The threonine requirement of broiler chickens during subclinical intestinal infection

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Besides the threonine (THR) requirement to obtain good performance of broilers, THR is a major component of intestinal mucins and digestive secretions. It is suggested that the THR requirement increases when intestinal metabolism is disturbed by increased microbial activity. Less threonine may be available for growth and performance decreases. A 20-d experiment was performed to determine the THR/LYS ratio in broilers during subclinical *Clostridium* infection. 612 Ross 308 day-old male broilers were allocated on weight to 36 litter pens. A wheat/barley-based diet was used with an AME of 12.1 MJ/kg and CP of 19%. Besides an uninfected and infected control, treatments consisted of diets varying in standardized ileal digestible THR/LYS ratios from 0.56 to 0.75 (0.65 is the current advice). Results of NE incidence were analysed by Fisher Exact and lesions severity and production parameters by ANOVA.
Results showed no effect of THR/LYS ratios on NE-incidence or lesion severity, but although higher THR/LYS ratios resulted in a similar reduction in FI as THR/LYS ratios, BWG and FCR were improved. Although not statistically proven, it seems that increasing THR in the diet prevents body protein be broken down and thereby alleviating the consequences of NE-infections in poultry on production performances.

Keywords: threonine; THR/LYS ratio; necrotic enteritis; broilers

Introduction

Clostridium perfringens, an α-toxin producing Gram-positive bacterium, is an enteric pathogen for poultry, causing necrotic lesions in the small intestinal mucosa. *Clostridium* infections are a potential challenge to broiler production especially since antimicrobial growth promoters are banned from poultry diets.

Besides the threonine (THR) requirement to obtain good performance of broilers, THR was found to be a major component of intestinal mucins and digestive secretions (Ball et al., 1999). Therefore, THR is considered to contribute to the intestinal metabolism and maintenance of a proper digestive function. It is suggested that the THR requirement of the intestinal tract may increase when intestinal metabolism is disturbed by increased microbial activity as is the case in a *Clostridium* infection. Less THR may be available for growth and performance decreases. At Schothorst Feed Research a broiler experiment was performed to determine the THR requirement of broilers during a subclinical intestinal infection.

Materials and methods

The experiment comprised seven treatments including three control treatments. The control treatments consisted of four replicate cages whereas the experimental treatments consisted of six replicate cages each (17 broilers per cage). Dietary treatments were based on varying THR/LYS ratios

from 0.56 to 0.75 with 0.65 being the current advice. Dietary treatments and the THR/LYS ratios of supplementation of the additives are given in Table 1.

Table 1 Description of the treatments and diet codes

Trt	Description	THR/LYS ratio	Dig. LYS	Feed code per period	
			g/kg	0 – 9 days	9 – 20 days
1.	negative control (birds not infected)	0.65	10.0	A	B
2.	negative control (birds solely infected with *Eimeria*)	0.65	10.0	A	B
3.	positive control (birds infected with *Eimeria* and *Clostridium)* [1)]	0.65	10.0	A	B
4.	As treatment 3	0.56	10.0	A	C
5.	As treatment 3	0.60	10.0	A	D
6.	As treatment 3	0.70	10.0	A	E
7.	As treatment 3	0.75	10.0	A	F

[1)] 10,000 of sporulated oocysts of *Eimeria maxima* in 1 ml and 1×10^8 cfu *Cl. perfringens* in 1 ml

700 Ross 308 male one-day-old broilers were purchased and housed until day 9 in a litter pen in which water and starter feed was supplied for *ad libitum* intake.

At day 9, broilers were individually weighed and 612 broilers were housed in battery (litter)cages in the laboratory facilities at Schothorst Feed Research, in such a way that the mean weight of 17 broilers per pen was similar in all 36 cages. Birds had free access to feed with exception of the 5 hours before inoculations and post mortem examinations (d9, d14, d15, d16 and d20). A commercial starter diet was fed until day 9. From day 9 on, a grower diet was formulated based on approximately 40% wheat and 25% barley (AMEn 2880 kcal/kg) and fed as pellets. The nutrient, mineral and vitamin composition of all diets were nutritionally adequate according to the CVB (2005) requirements for broilers. The starter and grower diet did not contain any coccidiostats or antimicrobial feed additive.

A lighting schedule of 20 hours light and 4 hour darkness was used throughout the experimental period. The ambient temperature was gradually decreased from 32ºC at arrival according a standard temperature schedule. At the hatchery the birds were vaccinated against IB. The experiment was finalised at day 20 of age.

FI were measured from 0-9 days and daily from day 9 to 20 and BW of the birds selected for lesion scoring was measured on days 15, 16 and the final day of the experiment. On these days coccidiose lesions and necrotic enteritis lesions in the small intestinal mucosa on four birds per cage were determined on a scale of 0 to 4 (0= no lesions, 1 to 3= increasing severity of lesions and 4= died of NE).

The incidence of NE-lesions (% of affected birds) was analysed by Fisher Exact Test and the severity of lesions and daily feed intake measurements were analysed by ANOVA (Genstat). The experiment was carried after approval of the experimental protocol by the Dutch Animal Experimental and Ethics Committee.

Results and discussion

Healthy one-day-old broilers arrived at the institute with an average body weight of 43 grams. The course of the experiment was without any problems. At first, the efficacy of the model was tested by comparing the NE incidence in both the positive and negative control group. It was shown that the model was effective because the NE incidence was significantly higher in the positive control group than in the negative control group (55% versus 0% at day 15 with P= 0.002). Second, an effect of varying THR/LYS ratios was tested by comparing the NE incidence as well as lesion severity among treatments (with treatment 3 being the positive control with advised THR/LYS ratio of 0.65). In Figure 1, the percentage of positive scored birds (birds with NE lesions) is given as well as the mean lesion score of all positive scored birds at 1 day p.i. The columns indicate the incidence of NE positive birds as a

percentage of total observed birds in a treatment. The severity of lesions is given above each column and indicates the severity of lesions observed in NE positive birds.

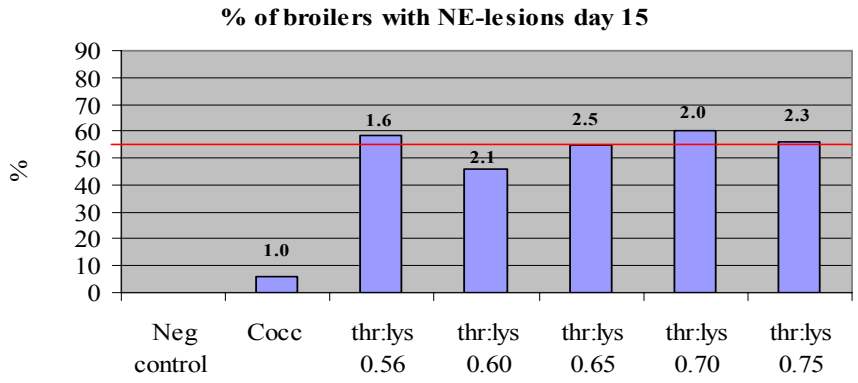

Figure 1 Percentage and severity of NE-lesions in broilers scored on day 15 (1 day p.i.). The horizontal line indicates the incidence of the positive control (treatment 3).

It was concluded that there was no significant treatment effect on the severity of necrotic lesions on day 15 ($P< 0.719$). No significant difference was observed among treatments, which means that the severity of necrotic lesions was similar in all experimental treatments. No significant treatment effects were observed on the NE incidence as well. Higher THR/LYS ratios did not result in a significantly lower incidence of necrotic lesions.

The daily FI was measured from day 9 to day 19. The reduction in FI after the successive inoculations with *Eimeria* and *Clostridium* is an indication of the severity of the infection, as the inflammatory response following the infection causes a loss in appetite and reduced FI. In this experiment a maximal reduction in FI of 47% was observed on day 15, whereas FI became almost normal in a period of five days p.i. When daily FI was compared among treatments, no statistical differences were observed until day 16. At day 16, there was a tendency for a quadratic effect of the treatments on FI. From these results it was clear that a THR/LYS ratio of 0.70 did result in the highest FI and, numerically seen, this started already at day 15 and prolonged until day 18. Although, no significant differences in BWG were observed among the treatments with infected birds, broilers fed a diet with THR/LYS ratio of 0.70 had consequently the highest BWG as well, which consequently resulted in the smallest reduction in growth (not significant). In Figure 2, the BWG of the healthy control was set on 100% and the relative reduction in growth and the relative increase of FCR of the other treatments are presented as a percentage of the control. In Figure 3, the results of FCR of the infected birds were compared to the healthy control in a similar way.
A higher severity of lesions normally correlates with a higher reduction in FI. The treatments having a high lesion severity showed indeed a lower FI due to the inflammatory response and consequently a lower BWG and higher FCR. Because Results showed no effect of THR/LYS ratios on NE-incidence or lesion severity, there was a similar reduction in FI for all THR/LYS ratios although higher THR/LYS ratios resulted in improved BWG and FCR.

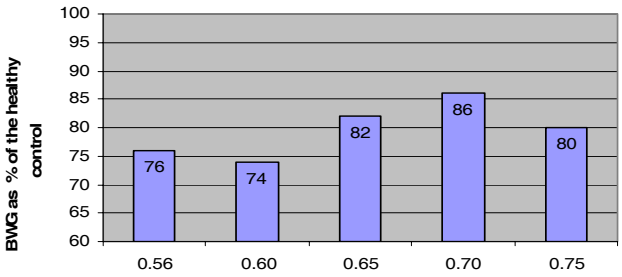

Figure 2 Body weight gain of the infected treatments with increasing THR/LYS ratios as a percentage of the uninfected control group.

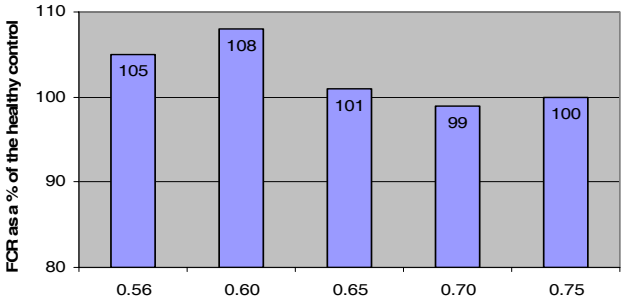

Figure 3 The feed conversion ratio of the infected treatments with increasing THR/LYS ratios as a percentage of the uninfected control group.

It can be concluded that higher thr:lys ratios do not prevent the severity and incidence of *Clostridium* lesions, but alleviate the consequences of infection. In case of a *Clostridium* infection the mucus production is increased as part of the inflammatory response thereby increasing the threonine requirement as threonine is a major component of intestinal mucins. The combination of a higher demand of threonine for mucus production and lower supply because of a reduction in feed intake will lead to a shortage of threonine. To increase the supply, body protein will be broken down and animal performance will decrease. This explains why higher thr:lys ratios did not prevent lesions and/or a reduction in feed intake, but seem to alleviate the reduction in body weight gain and consequently feed conversion ratio.

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