

Fighting antimicrobial resistance with immunoglobulins



By **Lea Poppe**, Regional Technical Manager On-Farm Solutions Europe, and **Dr. Inge Heinzl**, Editor

One of the ten global public health threats is antimicrobial resistance (AMR). Jim O'Neill predicted 10 million people dying from AMR annually by 2050 (O'Neill, 2016). The following article will show the causes of antimicrobial resistance and how antibodies from the egg could help mitigate the problem of AMR.

Global problem of AMR results from the incorrect use of antimicrobials

Antimicrobial substances are used to prevent and cure diseases in humans, animals, and plants and include antibiotics, antivirals, antiparasitics, and antifungals. The use of these medicines does not always happen consciously, partially due to ignorance and partially for economic reasons.

There are various possibilities for the wrong therapy

1. The use of antibiotics against diseases that household remedies could cure. A recently published [German study](#) (Merle et al., 2023) confirmed the linear relationship between treatment frequency and resistant scores in calves younger than eight months.
2. The use of antibiotics against viral diseases: antibiotics only act against bacteria and not against viruses. Flu, e.g., is caused by a virus, but doctors often prescribe an antibiotic.
3. Using broad-spectrum antibiotics instead of determining an antibiogram and applying a specific antibiotic.
4. A too-long treatment with antimicrobials so that the microorganisms have the time to adapt. For a long time, the only mistake you could make was to stop the antibiotic therapy too early. Today, the motto is “as short as possible”.

Let's take the example of neonatal calf diarrhea, one of the most common diseases with a high economic impact. Calf diarrhea can be caused by a wide range of bacteria, viruses, or parasites. This infectious form can be a complication of non-infectious diarrhea caused by dietary, psychological, and environmental stress ([Uetake, 2012](#)). The pathogens causing diarrhea in calves can vary with the region. In Switzerland and the UK, e.g., rotaviruses and cryptosporidia are the most common pathogens, whereas, in Germany, *E. coli* is also one of the leading causes. To minimize the occurrence of AMR, it is always crucial to know which pathogen is behind the disease.

Prophylactic use of antibiotics is still a problem

1. The use of low doses of antibiotics to promote growth. This use has been banned in the EU now for 17 years now, but in other parts of the world, it is still common practice. Especially in countries with low hygienic standards, antibiotics show high efficacy.
2. The preventive use of antibiotics to help, e.g., piglets overcome the critical step of weaning or to support purchased animals for the first time in their new environment. Antibiotics reduce pathogenic pressure, decrease the incidence of diarrhea, and ensure the maintenance of growth.
3. Within the scope of prophylactic use of antimicrobials, also group treatment must be mentioned. In veal calves, group treatments are far more common than individual treatments (97.9% of all treatments), as reported in a [study](#) documenting medication in veal calf production in Belgium and the Netherlands. Treatment indications were respiratory diseases (53%), arrival prophylaxis (13%), and diarrhea (12%). On top, the study found that nearly half of the antimicrobial group treatment was underdosed (43.7%), and a large part (37.1%) was overdosed.

However, in several countries, consumers request reduced or even no usage of antibiotics (“No Antibiotics Ever” – NAE), and animal producers must react.

Today's mobility enables the spreading of AMR worldwide

Bacteria, viruses, parasites, and fungi that no longer respond to antimicrobial therapy are classified as resistant. The drugs become ineffective and, therefore, the treatment of disease inefficient or even impossible. All the different usages mentioned before offer the possibility that resistant bacteria/microorganisms will occur and proliferate. Due to global trade and the mobility of people, drug-resistant pathogens are spreading rapidly throughout the world, and common diseases cannot be treated anymore with existing antimicrobial medicines like antibiotics. Standard surgeries can become a risk, and, in the worst case, humans die from diseases once considered treatable. If new antibiotics are developed, their long-term efficacy again depends on their correct and limited use.

Different approaches are taken to fight AMR

There have already been different approaches to fighting AMR. As examples, the annually published [MARAN Report](#) compiled in the Netherlands, the [EU ban on antibiotic growth promoters](#) in 2006, “[No antibiotics ever \(NAE\) programs](#)” in the US, or the annually published “[Antimicrobial resistance surveillance in Europe](#)” can be mentioned. One of the latest approaches is an advisory “One Health High-Level Expert Panel” (OHHLEP) founded by the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE), the United Nations Environment Program (UNEP), and the World Health Organization (WHO) in May 2021. As AMR has many causes and, consequently, many players are involved in its reduction, the OHHLEP wants to improve communication and collaboration between all sectors and stakeholders. The goal is to design and implement programs, policies, legislations, and research to improve human, animal, and environmental health, which are closely linked. Approaches like those mentioned help reduce the spread of resistant pathogens and, with this, remain able to treat diseases in humans, animals, and plants.

On top of the pure health benefits, reducing AMR improves food security and safety and contributes to achieving the [Sustainable Development Goals](#) (e.g., zero hunger, good health and well-being, and clean water).

Prevention is better than treatment

Young animals like calves, lambs, and piglets do not receive immunological equipment in the womb and need a passive immune transfer by maternal colostrum. Accordingly, optimal colostrum management is the first way to protect newborn animals from infection, confirmed by the general discussion on the [Failure of Passive Transfer](#): various studies suggest that calves with poor immunoglobulin supply suffer from diarrhea more frequently than calves with adequate supply.

Especially during the immunological gap when the maternal immunoglobulins are decreasing and the own immunocompetence is still not fully developed, it is crucial to have a look at housing, stress triggers, [biosecurity](#), and the diet to reduce the risk of infectious diseases and the need for treatments.

Immunoglobulins from eggs additionally support young animals

Also, if newborn animals receive enough colostrum in time and if everything goes optimally, the animals suffer from two immunity gaps: the first one occurs just after birth before the first intake of colostrum, and the second one occurs when the maternal antibodies decrease, and the immune system of the young animal is still not developed completely. These immunity gaps raise the question of whether something else can be done to support newborns during their first days of life.

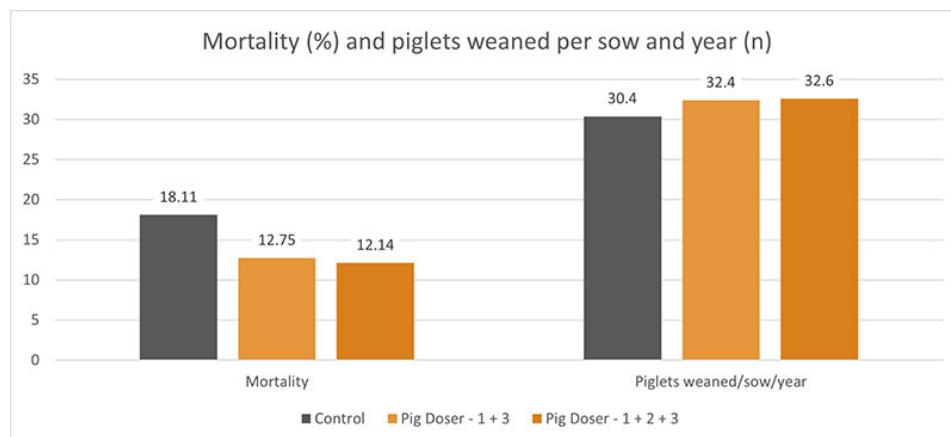
The answer was provided by Felix Klemperer (1893), a German internist researching immunity. He found that hens coming in contact with pathogens produce antibodies against these agents and transfer them to the egg. It is unimportant if the pathogens are relevant for chickens or other animals. In the egg, the immunoglobulins usually serve as an immune starter kit for the chick.

Technology enables us today to produce a high-value product based on egg powder containing natural egg immunoglobulins (IgY – immunoglobulins from the **y**olk). These egg antibodies mainly act in the gut. There, they recognize and tie up, for example, diarrhea-causing pathogens and, in this way, render them ineffective.

The efficacy of egg antibodies was demonstrated in different studies (Kellner et al., 1994; Erhard et al., 1996; Ikemori et al., 1997; Yokoyama et al., 1992; Marquart, 1999; Yokoyama et al., 1997) for piglets and calves.

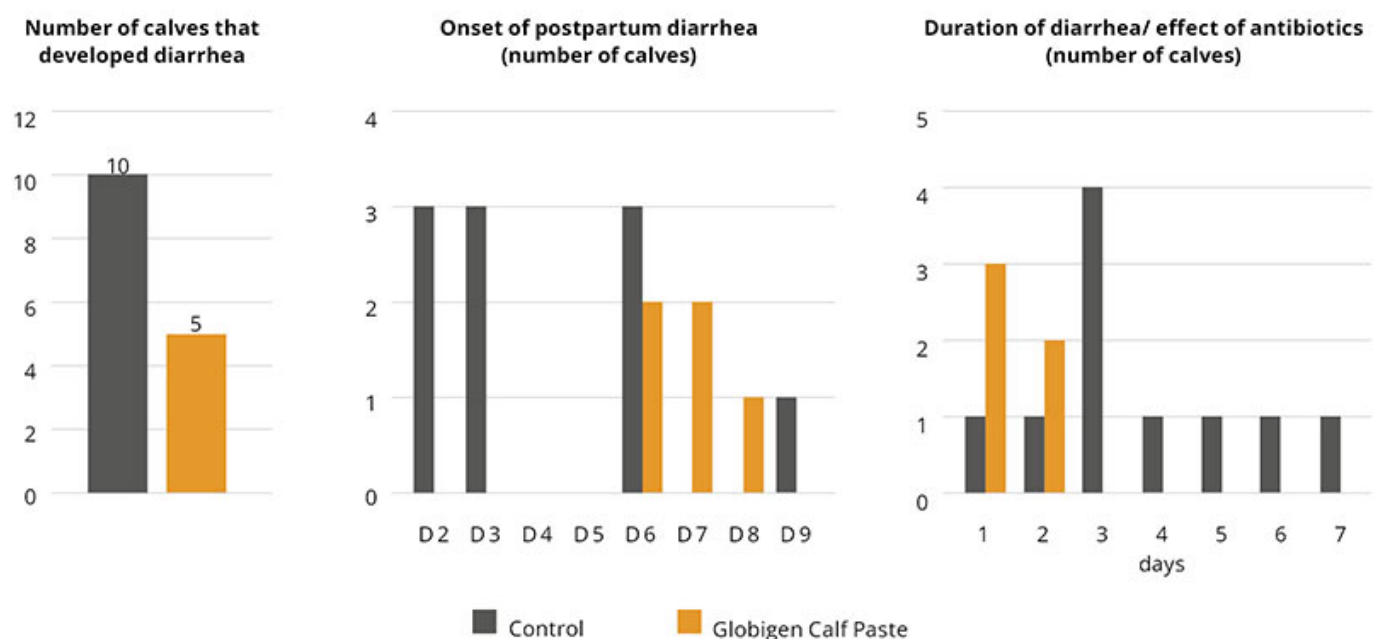
Trial proves high efficacy of egg immunoglobulins in piglets

One trial conducted in Germany showed promising results concerning the reduction of mortality in the farrowing unit. For the trial, 96 sows and their litters were divided into three groups with 32 sows each. Two of the groups orally received a product containing egg immunoglobulins, the EP -1 + 3 group on days 1 and 3 and the EP - 1 + 2 + 3 group on the first three days. The third group served as a control. Regardless of the frequency of application, the egg powder product was very supportive and significantly reduced mortality compared to the control group. The measure resulted in 2 additionally weaned piglets than in the control group.



Egg immunoglobulins support young dairy calves

IgY-based products were also tested in calves to demonstrate their efficacy. In a field trial conducted on a Portuguese dairy farm with 12 calves per group, an IgY-containing oral application was compared to a control group without supplementation. The test product was applied on the day of birth and the two consecutive days. Key observation parameters during a two-week observation period were diarrhea incidence, onset, duration, and antibiotic treatments, the standard procedure on the trial farm in case of diarrhea. On-farm tests to check for the pathogenic cause of diarrhea were not part of the farm's standards.



In this trial, 10 of 12 calves in the control group suffered from diarrhea, but in the trial group, only 5

calves. Total diarrhea and antibiotic treatment duration in the control group was 37 days (average 3.08 days/animal), and in the trial group, only 7 days (average 0.58 days/animal). Additionally, diarrhea in calves of the Globigen Calf Paste group started later, so the animals already had the chance to develop an at least minimally working immune system.

The supplement served as an effective tool to support calves during their first days of life and to reduce antibiotic treatments dramatically.

Conclusion

Antimicrobial reduction is one of the biggest tasks for global animal production. It must be done without impacting animal health and parameters like growth performance and general cost-efficacy. This overall demand can be supported with a holistic approach considering biosecurity, stress reduction, and nutritional support. Feed supplements such as egg immunoglobulins are commercial options showing great results and benefits in the field and making global animal production take the right direction in the future.

References upon request.

Cryptosporidia in calves - chickens can help



By **Lea Poppe**, Regional Technical Manager, EW Nutrition

Diarrhea due to infestation with cryptosporidia is one of the most pressing problems in calf rearing. These

protozoa, along with rotaviruses, are now considered the most common pathogens in infectious calf diarrhea. Due to their high resistance and thus limited possible control and prevention measures, they have now overtaken other pathogens such as coronaviruses, salmonellae, and *E. coli*.

Cryptosporidia show complex development

Cryptosporidia are single-celled intestinal parasites. In calves, *Cryptosporidium parvum* and *Cryptosporidium bovis* are most commonly found. *C. bovis* is normally considered nonpathogenic. Accordingly, the disease known as cryptosporidiosis is caused by *C. parvum*. The rapid tests for determining the diarrheal pathogens, which are increasingly widespread, are usually unsuitable for distinguishing between the individual strains, which can lead to false positive results.

Resistant in the environment, active in the animal

In the environment, cryptosporidia are distributed as oocysts. The oocysts are only about 5 μm in size and have a very resistant shell. They can remain infectious for up to 6 months in high humidity and moderate temperatures. Drought and extreme temperatures (below -18°C and above 65°C) cause the oocysts to die.

After oral ingestion, the oocysts are reactivated by conditions in the gastrointestinal tract (low pH and body temperature): As sporozoites, the parasites attach to the posterior small intestine, causing diarrhea symptomatology. There, they surround themselves with a special protective membrane, and the complex life cycle continues. Only a few days after infection, reproductive forms are detectable in the calf's intestine, and excretion of infectious oocysts in the feces begins.

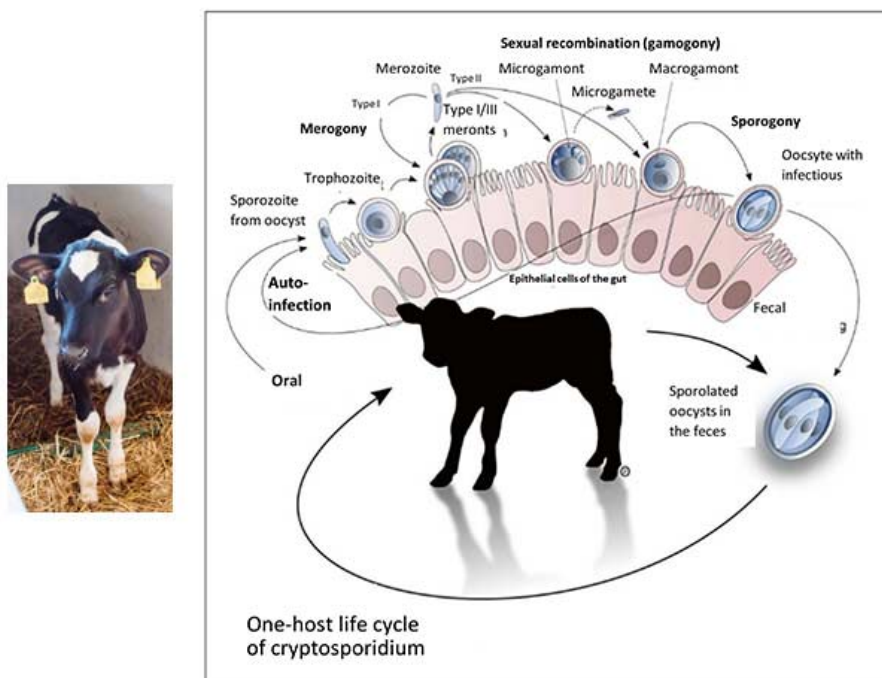


Figure 1 (Olias et al., 2018): Life cycle of cryptosporidia: ingested oocysts release four sporozoites that invade host enterocytes (intestinal epithelial cells). There, they develop into trophozoites before asexual and sexual reproduction ensues, and thin- and thick-walled oocysts are formed. Thick-walled oocysts are excreted through the intestine. Thin-walled oocysts may break apart, and the sporozoites may infect other enterocytes, resulting in relapse or prolonged diarrhea. Infestation of the cells leads to their destruction, resulting in villi atrophy or fusion.

Oocysts bring the disease to the animal

Cryptosporidiosis is transmitted either by direct contact of calves with feces from infected animals or indirectly by ingesting contaminated feed, bedding, or water. Each gram of feces excreted by calves showing symptoms may contain up to 100 million oocysts. According to experimental studies, as few as 17 orally ingested oocysts are sufficient to trigger infection. In addition, some multiplication forms can infect other intestinal cells directly within the intestine and thus further advance the disease by autoinfection.

Cryptosporidiosis caused by cryptosporidia often presents with typical diarrhea symptoms and occurs primarily in calves up to 3 weeks of age. Older calves may also be infected with cryptosporidia but usually show no symptoms. Pathogen excretion and, thus, the spread of disease within the herd is nevertheless likely due to the minimal infectious dose.

Damage to the intestinal wall leads to retardation of growth

Attachment of cryptosporidia to the intestinal wall is associated with an inflammatory reaction, regression and fusion of the intestinal villi, and damage to the microvilli. As a result, nutrient absorption in the small intestine is impaired, and more undigested nutrients enter the colon. The microflora starts a fermentation process with lactose and starch, leading to increased lactate levels in the blood and, thus, hyperacidity in the calf. Faintness, unwillingness to drink, recumbency, and growth disorders are the consequences.

Diarrhea often occurs late or not at all and, accordingly, is not considered the main symptom of cryptosporidiosis. When diarrhea occurs, it lasts about 1-2 weeks. The feces are typically watery, greenish-yellow, and are often described as foul-smelling. Due to diarrhea, there is a loss of electrolytes and dehydration.

Studies show: Cryptosporidia are the most prevalent diarrheal pathogens

Several studies in different regions, which examined calf diarrhea and its triggers in more detail, came to a similar conclusion: Cryptosporidia are one of the most common causes of calf diarrhea. In addition, mixed infections often occur.

Country or region	Number	Age/Health status	% Crypto-sporidia	% Rota viruses	Combined infections with crypto-sporidia	Others (%)	Source
Switzerland		2 - 21 DL Ill and healthy	43	46		1 case of E. coli	Luginbühl et al., 2012
Switzerland	63	1 - 4 DL Ill and healthy 7 - 20 DL 26 - 49 DL	34.4 54.0 33.3	3.1 28.6 13.3	2 EP - 1.6 4 EP - 3.2 2 EP - 19 3 EP - 3.2 4 EP - 0 2 EP - 30 3 EP - 11.7 4 EP - 6.7	Corona 4.7 E. coli 4.7 Giardia 1.6 Corona 0 E. coli 3.2 Giardia 6.3 Corona 0 E. coli 15 Giardia 35	Weber et al., 2016 Weber et al., 2016 EN
Switzerland	147	Up to 3rd WL; Diarrhea	55	58.7		5.5 % Rota 7.8 % BCV	Lanz Uhde et al., 2014
Sweden	782	1 - 7 DL Diarrhea	25.3		Detected with Giardia, E. coli, Rota, Eimeria		Silverlås et al., 2012
USA (East coast)	503	Pre-weaning	50.3				Santin et al., 2004

USA	30	2 weeks old 1-8 weeks old 3-12 months 12-24 months	96.7 45.8 18.5 2.2				Santin et al., 2008
Germany	521		32	9			Losand et al., 2021
Ethiopia	360		18.6				Ayele et al., 2018
Argentina	1073	n.m. / Ill and healthy	25.5				Lombardelli et al., 2019
UK	n.m.	Ill ??	37	25	20	Coccidia 8 E. coli 4 Corona 3 Co infections not including Cryptosporidia 3	APHA, SRUC, Veterinary investigation diagnosis analysis (VIDA) report (2014)

DL = days of life WL = weeks of life n.m. = not mentioned EP = enteropathogen

Cryptosporidia reduces profit

Infection with cryptosporidia and sometimes subsequent diarrhea entails treatment of the animals and generates costs (veterinarian, medication, electrolyte drinks). In addition, poorer feed conversion, lower growth, and animal losses result in lower production efficiency.

A [Scottish study](#) shows 34 kg less gain in the first six months of life compared to healthy calves in beef calves that experienced severe cryptosporidiosis in the first three weeks of life. Similar results are described in lambs, also a susceptible species to cryptosporidia. These studies suggest a long-term negative effect of cryptosporidia on growth performance and production efficiency.

Here's how you can support your calves against cryptosporidia

High resistance of the pathogens to environmental influences, a very low necessary infection dose combined with an elevated excretion of infectious oocysts, and the possibility of autoinfection make cryptosporidia tough opponents. This is also reflected in their worldwide distribution.

What is the treatment?

Suitable drugs for the treatment of cryptosporidiosis are currently unavailable on the market. The only medicine that can be used in case of cryptosporidiosis infestation may only be administered to calves that have had diarrhea symptoms for 24 hours or less. Accordingly, this agent is usually used only for prevention. Scientific studies on its effectiveness are contradictory; some suggest that it merely delays the onset of the disease. In addition, it is not always easy to use due to the exact dosage that must be followed. Doubling the dose (sometimes happening already due to incorrectly observed intervals between doses) can lead to a toxic overdose.

Accordingly, only the symptoms of the disease – diarrhea with its accompanying symptoms – can be treated. Electrolyte and water losses must be continuously compensated with the help of a [high-quality electrolyte drink](#). The buffer substances contained also reduce the hyperacidity of the blood caused by faulty fermentation in the intestines. For successful treatment, the electrolyte drink should be given in addition to the milk drink. Under no circumstances should the feeding of milk or milk replacer be discontinued because the sick calf urgently needs energy and nutrients. Opinions to the contrary are outdated.

As always: prevention is better than treatment

To make it more difficult for [cryptosporidiosis](#) to spread from the outset, it is worth looking at the risk factors. These include direct contact with other calves and general herd size. Furthermore, organic farms seem to have more problems with cryptosporidia. Weather also influences calves born during warmer and, at the same time, wetter weather periods (temperature-humidity index) often get sick.

Due to the limited possibilities for treatment, prevention is of greater importance. For other diarrheal pathogens such as rotavirus, coronavirus, and *E. coli*, it has become established practice to vaccinate dams to achieve better passive immunization of the calf. However, commercial vaccination against cryptosporidia is not currently available, making dam vaccination as unavailable as calf vaccination.

Accordingly, optimal colostrum management is the first way to protect the calf from cryptosporidia infection. This also confirms the general discussion on the [Failure of Passive Transfer](#): various studies suggest that calves with poor immunoglobulin supply suffer from diarrhea more frequently than calves with good supply, although a concrete link to cryptosporidia itself cannot always be established with certainty.

Furthermore, it is essential to break the chain of infection within farms. In addition to the separate housing of the calves, it is necessary to ensure consistent hygiene. One should take advantage of the pathogen's weakness as well as its sensitivity to high temperatures and ensure that the water temperature is sufficiently high when cleaning the calf pens and calving area. When disinfecting afterward, it is crucial to consider the spectrum of activity of the agent used, as not all are effective against cryptosporidia.

Egg immunoglobulins support animals against cryptosporidia

[Egg immunoglobulins](#) were initially designed to help chicks get started. In this process, hens form antibodies against pathogens they are confronted with. As studies have shown, this also works with cryptosporidia. Cama and Sterling (1991) tested their produced antibodies in the neonatal mouse model and achieved a significant ($P \leq 0.001$) reduction in parasites there. Kobayashi et al. (2004) registered decreased binding of sporozoites to the intestinal cell model and their decreased viability in addition to oocyst reduction.

In the IRIG Research Institute (2009, unpublished), feeding egg powder with immunoglobulins against cryptosporidia (10 g/day) to 15 calves reduced oocyst excretion. Before administration, calves excreted an average of 106.42 oocysts/g of feces. After administration of egg powder, only two calves still showed 103.21 oocysts/g feces, and the other 13 of the 15 calves showed no oocyst excretion. All these results are confirmed by positive customer feedback on [IgY-based feed supplements](#).

Egg immunoglobulins and optimal colostrum management as a key solution

Since there are no effective drugs against cryptosporidia, animals must be prophylactically protected against this disease as much as possible. In addition to optimal colostrum management, which means feeding high-quality colostrum ($\text{IgG} \geq 50\text{g/L}$) to the calf as soon as possible after birth, we have products with egg immunoglobulins available to support the calf as a prophylactic against cryptosporidia infestation and thus prevent significant performance losses, especially during rearing.

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Diarrhea? Egg powder to the rescue



Another tool to reduce the use of antibiotics is the use of [immunoglobulins from eggs](#). Trials showed that this product is effective to support a calf's start in life and also to offer support when challenged by various forms of diarrhoea.

The main cause for calf losses during the first two weeks of life is diarrhea. In general diarrhoea is characterised by more liquid being secreted than that being resorbed. However, diarrhoea is not a disease, but actually only a symptom. Diarrhea has a protective function for the animal, because the higher liquid volume in the gut increases motility and pathogens and toxins are excreted faster. Diarrhoea can occur for several reasons. It can be caused by incorrect nutrition, but also by pathogens such as bacteria, viruses and protozoa.

Bacteria in the gut

E. coli belong to the normal gut flora of humans and animals and can be mainly found in the colon. Only a fraction of the serotypes causes diseases. The pathogenicity of *E.coli* is linked to virulence factors. Decisive virulence factors are for example the fimbria used for the attachment to the gut wall and the bacteria's ability to produce toxins.

Salmonella in general plays a secondary role in calf diarrhea, however, salmonellosis in cattle is a notifiable disease. Disease due to *Clostridia* is amongst the most expensive one in cattle farming globally. In herbivores, clostridia are part of the normal gastro-intestinal flora, only a few types can cause serious disease. In calves, *Clostridium perfringens* occurs with the different types A, C, and D. *Rotaviruses* are the most common viral pathogens causing diarrhoea in calves and lambs. They are mainly found at the age of 5 to 14 days. *Coronaviruses* normally attack calves at the age of 5 to 21 days. *Cryptosporidium parvum* is a protozoa and presumed to be the most common pathogen causing diarrhoea (prevalence up to more than 60 %) in calves.

Undigested feed and incorrect use of antibiotics

Plant raw materials (mainly soy products) are partly used in milk replacers as protein sources. These products contain carbohydrates, that cannot be digested by calves which can lead to diarrhea. The transition from milk to milk replacer can also be a reason.

An early application of tetracyclines and neomycin to young calves can lead to a change in the villi, malabsorption and therefore to slight diarrhoea. Longer therapies using high dosages of antibiotics can also lead to a bacterial superinfection of the gut. The problem is that in a disease situation, antibiotics are

often used incorrectly. The use of antibiotics only makes sense when there is a bacterial diarrhea and not due to viruses, protozoa or poor feed management. To keep the use of antibiotics as low as possible, alternatives need to be considered.

Egg powder to add immunoglobulins

In order to achieve optimal results in calf rearing two approaches are possible. Firstly, the prophylaxis approach. This is the method of choice as diarrhoea can mostly be prevented. Therefore, it is necessary to supply the calf with the best possible equipment. As antibodies are one crucial but limiting factor in the colostrum of the “modern” cow, this gap needs to be minimised. A study conducted in Germany in 2015 demonstrated that more than 50% of the new-born calves had a deficiency of immunoglobulins in the blood. Only 41% of the calves showed an adequate concentration of antibodies in the blood (>10 mg IgG/ml blood serum). Immunoglobulins contained in hen eggs (IgY) can partly compensate for poor colostrum quality and serve as a care package for young animals. A trial was conducted with an egg powder product* on a dairy farm (800 cows) in Brandenburg, Germany. In total 39 new-born calves were observed until weaning (65th day of life). Before birth, the calves were already divided into control and trial group according to the lactation number of their mother cow. All calves were fed the same and received four litres of colostrum with ≥ 50 mg IgG /ml on the first day of life.

Control (n=20): no additional supplementation

Trial group (n=19): day 1 – 5: 100 g of the egg powder product per animal per day mixed into the colostrum or milk.

It was shown that the calves in the trial group showed a significantly higher (13%) weaning weight (105.74 kg compared to 93.45 kg in the control group) and 18% higher average daily gain (999 g compared to 848 g in the control group) (Figure 1 and Figure 2).

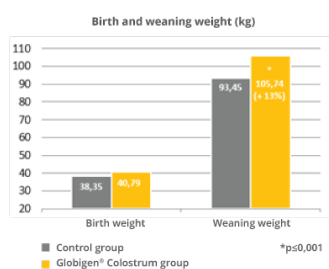


Figure 1: Effect of an egg powder product on weaning weight (kg)

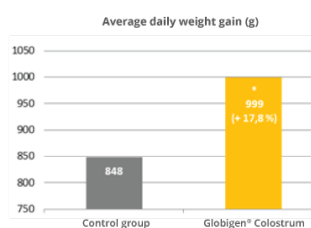


Figure 2: Effect of an egg powder product on ADG (g)

Support during acute diarrhea

When diarrhea occurs, the calf has to be treated. So the second approach is to find the best and quickest solution. It is not always necessary to use antibiotics, as they do not work against virus or protozoa. Egg antibodies can be an answer when combined with electrolytes as the following trial shows. On a dairy farm (550 cows) in Germany a feeding trial with a product based on egg powder and electrolytes** was conducted from December 2017 to May 2018. Two groups of calves were used. Before birth the animals were allocated into the two groups according to the calving plan and were examined from day one until weaning (77th day of life). All calves suffering from diarrhea (38 in total, 17 in the control and 21 in the trial group) were treated as follows:

Control (n=17): Application of electrolytes

Trial group (n=21): 50 g of the [egg powder](#) and electrolytes product twice daily, stirred into the milk replacer until diarrhea stopped.

If the diarrhea did not stop or even got worse, the animals were treated with antibiotics. It was shown that in the control group the antibiotic treatment necessary was nearly twice as long as needed in the trial group (Figure 3). This means also that nearly twice the amount of antibiotics were used. This leads to the conclusion that calves in the trial group had an improved health status compared to calves in the control group. A further result from the improved health status was an increase in performance in the trial group (Figure 4).

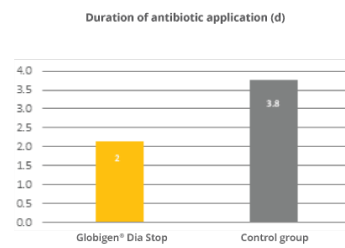


Figure 3: Duration of antibiotic application (d)

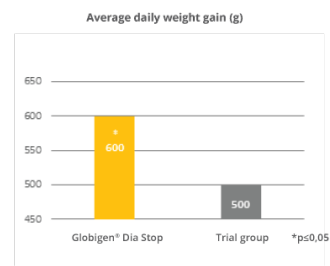


Figure 4: Effect on average daily weight gain (g)

The average daily weight gain of the trial group was 20% higher than in the control (600 vs. 500 g per day) leading to a significantly higher weaning weight (87.8 kg) than in the control (80.7 kg).

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