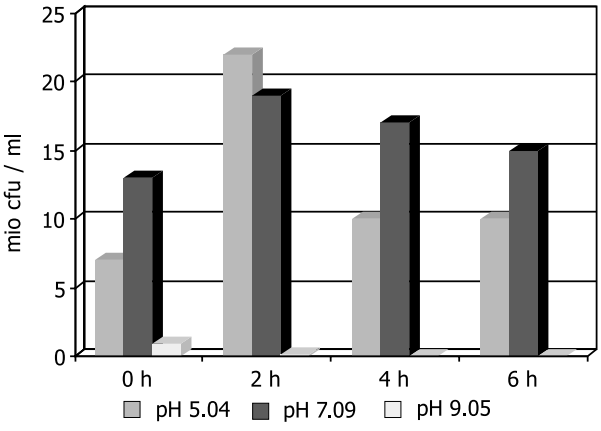
4 The water

The water used for drinking water vaccination is of special importance as transport medium for the vaccine. The critical requirements with regard to water quality are absence of undesirable substances such as heavy metals and organic matter and excellent bacteriological condition. Selection of the correct pH is also important, as are taste and odour, which must be acceptable to the birds to achieve the desired water intake.

It is advisable to use water of potable quality (eg as specified in the *German Drinking Water Order* of 21.05.2001) because it meets all the necessary requirements for water to be used for drinking water vaccination (the only exceptions are the nitrate and nitrite content of drinking water because these substances can impair the potency of live vaccines even at the maximum permitted concentration in drinking water) (BEHR et al., 2003). If the farm has its own water supply the water quality should be checked regularly in a suitable laboratory for hardness, salt content, nitrates and bacterial impurities.

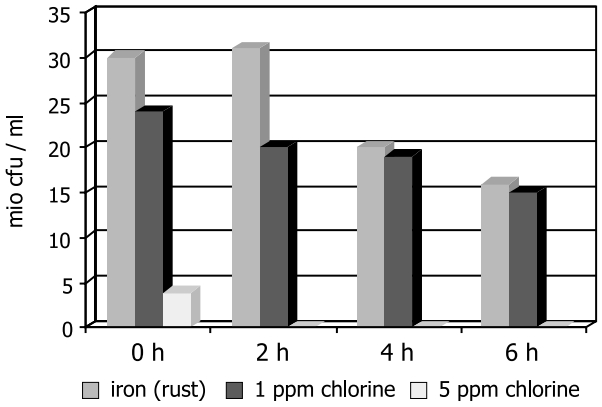
A high pH in the drinking water has an extremely deleterious effect on the stability of *Salmonella* live vaccines. Figure 1 shows that the vaccine strain is almost undetectable after just 2 hours at a pH of 9.05, whereas the organism was still clearly present after 6 hours in drinking water with an acid pH of 5.04. This is probably due to the fact that *Salmonella*, who are resident in the gut, have to withstand relatively low pH levels in the stomach and duodenum.

Figure 1: Stability of *Salmonella* live vaccine in drinking water at different pH levels (drinking water at room temperature)



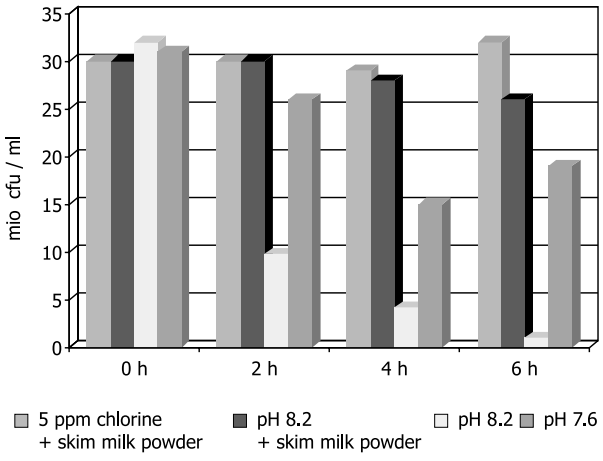
High concentrations of iron and chlorine also markedly reduce the survival rate of the vaccine strain in drinking water, as can be seen in Figure 2. As little as 5 ppm chlorine in the drinking water leads to instantaneous inactivation of the vaccine strain.

Figure 2: Stability of *Salmonella* live vaccine in drinking water of different quality (drinking water at room temperature)



As mentioned earlier, it is advisable to protect the vaccine by adding skimmed milk or skim milk powder to the drinking water. This recommendation also applies unreservedly to *Salmonella* live vaccines. Figure 3 shows that when skimmed milk was added the vaccine strain was stable for 6 hours, despite the presence of 5 ppm chlorine in the drinking water. Even the adverse effects of high pH levels can be partially neutralized in this way.

Figure 3: Effect of skim milk powder on the stability of *Salmonella* live vaccine in drinking water



5 Watering systems

Not every watering system is suitable for drinking water vaccination. Bell drinkers have proved successful for day-old chicks or nipple drinker systems for older birds. Open troughs are suitable only if the water does not flow out continuously. Drinkers and water lines must be thoroughly cleaned and disinfected prior to each new batch; hard and iron-containing water can clog up the drinking valves and nipples (install a filter, 40 to 50 micrometers).

The cleanliness of the watering system and absence of disinfectants, acids or other contaminants in drinkers and water lines are crucial to the success of the vaccination. It is good practice to refrain from chlorination or the use of any other water disinfectant, and the addition of acids, for two days prior to the proposed vaccination.

5.1 Bell drinkers

Bell drinkers are a suitable system for vaccination of day-old chicks. These drinkers are easy to clean, fill and install. When using bell drinkers for drinking water vaccination it is important to ensure that all birds can get to the drinkers during the two to three hours of the vaccination and can actually reach the water. The vaccine solution is mixed as described in chapter 3.1. In addition to the equipment for the preparation of the vaccine solution listed there, a measuring jug (1 litre capacity) is needed.

The estimation of the volume of drinking water required is based primarily on the number of bell drinkers as opposed to the number of chicks:

- The first step is to calculate the number of bell drinkers needed. According to guidelines in various management programmes and data from the literature, day-old chicks need between 0.5 and 2.5 cm of drinking space (Table 1). The number of birds per bell drinker can be calculated from the circumference of the drinker (diameter multiplied by 3.14) divided by the space requirement of one bird.
- The next step is to estimate the volume of water needed to fill the free water channel of the bell drinker that is accessible to the chicks. This is best done with a measuring jug.
- The volume of water thus determined is multiplied by the number of bell drinkers needed.
- The vaccine is then incorporated into the total calculated volume of water.

The amount of water provided is usually between 2 and 4 ml per chick, sufficient for two to three hours of drinking time.

Table 1: Drinking space requirements of chicks and laying hens (RAUCH and PETERSEN, 2003)

	Water channel space per bird (cm)	Birds per nipple
Week 1	0.5	6-7
Weeks 2-4	1.0	6-7
Weeks 5-8	1.5	5-6
Weeks 9-15	2.5	4-5
Weeks 16-20	3.0	3-5
Laying hens	3.0	2-6 (75-100 birds for each automatic circular drinker)

The recommendations by the state of Lower Saxony for the management of laying hens in deep litter and free-range systems (1997) propose the following drinking space allowances:

Troughs	- 2.5 cm of trough space per bird
Circular drinkers	- max. 80 birds per drinker (recomm.: 2.5 cm drinker space per bird)
Nipple drinkers	- max. 12 birds per nipple (recomm.: 6 birds per nipple)

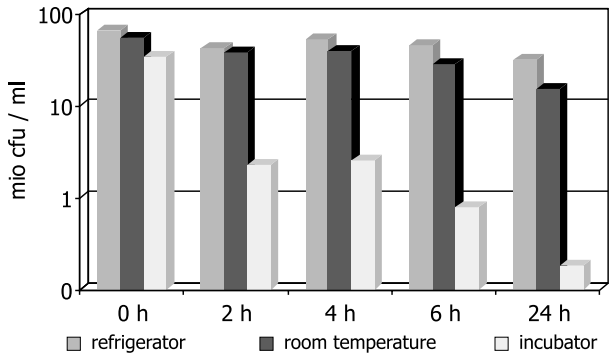
5.2 Nipple drinkers

Nipple drinker systems are widely used in the poultry industry. As regards drinking water vaccination, they are most suitable for vaccinating birds of more than 3 weeks old because that is the age when water intake becomes predictable. A problem in vaccination with "respiratory" vaccines, i.e. those that act mainly in the respiratory tract (IB, ND), is the often inadequate antigen contact with the mucous membranes of the respiratory tract. But the system is recommended for vaccination against *Salmonella*.

The water-vaccine mixture is prepared as described in chapter 3.1. A crucial factor is whether the vaccine solution is added to the drinking water via a tank or via a dose metering device because in one option the vaccine solution is added to the entire supply of drinking water prior to the actual vaccination and in the other option it is added during the vaccination.

Figure 4 shows that the stability of the vaccine is partially determined by the temperature of the drinking water. At higher temperatures the stability of the vaccine solution is reduced. It may therefore be necessary during the summer months or in hot regions of the world to cool the vaccine solution after mixing. If a dosing pump is used this can be done very simply by cooling the vaccine solution with ice cubes, but if a supply tank is used cooling is not such a simple matter.

Figure 4: Effect of drinking water temperature on the stability of *Salmonella* live vaccine



In either case the water is distributed in the barn through hosepipes and pipelines. At the beginning of the vaccination these often contain substantial amounts of (partly stagnant) drinking water. This water should be removed, preferably by flushing the entire watering system with vaccine solution (i. e. by opening the water line at the end until the milky-white vaccine solution comes through). To avoid disturbing the birds this is best done in the dark at dawn before they take their first drink.

6 Operation of the vaccination

Along with the choice of the right vaccine, the correct method of administration and proper mixing of the vaccine, the operation of the vaccination is of crucial importance.

It is often recommended that the amount of water used should be the volume that can be consumed by the birds in two hours. But chicks and laying hens have a two-peaked daily water intake rhythm, which often makes it difficult to estimate the amount of water needed. Literature data on the water requirement for drinking water vaccination also vary. To obtain reliable information on the amount of water to be used it is recommended to record the birds' water consumption hourly one day before a drinking water vaccination. A "practice run", i.e. a mock vaccination one day before the real vaccination, can also be an effective way of verifying the actual water consumption. If this is not possible the data in Tables 2 and 3 can be used for guidance:

Table 2: Water requirement of chicks in the first week of life in drinking water vaccination

Age	Amount of water (per bird)
2 - 3 days	3 ml
3 - 5 days	5 ml
5 - 7 days	7 ml

Table 3: Water requirement of broilers in drinking water vaccination (after JORDAN et al., 2001)

Age	Amount of water (1000 birds)
2 weeks	14 litres
3 weeks	21 litres
4 weeks	28 litres

The water requirement of commercial layers during grow-out can be estimated with the following formula:

Amount of water needed =
1 litre of water per 1000 birds x age in days

The water requirement peaks at 6 weeks (1 x 42 = 42 litres of water for 1000 birds); this volume is therefore also sufficient for birds aged 8, 10 or 12 weeks. Water consumption is highest at dawn, which is why the vaccination should take place at that time.

6.1 Primary vaccination

Salmonella live vaccines should be used from day-old. The earlier the vaccine is administered the greater the certainty that the immune system of the gut comes into close contact with the vaccine strain, leading to the development of specific cell-associated immunity. This is why the primary vaccination should take place immediately after arrival of the chicks on the farm.

It is good practice to fill the bell drinkers just before placing the chicks in the barn so that the birds can start drinking immediately. Chicks away from the water source should be encouraged to walk to the drinkers. Water and feed should not be offered simultaneously because it has been found time and again that food exerts a great attraction for many birds, distracting them from drinking water.

There are some exceptions to vaccination at day-old:

1. Birds premedicated with antibiotics cannot be vaccinated with *Salmonella* live vaccines at day-old. Gentamicin medication in conjunction with Marek vaccination for example prevents adequate colonisation of the vaccine strain on the first day. In this case it is necessary to wait several days to ensure the efficacy of the vaccine.

2. Occasionally day-old chicks arrive on the farm exhausted, especially on hot days or after travelling long distances. As these birds refuse to drink any water during the first few hours after arrival, it is preferable in this case either to vaccinate later or to choose an alternative route of administration for the vaccine.
3. Finally, sick birds or birds likely to need antibiotic medication in the first two to three days of life should not be vaccinated. In all these cases vaccination should be postponed until a later date.

6.2 Booster vaccinations

The vaccinations in weeks 6 to 8 and weeks 16 to 18 place fewer demands on management than the primary vaccination, but should still be performed with the necessary care. Water consumption is usually known exactly, the birds have become accustomed to a steady diurnal rhythm and the timing of the vaccination is also more variable than for the primary vaccination. But the rules mentioned earlier also apply for these vaccinations:

- Cleanliness when mixing the vaccine,
- Good water quality,
- No disinfectants in the drinking water,
- No antibiotic residues in the birds or in the drinking water,
- Vaccinate only healthy birds,
- Ensure an adequate water supply,
- and sufficient space at the drinkers.

Nipple drinkers are highly suitable for vaccination of growing birds. It is advisable to vaccinate after a dark period of several hours during which the birds have been deprived of water for some time (which depends on the birds' condition and the house temperature; two hours is normally sufficient). The preparations prior to vaccination (flushing of the water lines) should take place in the dark so that the birds have immediate access to the vaccine-medicated drinking water as soon as the lights are turned on.

7. References

BEHR, K. P., M. PÖPPEL, G. REETZ (2003): Jahrbuch für die Geflügelwirtschaft, p 151

JORDAN, F. et al. (2001) in: Poultry Diseases, 5th Ed., Saunders, p 58

RAUCH, H. W., J. PETERSEN (2003): Jahrbuch für die Geflügelwirtschaft, p 76

SCHOLTYSSEK, S. (1987) in: Geflügel, Stuttgart (Hohenheim): Ulmer, p 386

Verordnung zur Novellierung der Trinkwasserverordnung in der Fassung vom 21. Mai 2001

Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe (Trinkwasserverordnung - TrinkwV) in der Fassung vom 5. Dezember 1990

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