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Malabsorption syndrome in broilers

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Malabsorption syndrome (MAS) is a multifactorial disease that causes intestinal disorders in broilers due to infection of the gastrointestinal tract with different infectious agents. The exact aetiology is unknown, although several viruses are isolated from MAS affected chickens. None of these isolated infectious agents alone inducted the malabsorption syndrome. MAS in broilers is characterised by poor growth and lesions in the GI-tract, mainly in the small intestine. Experimentally, MAS can be induced in one-day old broilers by oral inoculation of homogenates obtained from digestive tract tissues of MAS affected broilers. Susceptibility to the MAS syndrome differs between broiler lines. The susceptibility to MAS is correlated with the severity of the lesions, apoptosis and heterophil infiltration of the jejunum. Susceptibility to MAS is also related to the frequency of CD4 and CD8 positive T-cells in the intestinal villus and the mRNA expression level of different cytokines in control and in MAS induced broilers. With the use of micro-arrays differences in gene expression levels between broiler lines that differ in MAS susceptibility were observed. From these experiments genes that are immune and food absorption related were identified. If some of these genes or the T-cell population in the gut and the other MAS susceptible related parameters could predict or prevent MAS susceptibility in broilers needs to be further investigated but can be interestingly for breeding programmes.

Keywords: malabsorption; susceptibility; pathogenesis; immune response; gene expression

Introduction

The broiler industry encounters several diseases that are complex or of unknown origin. One of these is malabsorption syndrome (MAS) which is a multifactorial disease that causes intestinal disorders in broilers and is associated with different infectious agents, among others viruses and bacteria. This syndrome was first reported in the 1940s (Robertson *et al.*, 1949) but appeared more prominent in de 1970s (Olsen, 1977; Kouwenhoven *et al.*, 1986a). Since then the syndrome has been reported from around the

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world. The same syndrome is also described by other terms, based on clinical signs or pathological findings, such as infectious proventriculitis, helicopter disease, runting-stunting syndrome, infectious stunting syndrome, pale bird syndrome or brittle bone disease (Kouwenhoven *et al.*, 1978a; Bracewell and Randall, 1984; McNulty *et al.*, 1984). Brittle bone disease refers to the condition of stunted broilers that may have osteoporosis and fractures of the femoral heads. Pale bird syndrome is characterised by pale shanks and excessive quantities of pigment in faeces because of carotene malabsorption in chickens that are fed with maize or feed containing carotene. Helicopter chickens show retarded feathering characterized by retention of down feathers, especially on the head, incomplete feathering or alopecia especially over dorsum and wings, and splitting of primary wing and tail feathers. The incidence of helicopter disease is usually low but may reach higher levels in some flocks. Kouwenhoven *et al.* (1992) described a syndrome similar to MAS, wet litter syndrome, in older broilers from 3 weeks of age. Most likely wet litter syndrome is a subset of MAS. Nevertheless, their definitive aetiology and pathogenesis are still unknown. In this review we will focus on the pathobiology of the MAS syndrome.

MAS is a syndrome seen in broiler chicken, that is found world wide and continues to cause significant economic losses in the broiler industry due to a decreased body weight gain, increased mortality, downgraded carcass quality and secondary diseases. Broilers are most susceptible within the first two weeks of age. MAS is characterised by the widespread occurrence of stunting and uneven growth in a flock with a high culling rate, diarrhoea with undigested feed resulting in wet litter, retarded feathering, pigment loss and bone abnormalities, which occurs in the first three weeks of age, (Reece *et al.*, 1984; Reece and Frazier, 1990, Mc Nulty and Mc Farran, 1993). Accompanying clinical signs vary including distended abdomens, depression and early mortality.

There is a wide variety in clinicopathological changes due to MAS in literature. It is still unknown whether the underlying pathophysiology is based on either maldigestion or malabsorption or both. Lesions that are found can cause an impairment of digestion by insufficiency of digestive secretions and/or an impairment of absorption because of insufficient absorptive capacity. Both will result in reduced weight gain. MAS affected chickens have grossly pale and distended small intestines containing mucoid contents. The principal lesions are found in the mucosa of the small intestine and are characterized by cyst formation in the crypts of Lieberkühn and atrophy of the intestinal villi (Songserm *et al.*, 2000). Pancreatic and proventricular lesions are reported in field cases (Sinclair *et al.*, 1984), however, these lesions are rarely detected in experimentally induced MAS (Reece and Frazier, 1990; Songserm *et al.*, 2002b). Factors that influence the induction and severity of signs of MAS are the genetic background of the broiler, the condition of the one-day-old chicken, age and condition of the hen mother, nutritional, environmental stress and management disorders (Robb *et al.*, 1982; Smart *et al.*, 1988; Rebel *et al.*, 2004).

Experimental model

Kouwenhoven and colleagues (Kouwenhoven *et al.*, 1978b) showed that MAS can be experimentally induced by inoculating one-day-old broiler chickens with homogenised intestinal material collected from clinical cases of MAS affected chickens. Since then this procedure remained the only way to reproduce MAS experimentally. In *Figure 1* lesions in the jejunum are shown from experimental induced MAS. Using this model the pathobiology was studied of different intestinal homogenates obtained from chickens affected with malabsorption syndrome from different field cases in the Netherlands and Germany (Songserm *et al.*, 2000). MAS was induced by oral inoculation of newly hatched

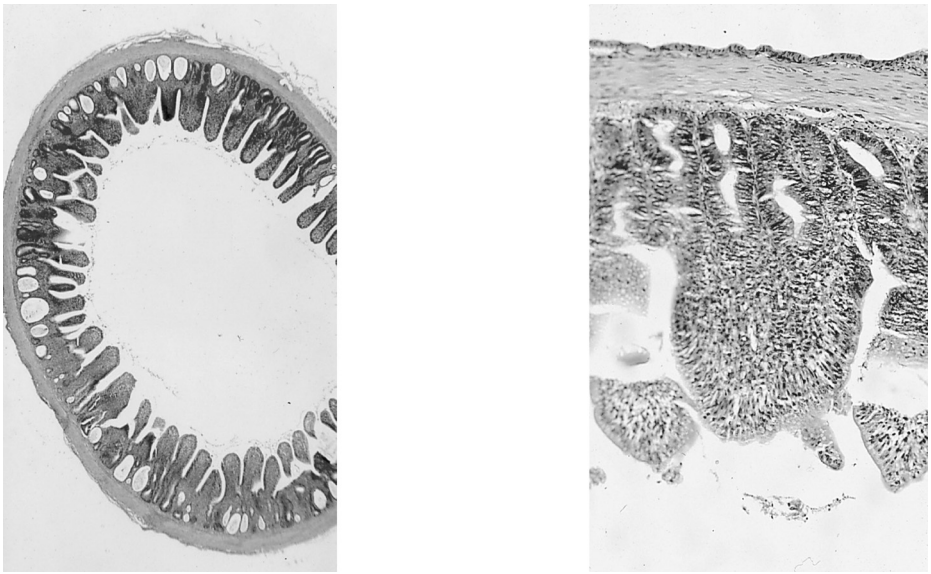

Figure 1 Jejunum of MAS affected chicken at 7 days post inoculation.
Left picture shows dilated crypts and villus atrophy in the jejunum, right picture shows fusion of villi in the jejunum.

chickens with 0,5 ml of intestinal homogenate obtained from two-week old MAS affected stunted chickens. The MAS homogenate was prepared by homogenising the small intestine (duodenum including the pancreas, jejunum and ileum) as a 25% suspension in tryptose phosphate broth (TPB). After homogenising this in a blender, it was centrifuged at 2000 xg for 10 min. and the supernatant was collected and frozen at -70°C until use. The frozen material was thawed immediately before inoculation of the chickens (Songserm *et al.*, 2000). It was observed that not all inoculated homogenates gave the same severity in lesions in the small intestine and the same growth retardation. However, the lesions, growth retardation and retarded feathering that were found experimentally with some homogenates, were highly similar to the ones found in chickens in the field. Therefore to compare experimental received results between different animal experiments the same origin of the MAS homogenate need to be used.

Aetiology

The Malabsorption syndrome has been termed a multifactorial disease since the identical syndrome could not be successfully reproduced using only one factor or agent derived from affected chickens. Some groups suggest that the syndrome is not specific for any disease (Goodwin *et al.*, 1993; Montgomery *et al.*, 1997) Meanwhile other groups agree that it is an infectious disease because of its transmissible nature (Olsen, 1977; Kouwenhoven *et al.*, 1978b). The MAS aetiology is mainly associated with pathological changes of the gut. Despite many efforts to elucidate the exact cause(s) of MAS, the aetiology of MAS is yet not established although several viruses and bacteria were suspected as aetiological causes

Initially, reovirus was believed to be the major causative agent of MAS and several enteric reovirus strains were identified in MAS affected chickens (Rekik *et al.*, 1987;

Kouwenhoven *et al.*, 1988; van Loon *et al.*, 2001; Kant *et al.*, 2003). In addition, induction of a mild form of intestinal lesions was reported in SPF chickens with enteric reovirus infection (Shirai *et al.*, 1990; Songserm *et al.*, 2003). However, neutralisation of reovirus from the infective homogenate or vaccination of breeder hens against reovirus did not reduce the severity of MAS (Eidson *et al.*, 1985). Other virus types, which have been associated with MAS, are adenovirus, enterovirus-like virus, rotavirus, parvovirus and togavirus-like partikels (Kouwenhoven *et al.*, 1978b; McNulty *et al.*, 1984). The virulence of these viruses may be highly variable, especially reovirusses that have different strains and serotypes (Kant *et al.*, 2003). The capacity to induce intestinal lesions differs and so are the predilection sites or targets of these viruses.

Several bacteria have been associated with the syndrome as well, like Escherichia coli, Proteus micabilis, Enterecoccus faecium, Staphylococcus cohnii, Clostridium perfringes, Bacteroides fragilis and Bacillus licheniformis (Montgomery *et al.*, 1997). However, most of them can also be present in the microflora of the GI-tract of healthy chickens. Possibly, bacteria play a role in the syndrome as secondary agents that can aggravate the lesions in the intestine resulting in malabsorption and maldigestion.

However, none of these agents is capable of causing all signs of MAS by itself. Kouwenhoven *et al.*, (1986a; 1986b) suggested that a combination of virus and bacteria is involved in the etiology of MAS. Smart *et al.* (1988) stated that bacteria alone did not cause MAS. Montgomery *et al.* (1997) attempted to reproduce MAS in one-day-old chicken by using several combinations of agents isolated from MAS affected chickens. Although they could reproduce weight gain depression, the intestinal lesion was not the same as found in MAS chickens. Kouwenhoven *et al.* (1988) could not reproduce MAS by using intestinal homogenate containing only reovirus and other agents after methanol or chloroform treatment. A combination of enterovirus and gut content could induce MAS, although intestinal lesions were not investigated.

In an experimental model Songserm *et al.* (2002b) also studied the aetiology of MAS. From intestinal homogenate of MAS affected chickens' reovirus, haemolytic *E. coli, Pasteurella hemolytica* and *Enterococcus durans* were isolated. The effects on weight gain depression and occurrence of intestinal lesions as cystic crypts of Lieberkühn and atrophy of intestinal villi in chickens were compared after inoculation of the 1-day old chickens with the individual infectious agents. None of these pathogens alone reproduced MAS in broilers (Songserm *et al.*, 2002b; 2003).

To confirm the infectious origin of the syndrome, chicken were inoculated either with intestinal homogenates from healthy chickens or formalin-treated intestinal homogenates from healthy chickens or formalin treated intestinal homogenate from MAS affected chickens, but no weight gain depression or intestinal lesions were observed in any of the groups. However, when reovirus, isolated from MAS affected chickens, was inoculated in combination with formalin treated homogenates of MAS affected intestine or with homogenates of healthy chickens the intestinal lesions and body weight gain depression were reproduced, although changes were not as severe as when non formalin treated homogenate of MAS affected intestine was used. Hemolytic E. coli in combination with reovirus and formalin treated homogenate from MAS affected intestines did not induce weight gain depression although this combination caused the intestinal lesions as described for MAS affected intestines. These lesions were not as severe as the lesions caused by the intestinal homogenate of MAS affected chickens (Songserm *et al.*, 2002b). These results suggested that reovirus in combination with substance(s) in the intestinal homogenates, from healthy or MAS affected chickens, play a role in weight gain depression.

Thus far, a single causal agent for MAS could not be established and therefore MAS is recognised as a multifactorial disease involving a combination of pathogens.

Pathogenesis

The jejunum is the part of the intestine that is most affected by MAS. MAS affected chickens develop severe enteritis with cystic deformation of the crypts of Lieberkühn and atrophy of the intestinal villi as described by Reece and Frazier (Frazier and Reece, 1990; Reece and Frazier, 1990). In the acute phase, dilated crypts of Lieberkühn or small cytic crypts are present. In this stage occasionally hypertrophy of globet cells is observed. At a latter stage, the crypt cells become more degenerated and detached from the crypt wall resulting in flattening of the crypt wall and larger cysts. The cysts get filled with cellular debris and degenerated cells. Villus atrophy is more pronounced when the cyst are larger. The actual pathogenesis of these mucosal lesions is not clear. Some authors suggested necrosis of the crypt epithelium due to viral and bacterial infections (Frazier and Reece, 1990).

Many authors suggested impaired enzymatic digestion due to disturbances of the exocrine pancreatic function as a primary factor in MAS pathogenesis (Sinclair *et al.*, 1984; Szabo *et al.*, 1989). Reduced enzyme activities such as glutamyltransferase, leucylaminopeptidase, amylase, trypsin, chymotrypsin, lipase, and other enzymes were reported in MAS affected chickens (Mazurkiewicz *et al.*, 1993). An increase in plasma alkaline-phosphatase (AP) level was earlier considered a clinico-pathological feature in MAS (Kouwenhoven *et al.*, 1988). The disturbances of the digestive enzymes can be a secondary effect of the intestinal lesions rather than being the primary factor in the pathogenesis. Nevertheless, it is possible that the pathogenesis of MAS varies between cases depending on the infectious agents involved.

Villus atrophy can be caused by an increased epithelial apoptosis or inhibition of cell proliferation. Normally, the gut mucosa is maintained by regular renewal of the surface epithelium by proliferation of stem cells at the base of crypts, migrating of these cells to the tip of the villus, and then apoptosis (Pritchard and Watson, 1996; Mayhew *et al.*, 1999). A disturbance of this regular intestinal renewal, stem cell proliferation and apoptosis, could lead to intestinal damage as is shown for different gastrointestinal tract diseases as rotavirus infection and ulcerative colitis (Iwamoto *et al.*, 1996; Guy-Grand *et al.*, 1998; Shirin and Moss, 1998; Boshuizen *et al.*, 2003). A disturbance in maintenance of the intestine by apoptosis and proliferation could play a role in the pathogenesis of the mucosal lesions in MAS.

Zekarias *et al.* (2005), studied the early pathogenesis of the mucosal lesions in experimentally MAS affected chickens by comparing the leukocyte response in the intestinal mucosa, epithelial apoptosis and proliferation. The intestinal mucosal lesions in MAS affected chickens developed vacuolar degeneration and apoptosis of the villous epithelial cells at day 1 post inoculation. This was accompanied with crypt hyperplasia and heterophil infiltration. Acute heterophilic inflammation was a major feature in the early phases of MAS development preceding crypt epithelial apoptosis. Subsequently, there was cystic dilation of crypts and villous atrophy. Heterophil infiltration can be beneficial in host defence (Kogut *et al.*, 1994). However, tissue damage often results from the accumulation of heterophils in tissues (Madara *et al.*, 1991; Harmon, 1998). Infiltration of the intestinal mucosa by polymorphonuclear leukocytes (PMNL) in association with epithelial apoptosis is observed in other gastrointestinal infections (Madara *et al.*, 1991; Iwamoto *et al.*, 1996). The exact role of heterophils in the pathogenesis of epithelial apoptosis is not clear. The severity of MAS was correlated with the severity of the lesions in the jejunum and the level of heterophils infiltration.

MAS Susceptibility

PATHOBIOLOGY

Shapiro *et al.* (1998) have shown that broilers are more susceptible to MAS than are Leghorn chickens and turkey poults. Contrary to layer chickens, broilers have a faster development of intestinal morphology and activities (Yamauchi and Isshiki, 1991; Yamauchi *et al.*, 1992). The difference in severity of the intestinal lesions and the reduction in body weight gain among genetic lines of commercial broiler chicks reflected the difference in susceptibility to MAS between broiler lines (Songserm *et al.*, 2002a). The crypt and villi apoptosis and formation of cystic crypts were more severe in the susceptible broiler chickens

It is interesting to note that the age of susceptibility for MAS, the first two weeks of age, is in the same period in which fast development and differentiation of enterocytes in broilers takes place. Uni *et al.* (1995) have shown that villus volume and enterocyte density are different in two broiler lines. Zekarias *et al.* (2002) have investigated whether differences in intestinal morphology and organ development reflected susceptibility differences to MAS between broilers lines. Broiler lines were used that differ in MAS susceptibility to investigate whether an association between susceptibility and specific traits in the genetic background could be established. Differences in the development of organs or the body weight gain is not the cause of the MAS susceptibility.

In another study Zekarias *et al.* (2005)showed that in the onset of MAS there was an association between heterophil influx and the onset of apoptosis of the crypt epithelium. MAS induced chickens showed crypt hyperproliferation and increased epithelial turnover. In this study it appeared that the difference in susceptibility to MAS was related to the influx of heterophils and the onset of apoptosis. Difference in heterophil recruitment could be a major factor in the susceptibility differences between the two broiler lines. The intestinal heterophil infiltration could be triggered by pro-inflammatory cytokines. These cytokines could be produced by the affected villous epithelium and leukocytes in the lamina propia (Madara *et al.*, 1991; Jung *et al.*, 1995). Interestingly, the heterophil infiltration was significantly more pronounced in the susceptible broilers than the resistant broilers. We have reported previous that at 1 day of age the chickens of line S have higher proportion of circulating heterophils in peripheral blood (Zekarias *et al.*, 2002). This may be related to the greater heterophil infiltration into the intestinal mucosa in the MAS affected chickens of the MAS susceptible line compared to chickens of less MAS susceptible line. Concurrent with the epithelial apoptosis, there is hyperproliferation of crypt epithelium in MAS affected chickens. The crypt hyperproliferation could be a reaction to the epithelial apoptosis or could be stimulated by inflammatory cytokines (Stappenbeck *et al.*, 1998).

IMMUNE RESPONSE

Intensive genetic selection for fast growth rate in chickens over the last 40 years has decreased the time for a broiler to reach its slaughter weight enormously. The difference in growth rate between strains occurs already within the first weeks of life (Gavin and McDevitt, 1999). In quails it was described that the digestive organs in the embryo of a fast growing strain develop more rapidly in time than of the slow growing strain (Lilja and Olsson, 1987; Lilja and Marks, 1991). Due to the intensive selection of broiler chickens for fast body weight gain and other production traits several unfavourable indirect selections may have occurred. These indirect selections may result in a decrease in general resistance leading to disease susceptibility and or a bad adaptation capacity against enteric disorders. It is described that genetic selection for superior growth affects the cell mediated immune responses and the ratio of CD4+ to CD8+ T-cells in turkeys (Bayyari *et*

al., 1997; Li *et al.*, 2000). Thus genetic background may play a role in susceptibility to MAS.

Differences in immune competence in particular in the gut mucosal immunity and the reactivity patterns during infection could be sources for the differences in MAS susceptibility. Since chickens are susceptible to MAS immediately after hatching, at which time the gut mucosa and the systemic immune system are less well developed, differences in the development of the innate and adaptive immunity at early age could be a crucial factor in susceptibility differences to MAS (Bar Shira *et al.*, 2003). Broilers that differ in MAS susceptibility differed significantly in their frequency of CD4/CD8 positive intestinal cells and amount of goblet cells in the intestine, both under challenge and non-challenge conditions (Songserm *et al.*, 2002a; Zekarias *et al.*, 2002). The "resistant" broiler line at day 1 of age had higher percentages of peripheral blood leukocytes, especially lymphocytes. At 3 and 8 days the "resistant" line had less CD8 positive T cells in the small intestinal villi detected with immunohistochemistry, while the amount of CD4 positive cells was slightly higher in the "resistant" line as compared to the susceptible chickens (*Figure 2*) (Zekarias *et al.*, 2002). When MAS was inoculated in these broiler lines the number of CD8 positive cells in the small intestinal villi in the susceptible line was higher as compared to the "resistant" line (Songserm *et al.*, 2002a). With the use of real-time PCR the mRNA expression levels of IFN-gamma, IL-2, IL-6, IL-8 and IL-18 in the intestine were investigated (Rebel *et al.*, 2005).

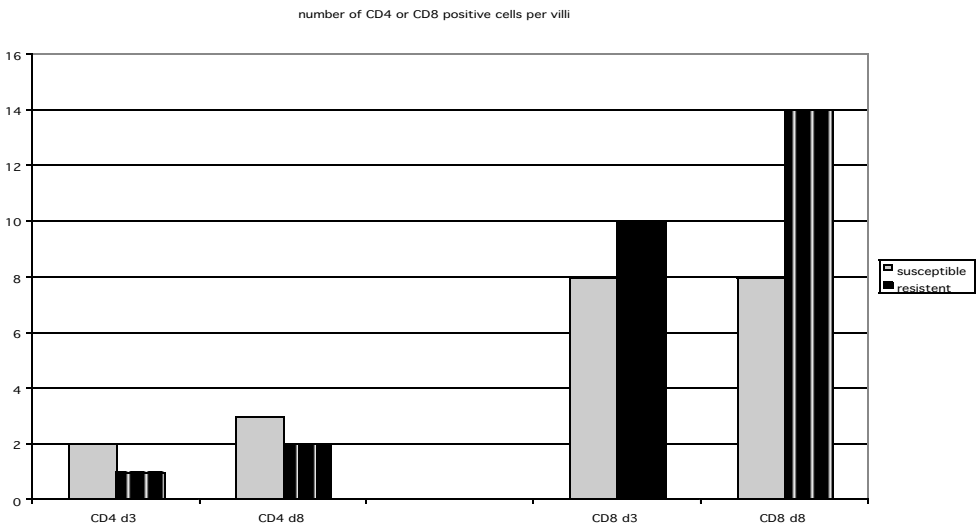

Figure 2 Mean number of CD4 or CD8 positive cells per villus in the jejunum at the age of 3 or 8 days in control chickens. The two control broiler lines showed are either susceptible or more resistant for MAS. Adapted from (Zekarias et al., 2002).

Due to the differences observed in proportions of CD4 positive and CD8 positive cells in the intestine and the differences in heterophil infiltration in the intestine after MAs induction of the two broiler lines we hypothesised that these two broiler lines differed in their immune reaction and might therefore differ in their susceptibility to MAS. To study such difference in immune reactivity the cytokine responses of intestinal cells in control and MAS stimulated "resistant" and susceptible broilers were investigated. With the use

of a real time PCR the mRNA expression levels of IFN-gamma, IL-2, IL-6, IL-8 and IL-18 in the intestine were investigated. The "resistant" chickens had at day 1 and 3 higher mRNA basic levels in the jejunum of the non-inoculated control chickens of IL-2, IL-6, IL-18 and INF-gamma as compared to the jejunum of the susceptible chicken line (*Figure 3*). The broilers of the susceptible line reacted with higher transcription of mRNA levels at 3 or 5 days post infection of INF-gamma, IL-2, IL-6 and IL-8 in the jejunum after MAS induction compared to the resistant line. From the T-cell profiles together with the cytokine mRNA profiles of the intestines it was concluded that the susceptible line reacted with a more cell mediated T-helper response to a MAS infection compared to the "resistant" line. This is in agreement with the recruitment that was observed of cytotoxic T-cells and heterophils (Songserm *et al.*, 2002a; Zekarias *et al.*, 2005). Such insufficient or uncontrolled reactivity at the mucosal surfaces might lead to the severe damage that was observed in the intestine of the sensitive line.

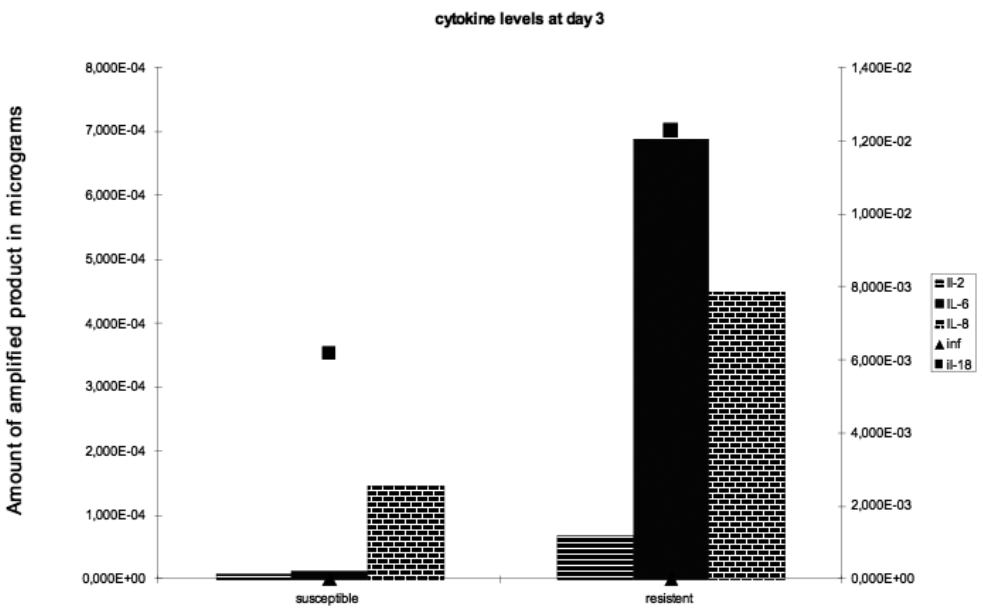

Figure 3 Mean cytokine mRNA expression levels of three day old chicks of the same chicken lines as shown in Figure 2. Note the difference in expression levels of the analysed cytokines in control situation of two different broiler lines.

GENE EXPRESSION

Examination of the host gene expression response upon encounter with pathogens may provide insight into the cellular events following an infection. In addition it may shed light on the basic mechanisms underlying differences in the susceptibility of the host. Genes associated with disease susceptibility may be discovered by comparing on a genome-wide scale susceptible and 'resistant' lines under control and challenge conditions (Yonash *et al.*, 1999; Liu *et al.*, 2001). Identifying potential important genes for disease susceptibility in chickens may be done with a number of different techniques. cDNA microarrays however are a recommended technique to study mRNA expression profiles of many different genes simultaneously (Meltzer, 2001). Gene expression technology is a powerful

tool that has already been used to expand the understanding of host-pathogen interactions. A number of reports have been published about host transcriptional responses to infectious agents as Salmonella using gene arrays (reviewed in (Rosenberger *et al.*, 2001). Also studies have been done to investigate gene expression in relation with host susceptibility. Genes are differentially expressed between chicken lines that differ in their susceptibility to an Eimeria Acervulina infection or to Marek's disease (Choi *et al.*, 1999; Kaiser *et al.*, 2003). Nowadays expressed sequence tags (ESTs) from chickens are available in the public database (Wong *et al.*, 2004) and chicken gene expression arrays have been generated (Min *et al.*, 2003; Caldwell *et al.*, 2004). One of the described arrays is a chicken jejunum cDNA microarray. This microarray consisted of ESTs of a normalised and subtracted chicken jejunum cDNA library. Randomly chosen clones were sequenced for control purposes. New ESTs were found and multiple ESTs not identified in the chicken intestine before were observed (Van Hemert *et al.*, 2003). In order to study host specific differences that could be associated with MAS susceptibility differences in intestinal gene expression of two broiler lines were studied.

The gene expression was investigated at six different timepoints post inoculation under MAS challenge conditions and in age matched non-challenged chickens (Van Hemert *et al.*, 2004). Marked differences were observed in mRNA expression profiles between two broiler lines that differ in MAS susceptibility, in age matched non-challenged chickens as well as in the MAS affected animals. Differences in gene expression between non-challenged chickens which differ in MAS susceptibility were detected in chickens at 11 days of age. These genes were not differentially expressed at day 11 post infection (age 11 days old) in chickens of either line after inoculation with a MAS homogenate. Possibly these genes are involved in intestinal development and both chicken lines regulate their intestinal development in a different matter. After MAS induction more genes at different time points post MAS inoculation were up- or downregulated in the jejunum of the susceptible broiler line when compared to the genes of the intestine from a MAS induced resistant broiler line (*Table 1*) (Van Hemert *et al.*, 2004). In the MAS affected situation, 15 genes differed more than fourfold in expression between the MAS susceptible and MAS resistant broiler line. These genes were differentially expressed at day 1, 7 or 11 post MAS induction. Some of these genes were expressed at a higher level in the susceptible line while others had a higher expression level in the resistant line. All these genes lacked significant expression differences in non-challenged age matched broilers between the two lines. Thus differences in MAS susceptibility could be due to differences in gene regulation upon MAS induction or to a difference in intestinal development of the two tested chicken lines.

Table 1 Total number of differentially expressed genes[1] in Malabsorption affected chickens from 8 hours until 11 days post inoculation.

Number of genes upregulated after a mas infection	
Susceptible line	78
Resistant line	51
Number of genes downregulated after a mas infection	
Susceptible line	43
Resistant line	12

[1]A gene was declared differentially expressed when the mean value of the ratio was >2 or <-2 and the gene was identified with significance analysis of microarrays with a False discovery rate <2%.
Adapted from (Van Hemert *et al.*, 2004)

Concluding remarks

Susceptibility to MAS is not related to the development of organs but is probably related to the number and proportion of CD4 and CD8 positive cells and to levels of mRNA cytokines. Also, MAS susceptibility is correlated with the cellular reaction upon MAS induction. The heterophil influx and the possibly correlated onset of epithelial cell apoptosis, the cytokine reaction profiles and a correlated the direction of immune reaction and the differences in gene regulation are all reactions induced by MAS that differed in broiler lines with different MAS susceptibility. When we used cytokine profiles together with propotions of CD8 and CD4 cells in the intestine, together with percentages of heterophils and lymphocytes in the blood in non-challenged broilers of the age of day 1 until day 5 we were able to predict MAS susceptibility (own observations). When these parameters could be mangerial modified, broilers could be less affected by MAS. A particular management influence could be encompass specific feeding of either the mother hen or the broiler. With nutritional measures, it is possible to change the immune response or the intestinal development of the broiler chick (Davis and Sell, 1983; Coskun *et al.*, 1998; Erf *et al.*, 1998; Uni *et al.*, 1998). It is also possible to change the severity to a MAS induction when changing the diet of the mother hen (Rebel *et al.*, 2004). At this time it is not known if breeder feed can change the cytokine profile in the intestine or the percentage of CD4 CD8 positive cells in the intestine in a direction that it also influences MAS susceptibility. It is known however that the gene expression in the jejunum of the chick is influenced by the diet of the mother-hen (own observations). Thus with breeding programmes or with diet of the mother hen (prenatal-programming) the MAS susceptibility of the chicks can be influenced.

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