Mind the immunity gap: egg immunoglobulins bolster piglets' immune system

In contrast to humans, piglets do not receive any maternal immunoglobulins via the placenta. It is therefore of vital importance for these young animals to receive maternal antibodies via the colostrum as soon as possible after birth. Otherwise, they are more vulnerable to illnesses in their early stages of life.

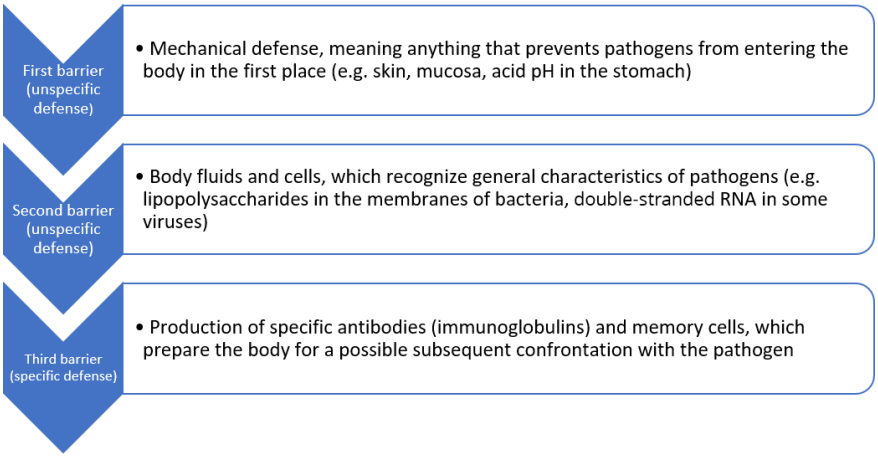
In this article, we look in-depth at how the immune system works and which role antibodies play in it. We then consider why immunoglobulins from the egg (IgY) might potentially be a powerful tool for supporting young animals immunologically, allowing producers to maintain young animals' health and to promote their performance.

How the immune system defends the

body: three barriers

The immune system aims to prevent pathogens such as viruses, bacteria, and fungi from entering the body or to eliminate them when they have already entered. Furthermore, it seeks to prepare the body for quicker reactions, in case of subsequent infections, by building an immunological memory. Generally, in case of an attack by pathogens, there are three barriers against the “enemy” (Figure 1).

Figure 1: The three barriers of the immune response



First barrier: the immediate, physical defense upon contact with pathogens

The animal body has several anatomical features that prevent pathogens from entering in the first place, such as cilia and mucus. Skin, intestines and nose lining are colonized by a community of beneficial micro-organisms that form a physical barrier against pathogens. Other barriers include the urinary system, the acid pH of the stomach, as well as tears and saliva, which contain antibacterial lysozymes.

Second barrier: the unspecific, native defense that does most of the work

If the mechanical mechanisms of defense were not successful, the unspecific, innate immune defense enters into play (Murphy and Weaver, 2018, 47ff.). At this stage, the body needs to differentiate between “known” and “alien” agents, and between “potentially harmful” and “harmless” ones.

To identify alien, potentially harmful agents, the unspecific defense looks for so-called PAMPs (pathogen associated molecular patterns). These are general characteristics often displayed by pathogens, such as lipopolysaccharides in the bacterial membrane or double-stranded RNA in viruses. Everything that shows PAMPs is heavily targeted.

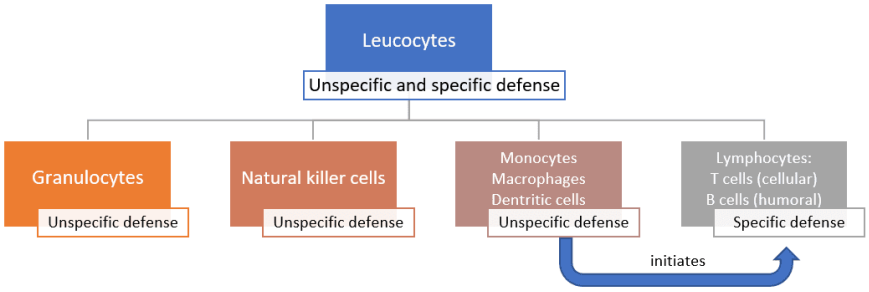
The unspecific defense can be further divided into the humoral and the cellular defense. **The humoral defense** consists of substances dissolved in the body fluids, such as enzymes, reactive oxygen compounds, signal molecules and a whole cascade of proteins. Some of these substances kill pathogens directly; others “mark” the pathogens and “call for” the help of leucocytes.

The **cellular defense** consists of different leucocytes, also known as white blood cells (because they do not contain any red hemoglobin). The main task of leucocytes is the defense of the body against pathogens, hence many leucocytes are capable of phagocytosis (the ingestion of other cells). To prevent phagocytes from accidentally ingesting the body’s own cells, these own cells are marked with the so-called major histocompatibility complex (MHC). This acts as a red flag, saying “I belong to the body!”.

The cellular defense consists of:

- Neutrophil granulocytes (60-70% of the leucocytes), which mainly act against bacteria
- Eosinophil and basophil granulocytes (1.5% of the leucocytes), which mainly act against parasites
- Natural killer cells, which mainly act against viruses
- Monocytes (3-8% of the leucocytes; they differentiate into macrophages and dendritic cells)

Figure 1: The three barriers of the immune response



The monocytes, as well as their macrophage and dendritic cell "offspring", are the bridge to the next step, the specific defense. When these phagocytes digest pathogens, minuscule protein structures (antigens) of the pathogens remain. These antigens are unique to each pathogen. During a process called antigen presentation, the antigens are tied to the cell's MHC and transported to the cell surface. This triggers the production of specific antibodies, the immune system's third barrier.

Third barrier: the specific immune defense that creates antibodies and immunological memory

The specific (also called adaptive or acquired) immune response kicks in a few days after contact with specific pathogens and is mostly carried out by lymphocytes called T and B cells (Murphy and Weaver, 2018, 177ff.). They are active at the cellular and the humoral level, respectively.

T cells possess receptors on their surface through which they can recognize the antigens presented to them by phagocytes. What they do subsequently depends on the subtype of the T cell:

- Cytotoxic T cells (CD8+) directly destroy the antigen-phagocyte-combination
- T helper cells (CD4+) attract other cells that can destroy the pathogens (e.g. macrophages) and stimulate B cells to produce antibodies against them

B cells also possess receptors through which they can recognize antigens. Once they spot an antigen (and T helper cells "confirm" that an immune response is required), they divide and mature into so-called plasma cells. Plasma cells, in turn, secrete plenty of antibodies (or immunoglobulins) into the bloodstream and the lymphatic system. Antibodies are protein structures that lock onto and neutralize antigens through different mechanisms.

The chemical reaction between antibodies and antigens is the body's most powerful immune response through which it can protect itself from pathogens and their toxins. Antibody production continues for several days to remove the antigens, and antibodies usually remain in circulation for a few months.

Moreover, certain T and B cells memorize the first attack of a pathogen and turn into memory cells. The T memory cells CD4+CD8+, for instance, match the antigens from certain past, latent, and particularly persistent viral infections. This immunological memory, created by acquired immunity, can be thought of as a library of antibodies that the body adds to whenever it deals with a new pathogen or receives a vaccine. In case of a subsequent contact with the pathogen, the right antibody "model" already exists and mass production can start up very quickly.

Why young animals' immune defense is so vulnerable – and what IgY can do about that

Building one's immunological memory takes time. A lot of new-born animals are in a vulnerable position: they have not had time yet to acquire immunity of their own, but they are also particularly fragile and susceptible to being attacked by commonly occurring pathogens such as corona and rotaviruses, *E. coli*

and *clostridia*. The toxins that *E. coli* and *clostridia*, for instance, release, may cause diarrhea, edema, endotoxic shock, and even death.

To be protected during the first critical days of their lives, new-born animals thus need to receive a foundational stock of antibodies (passive immunity) from their mother. Humans receive maternal immunoglobulins via the placenta. Piglets, because a sow's placenta is constructed differently, are dependent on receiving them through the colostrum after birth. If this is not the case – due to inadequate quantity or quality of the colostrum – they need to receive immune support in a different way.

Egg-yolk antibodies have been proposed as a powerful tool for supporting young animals during the critical period after birth. These special proteins support the colostrum supply and guarantee that every animal in the herd has some degree of protection. This protection mostly takes place in the gut. The IgY recognize and tie up pathogens and render them ineffective.

Trial: can egg immunoglobulins support piglet immunity?

In 2009, research at the National Veterinary Research Institute in Pulawy, Poland, was conducted to probe this hypothesis. The objective of the trial was to evaluate whether an oral application of egg immunoglobulins would have a quantifiable, positive influence on the immune system of the piglets. Different immunological parameters were measured, including different types of leucocytes.

Trial design

The test consists of 6 litters with 67 piglets in total divided into two groups. The control group (n=32) received the prophylaxis customary on the farm; the trial group (n=35) additionally received a product based on egg powder (EP)[1], applied at the inclusion rate recommended by the producer. Blood samples were taken on days 0 (before application of the product), 7, 14, and 28. They were analyzed with respect to the percentages of different types of lymphocytes.

Trial results

For the group receiving egg powder, the number of leucocytes in peripheral blood was significantly elevated compared to the control group on the 7th day of life (table 1). The amounts of lymphocytes and monocytes – indicators for the specific immunological defense – were also significantly increased on day 7, whereas the total amount of granulocytes – indicator for the innate, unspecific immune defense – remained constant. Hence, already during the first days, the piglets supplied with EP disposed of a higher level of adaptive (specific) immune defense, compared to the animals in the control group. In addition, there was a significant increase in the number of CD4-positive (CD4+) and CD4-CD8-double positive (CD4+CD8+) T cells in the EP group, compared to the control animals, indicating an active stimulation of the immune system.

Except for CD4+CD8+ T cells (which remained elevated in the EP group), on day 14, the differences in cellular immune response were no longer significant. This is most probably the case because by that time the immune system of the control group had activated its own protective response. The EP product therefore supported the young animals precisely when it was necessary, during the critical first days of life.

Table 1: Hematological parameters measured in piglets after prophylactic application of an egg powder based product (EP1)

Parameter evaluated	Unit	Average values± SD				
		Day 0	7th day of life		14th day of life	
			EP	Control	EP	Control
Erythrocytes	10^{12}/L	4.77 ±0.77	4.90 ± 1.61	4.56 ± 0.18	5.94 ± 0.45	5.78 ± 1.16
Hemoglobin	mmol/L	9.15 ± 1.57	9.47 ± 2.71	9.55 ± 1.63	12.03 ± 0.67	11.30 ± 2.04
Leucocytes	10^9/L	7.97 ± 3.41	**11.73[2)] ± 2.08**	**7.05[2)] ± 0.64**	10.98 ± 3.26	9.45 ± 2.58
Lymphocytes	10^9/L	2.62 ± 0.73	**7.03[2)] ± 1.97**	**3.1[2)] ± 0.57**	5.35 ± 1.76	4.88 ± 1.68
	%	36 ± 6.00	**60[2)] ± 5.77**	**45[2)] ± 4.24**	50 ± 5.74	52 ± 7.53
Monocytes	10^9/L	0.85 ± 0.24	**1.63[2)] ± 0.15**	**0.95[2)] ± 0.21**	1.25 ± 0.41	1.03 ± 0.29
	%	10.75 ± 2	13 ± 1.57	13 ± 1.44	11 ± 0.82	10 ± 0.82
Granulocytes	10^9/L	4.5 ± 2.52	3.07 ± 0.21	3.00 ± 0.14	4.38 ± 1.35	3.53 ± 1.01
	%	53.25 ± 8	26[2)] ± 4.73	42[2)] ± 5.66	40 ± 5.45	38 ± 8.17
CD4+	%	12.47 ± 6.14	**18.00[3)] ± 5.22**	**12.82[3)] ± 4.94**	21.52 ± 5.86	22.20 ± 3.77
CD8+	%	24.83 ± 1.77	32.45[3)] ± 3.65	35.08[3)] ± 2.57	33.88 ± 5.13	33.65 ± 3.71
CD4+CD8+	%*	6.27 ± 4.35	**12.98[3)] ± 5.67**	**8.55[3)] ± 4.10**	**15.85[1)] ± 2.95**	**9.62[1)] ± 3.62**
** in percent of the lymphocytes* *Statistical significance: 1) α ≤ 0.01; 2) α ≤ 0.05; 3) α ≤ 0.1*						

[1]Ig-PRO P (EW Nutrition)

The improvement of immune status, as indicated by the presence of the specific immune cells, was confirmed by the results for the incidence of diarrhea and mortality (table 2). The animals of the control group showed a nearly 1.5 times higher incidence of diarrhea and a 1.6 times higher rate of mortality. Another explanation of these results could be the mode of action of egg immunoglobulins: by neutralizing the pathogens directly in the gut, they prevent them from causing diarrhea in the first place.

Table 2: Incidence of diarrhea and mortality

		Piglets showing diarrhea symptoms		Piglets dead due to diarrhea	
	N/litter, ø	N/litter, ø	%	N/litter, ø	%
EP group	10.97	1.95	18.76	0.37	3.37
Control group	10.94	2.94	27.21	0.59	5.39

In conclusion, this trial demonstrates that immunoglobulins from eggs (IgY) effectively support the immune system of piglets during the critical period of the first days of life.
Thanks to the stimulation of the young animals' specific immune defense and the direct neutralization of pathogens in the gut, the incidence of diarrhea – one of the main causes of losses during the first weeks of life – decreases. Hence, mind the immunity gap: providing piglets with a suitable egg powder based product sets them up for long-term health, growth, and performance.

By I. Heinzl, Editor, EW Nutrition

References:

Foley, J. A., and D. E. Otterby. "Availability, Storage, Treatment, Composition, and Feeding Value of Surplus Colostrum: A Review 1, 2." Journal of Dairy Science 61, no. 8, 1033-1060. doi.org/10.3168/jds.S0022-0302(78)83686-8.
Heinzl, Inge, and Fellipe Barbosa. "Egg Antibody Technology for Nursery Pig Application." Swineweb.com. June 24, 2019. Accessed July 17, 2019. http://www.swineweb.com/egg-antibody-technology-for-nursery-pig-application/.
Marquardt, Ronald R., L. Z. Jin, Jung-Woo Kim, Lin Fang, Andrew A. Frohlich, and Samuel K. Baidoo. "Passive Protective Effect of Egg-yolk Antibodies against Enterotoxigenic Escherichia Coli K88 Infection in Neonatal and Early-weaned Piglets." FEMS Immunology and Medical Microbiology 23, no. 4 (1999): 283-288. https://doi.org/10.1111/j.1574-695X.1999.tb01249.x.
Murphy, Kenneth M., and Casey Weaver. 2018. Janeway Immunologie. 9th ed. Translated by Lothar Seidler. Berlin: Springer.

Nascimbeni, Michelina, Eui-Cheol Shin, Luis Chiriboga, David E. Kleiner, and Barbara Rehermann. "Peripheral CD4 CD8 T Cells Are Differentiated Effector Memory Cells with Antiviral Functions." Blood 104, no. 2 (2004): 478-486. doi:10.1182/blood-2003-12-4395.
Yokoyama, Hideaki, Robert C. Peralta, Roger Diaz, Sadako Sendo, Yutaka Ikemori, and Yoshikatsu Kodama. "Passive Protective Effect of Chicken Egg Yolk Immunoglobulins." Infection and Immunity 60, no. 3 (March 1992): 998-1007. https://iai.asm.org/content/iai/60/3/998.full.pdf.

Optimal conditions in the farrowing unit put piglets in pole position

The most important parameters for a pig producer are the number of healthy pigs weaned/sow/year and their weaning weight. Due to improved genetics, it is possible today to find production systems that deliver more than 30 pigs weaned/sow/year. Strategies to increase sow productivity need to take into account the management, feeding, and health of both the piglets and the sows.

Pigs' start in life – limited energy reserves and practically no immune protection

It is generally known that pigs are born physiologically immature. Their energy reserves are limited. They only possess 1-2 % fat, the main part of which is subcutaneous or structural fat protecting organs, joints and skin. Thus, the young pigs depend on the glucose of the glycogen deposits in the liver as main source of energy. This energy supply only meets their requirements for the first few hours.
Besides that, pigs cannot count on maternal antibodies. Unlike in humans, a sow's placenta is not built to enable the transfer of these protective cells within the womb. At birth, the amount of protective cells in a pig's intestine, the main site of pathogenic contamination, is therefore virtually zero. As they are born without any immune protection, new-born pigs rely on an early supply of antibodies from the maternal colostrum. During the first 24-36 hours after birth, antibodies are absorbed in the intestine and pass directly to the bloodstream. The intestinal barrier then closes. Importantly, the content of antibodies in the colostrum decreases with every hour after birth.

Prevention – the best way to protect the progeny!

Given this difficult situation in the early stages of life, it is clear that the farrowing unit should be as comfortable as possible for the young animals:

- It should be warm, as low temperatures contribute to hypoglycemia. The search for body heat at the sow additionally increases the risk of crushing, one of the main causes of pig losses. The problem arising here is that sows and the new-born pigs have different temperature requirements. One good solution is a heat lamp, installed specifically for the piglets.
- It should be clean, and pathogenic pressure should be as low as possible. Due to their poor immune status, young pigs are susceptible to diarrhea-causing pathogens like E. coli and Clostridium perfringens during their first days of life. In order to meet hygiene requirements, the first step is a careful cleaning and disinfection of the farrowing unit prior to placing the sows/gilts.

Sows' manure – the first source of contamination

Cleaning both the farrowing unit and also the sows/gilts before placing them is helpful. Producers, however, have to understand that a sow is continuously shedding pathogens through her feces and that her young come into contact with them. In fact, sow manure is the first source of contamination for new-born pigs.
There are several methods to decrease pathogens within the sow's gut. Feeding them natural substances such as probiotics or phytomolecules (also known as secondary plant compounds) in order to improve gut health is one possibility: beneficial microbes such as lactobacilli or bifidobacteria compete with pathogens such as E. coli or clostridia for nutrients and prevent their proliferation. Phytomolecules such as carvacrol and cinnamaldehyde, on the other hand, were found to have antimicrobial properties.
Could feeding them egg immunoglobulins be another possibility?

Egg immunoglobulins – the key to reduce pathogenic pressure?

Yokoyama et al. (1992 and 1997) already showed that immunoglobulins from eggs applied to piglets bind to pathogens within their intestinal tract. If they also bind pathogens in the sow's gut – generating

harmless complexes – this could be the key to reduce pathogenic pressure in the farrowing unit.

Trial

Method
To evaluate this possibility a trial was conducted in Japan. Two groups of eight sows were used. The sows of the control group received standard lactation feed. The trial group was also fed standard feed, but additionally received a supplement containing egg powder product (EPP) with immunoglobulins* at a dosage of 5g/sow twice daily during the last ten days before and the first seven days after delivery. The feces of the sows were obtained by rectal stimulation (in order to rule out contamination from the environment) on day 10 before and day 7 after delivery. The amount of colony-forming units (CFU) of total E. coli, E. coli O141 and Clostridium perfringens was determined.

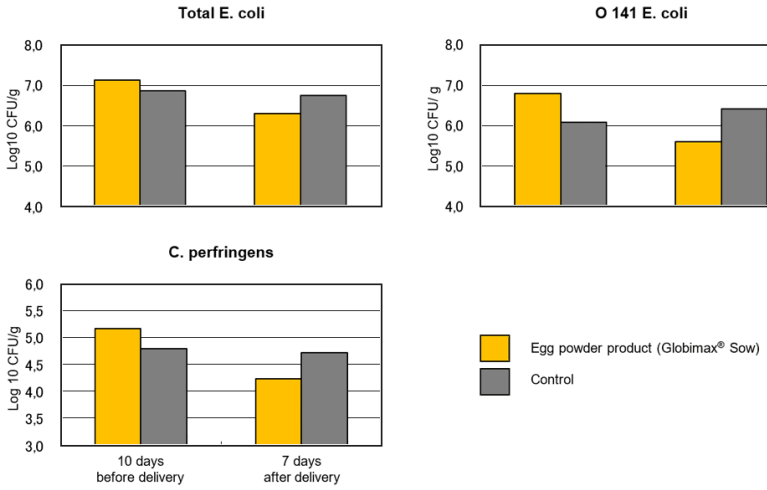
Results
The results are shown in figure 1. At the beginning of the trial, before the application of the EPP, both groups showed nearly the same level of the pathogens evaluated, with a slight disadvantage for the EPP group. After 17 days of using the EPP, the sows of the EPP group showed lower levels of pathogens in their excrements than the sows of the control group. A reduction in the colony-forming units of total Escherichia coli (from 107.12 to 106.3), Escherichia coli O141 (from 106.8 to 105.6) and of Clostridium perfringens (from 105.17 to 104.24) could be seen.

*The product used in this trial was Globimax® Sow, EW Nutrition.

Egg immunoglobulins – a tool to optimize conditions in the farrowing unit

It is important for pig producers to understand how they can combat adverse influences on their animals' performance. The results of this trial showed that supplementing the standard sows' diets with the EPP substantially reduced the amount of pathogenic colonies in sow's manure. Reducing pathogenic pressure in the farrowing unit is central to reducing the incidence of diarrhea and pre-weaning mortality. Giving young pigs the best possible start in life sets them up for delivering the best possible performance – and more healthy and heavy pigs weaned/sow/year means a more profitable farm.

Figure 1: Amounts of total E. coli, O 141 E. coli and Clostridium perfringens in the feces of sows 10 days before delivery (before the first application of EPP) and 7 days after delivery (after the last application of EPP)



By Inge Heinzl, Editor, EW Nutrition

References:

Yokoyama, H., Peralta, R. C., Diaz, R., Sendo, S., Ikemori, Y. and Kodama, Y. (1992): Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic Escherichia coli infection in neonatal piglets. Infection and Immunity, March edition, 998-1007.
Yokoyama, H., Hashi, T., Umeda, K., Icatlo, F. C., Kuroki, M., Ikemori, Y. and Kodama, Y. (1997): Reduced intestinal colonization with F18-positive enterotoxigenic Escherichia coli in weaned pigs fed chicken egg antibody against the fimbriae. FEMS Immunology and Medical Microbiology (18), 153-161.

Phytomolecules: A tool against antibiotic-resistant E. coli

Diseases caused by E. coli entail use of antibiotics in animal production

E. coli infections are a major problem in pig production. Especially young animals with an incompletely developed immune system are often unable to cope with the cavalcade of pathogens. In poultry, E. coli are

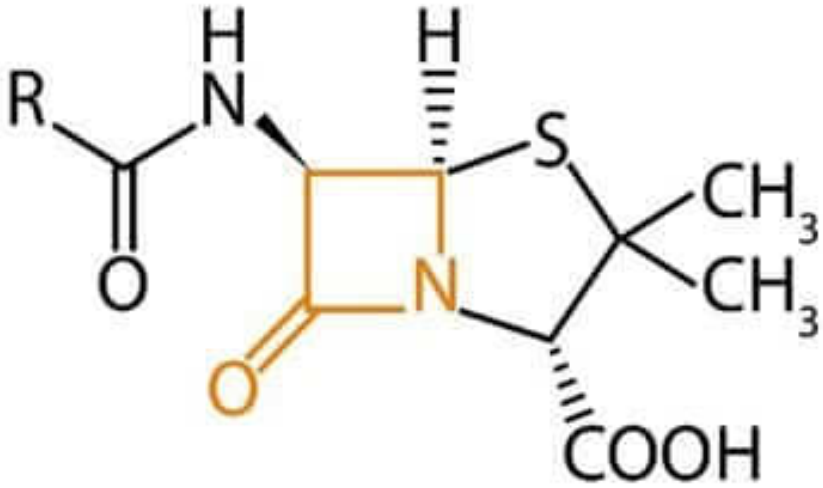
responsible for oedema, but also for respiratory diseases. In young piglets, E. coli cause diarrhoea , oedema, endotoxic shock and death. In order to cure the animals, antibiotics often must be applied. Besides this curative application, antibiotics were and in many countries still are used prophylactically and as growth promoters.

The excessive use of antibiotics, however, leads to the occurrence of antimicrobial resistance (AMR): due to mutations, resistance genes are created which enable enterobacteria such as Salmonella, Klebsiella and E. coli to produce enzymes (ß-lactamases) in order to withstand ß-lactam antibiotics. In case of an antibiotic treatment, the resistant bacteria survive whereas the other bacteria die.
The major problem here is that these resistance genes can be transferred to other bacteria. Harmless bacteria can thus transfer resistance genes to dangerous pathogens, which then cannot be combatted with antibiotics anymore. In this article we explore in detail how AMR happens and how phytomolecules, which have antimicrobial properties, could be a key tool to reduce the need for antibiotics in animal production.

How ß-lactam antibiotics work

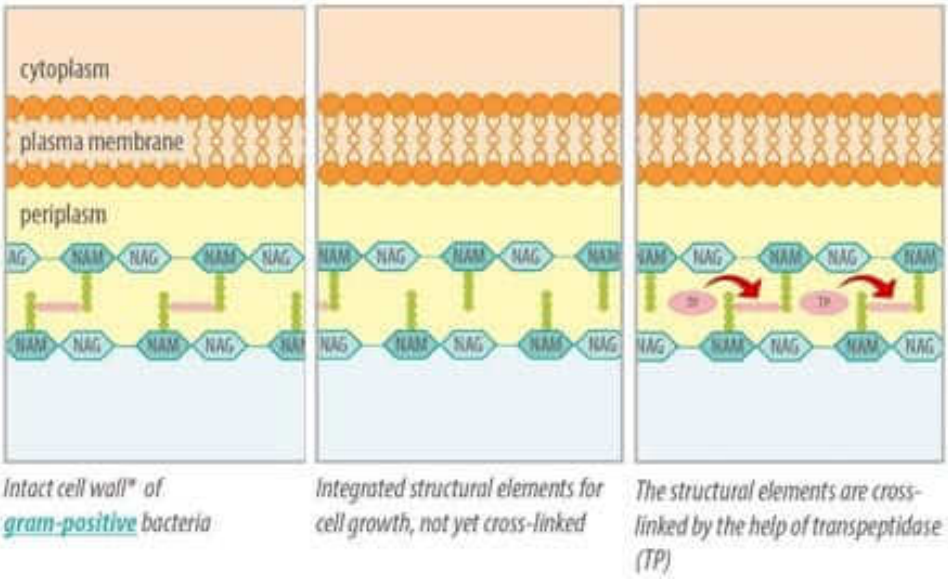
The group of ß-lactam antibiotics consists of penicillins, cephalosporins, monobactams, and carbapenems. These antibiotics are characterised by their lactam ring (Figure 1).

Figure 1: An antibiotic with a ß-lactam ring (in orange)



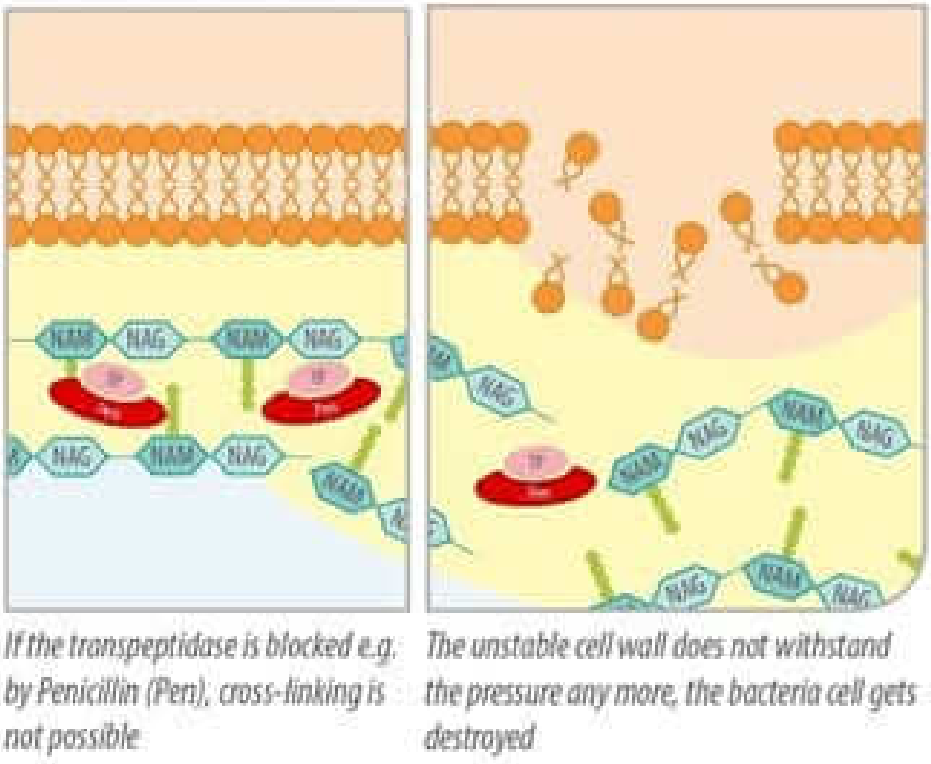
If bacteria are growing, the cell wall also has to grow. For this purpose existing conjunctions are cracked and new components are inserted. In order for the cell wall to remain a solid barrier, the new components must be interconnected by crosslinks. For the creation of these crosslinks an enzyme is essential, the transpeptidase (figure 2).

Figure 2: building up a stable cell wall with the help of transpeptidase



Due to their structure, ß-lactam-antibiotics also fit as binding partner for transpeptidase. They bind to the enzyme and block it (Kohanski et al., 2010). The crosslinks cannot be created and the stabilization of the cell wall is prevented. Disturbance of cell wall stability leads to the death of the bacterial cell, hence ß-lactam antibiotics act bactericidal.

Figure 3: blocked by ß-lactam antibiotics, transpeptidase cannot serve as enzyme for building the cell wall



The challenge: E. coli producing ß-lactamases

Resistant bacteria, which are able to produce ß-lactamases – enzymes that destroy the ß-lactam ring – prevent their own destruction. Divers point mutations within the ß-lactamase genes lead to the occurrence of “extended-spectrum-beta-lactamases“ (ESBL). ESBL are able to inactivate most of the ß-Lactam-antibiotics.

Another mutation leads to so-called AmpC (aminopenicillin and cephalosporin) ß-lactamases. They enable

the *E. coli* to express a resistance against penicillins, cephalosporins of the second and third generation as well as against cephamycins.

Phytomolecules – an alternative?

One approach to reduce the use of antibiotics is the utilization of phytomolecules. These secondary metabolites are produced by plants to protect themselves from moulds, yeasts, bacteria and other harmful organisms.

The use of plants and their extracts in human and veterinary medicine is well-established for centuries. Besides digestive and antioxidant characteristics they are well known for their bacteriostatic and bactericidal effects.

Consisting of a high number of chemical compounds, they attack at diverse points and their antimicrobial effect is not caused by only one single specific mechanism. This is crucial because it is therefore very unlikely that bacteria can develop resistances to phytomolecules like they do to antibiotics.

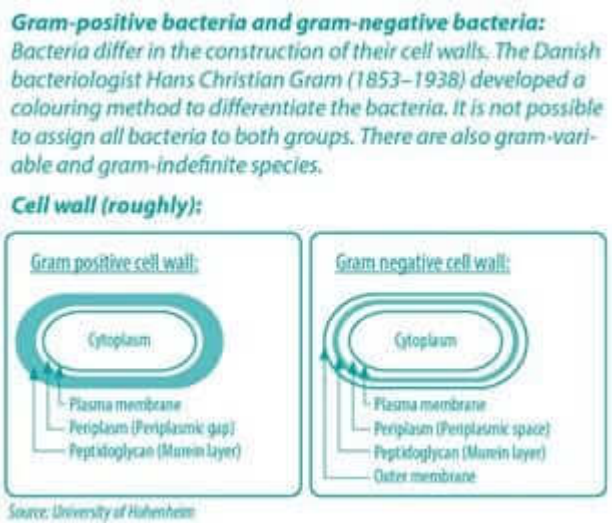
How phytomolecules work

Mostly, phytomolecules act at the cell wall and the cytoplasm membrane level. Sometimes they change the whole morphology of the cell. This mode of action has been studied extensively for thymol and carvacrol, the major components of the oils of thyme and oregano.

They are able to incorporate into the bacterial membrane and to disrupt its integrity. This increases the permeability of the cell membrane for ions and other small molecules such as the energy carrier ATP (Adenosin-tri-phosphate). It leads to the decrease of the electrochemical gradient above the cell membrane and to the loss of energy equivalents of the cell.

A special challenge: gram-negative bacteria

Gram-negative bacteria such as *E. coli* and *Salmonella* pose a special challenge. The presence of lipopolysaccharides in the outer membrane (OM) provides the gram-negative bacteria with a hydrophilic surface (Nikaido, 2003; Nazarro et al., 2013) (*see also blue infobox*).



The cell wall therefore only allows the passage of small hydrophilic solutes and is a barrier against macromolecules and hydrophobic compounds such as hydrophobic antibiotics and toxic drugs. The bypassing of the OM therefore is a prerequisite for any solute to exert bactericidal activity toward gram-negative bacteria (Helander et al., 1998).

Based on their trial results Helander et al. (1998) (1998) concluded that trans-cinnamaldehyde and partly also thymol and carvacrol gain access to the periplasm and to the deeper parts of the cell. Nikaido (1996) also concluded that OM-traversing porin proteins allow the penetration of lipophilic probes at significant rates.

Evaluating phytomolecules I – in vitro trial, Scotland

A trial conducted in Scotland evaluated the effects of Activo Liquid, a mixture of selected phytomolecules and citric acid, on ESBL-producing *E. coli* as well as on *E. coli* that generate AmpC.

Material and methods

For the trial two strains for each group were isolated from the field, a non-resistant strain of *E. coli* served as control. Suspensions of the strains with 1×10^4 CFU/ml were incubated for 6-7 h at 37°C (98.6°F) together with diverse concentrations of Activo Liquid or with cefotaxime, a cephalosporin. The cefotaxime group saved as a control for differentiating resistant and non-resistant *E. coli*.

The suspensions were put on LB agar plates and bacteria colonies were counted after further 18-22h incubation at 37°C.

Results

The antimicrobial efficacy of the blend of phytomolecules depended on the concentration at which they were used (see table 1). A bacteriostatic effect could be shown at dilutions up to 0.1 %, a bactericidal effect at higher concentrations.

Table 1: Effect of phytomolecules against resistant E. coli producing ESBL and AmpC in poultry

Poultry Microbiology Laboratory, Edinburgh, Scotland	Cefotaxime	Phytomolecules (Activo® Liquid)			
	30 µg / ml	0.1 %	0.2 %	0.4 %	0.5 %
E. coli ESBL 1 (Poultry)	-	+	++	++	++
E. coli ESBL 2 (Poultry)	-	+	++	++	++
E. coli AmpC 1 (Poultry)	-	+	++	++	++
E. coli AmpC 2 (Poultry)	-	++	++	++	++
E. coli non-resistant	+	+	++	++	++

\- no effect
\+ growth inhibiting (bacteriostatic)
++ bactericidal

Evaluating phytomolecules II – in vitro trial, Germany

A further trial was conducted in Germany (Vaxxinova, Münster), confirming the preceding results.

Material and methods

Four ESBL producing *E. coli* all isolated from farms and a non-resistant reference strain as control were tested concerning their sensitivity against Activo Liquid. Every bacteria strain (Conc.:1×10^4 CFU/ml) was subjected to a bacterial inhibition assay in an appropriate medium at 37°C for 6-7 hours.

Results

In this trial Activo Liquid also showed a dose-dependent efficacy, with no or just a bacteriostatic effect up to a concentration of 0.1 %, but bactericidal effects at a concentration of ≥0.2 % (table 2).

Table 2: Effect of phytomolecules against resistant ESBL producing E. coli in pig and in poultry

Vaxxinova GmbH, Münster	Phytomolecules (Activo® Liquid)			
	0.1 %	0.2 %	0.4 %	1 %
E. coli ATCC25922	+	++	++	++
E. coli ESBL 1 (Pig)	-	++	++	++
E. coli ESBL 2 (Pig)	+	++	++	++
E. coli ESBL 3 (Poultry)	+	++	++	++
E. coli ESBL 4 (Poultry)	-	++	++	++

- no effect
+ growth inhibiting (bacteriostatic)
++ bactericidal

Phytomolecules: a promising outlook

E. coli infections have devastating effects on animals, from diarrhea to edema, enterotoxic shock and even death. Antibiotic treatments have long been the only practicable answer. However, their excessive use – for instance, the metaphylactic application to thousands of animals in a flock – has led to the development of resistant strains. There is evidence that a reduction of antibiotic use reduces the occurrence of resistances (Dutil et al., 2010).

The results of the two in vitro trials in Scotland and Germany demonstrate the bactericidal effects of phytomolecules on *E. coli* that produce ESBL and AmpC. Using phytomolecules could thus reduce the use of antibiotics and therefore also the occurrence of AMR.

While it is theoretically possible for bacteria to also become resistant against phytomolecules, the probability of this happening is very low: unlike antibiotics, phytomolecules contain hundreds of chemical components with different modes of action. This makes it exceedingly difficult for bacteria to adapt and develop resistance. To tackle the problem of antibiotic-resistant *E. coli*, antimicrobial phytomolecules therefore offer a promising, sustainable and long-term solution.

By Dr. Inge Heinzl, Editor, EW Nutrition

Literature

Dutil, Lucie, Rebecca Irwin, Rita Finley, Lai King Ng, Brent Avery, Patrick Boerlin, Anne-Marie Bourgault, Linda Cole, Danielle Daignault, Andrea Desruisseau, Walter Demczuk, Linda Hoang, Greg B. Horsman, Johanne Ismail, Frances Jamieson, Anne Maki, Ana Pacagnella, and Dylan R. Pillai. 2010." Ceftiofur Resistance in Salmonella enterica Serovar Heidelberg from Chicken Meat and Humans, Canada." Emerg Infect Dis 16 (1): 48-54.

Helander, Ilkka M., Hanna-Leena Alakomi, Kyösti Latva-Kala, Tiina Mattila-Sandholm, Irene Pol, Eddy J. Smid, Leon G. M. Gorris, and Atte von Wright. 1998. "Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria." J. Agric. Food Chem 46: 3590-595.

Kohanski, Michael A., Daniel J. Dwyer, and James J. Collins. 2010. "How Antibiotics Kill Bacteria: From Targets to Networks." Nature Reviews 8: 423-435.

Nazarro, Filomena, Florinda Fratianni, Laura De Martino, Raffaele Coppola, and Vincenzo De Feo. 2013. "Effect of Essential Oils on Pathogenic Bacteria." Pharmaceuticals 6 (12): 1451-1474.

Nikaido, Hiroshi " Molecular Basis of Bacterial Outer Membrane Permeability Revisited. 2003. " Microbiology and Molecular Biology Reviews, 67 (4): 593-656.

Rodriguez, Tori. 2015 "Essential Oils Might Be the New Antibiotics." The Atlantic.

http://www.theatlantic.com/health/archive/2015/01/the-new-antibiotics-might-be-essential-oils/384247/

Rüben, Christiane. 2009. "Antimikrobielle Wirksamkeit von chemischen Einzelkomponenten ätherischer Öle gegenüber ausgewählten Lebensmittelverderbniserregern". PhD diss, TeHo Hannover.