PRELIMINARY STUDY TO DETERMINE THE THREONINE REQUIREMENT OF SPECIFIC HUMORAL IMMUNE RESPONSE IN REARING DUCKS

Veronika HALAS [1], Zsolt SZEGEDI [2], József NAGY [3]

ABSTRACT

Dietary threonine supply is reported to determine the quality of immune response in challenged animals, however, few data on threonine requirement of ducks is available. Therefore, the aim of the present trial was to study specific humoral immune response of ducks fed surplus threonine (+15 or +30%) above to the recommendation of the breeding company. For that purpose a total of 1050, 7 weeks old breeding ducks were used in the trail. The birds were assigned into 3 groups and fed with a commercial growing duck breeder diet supplemented with different doses of threonine. Dietary threonine levels were 0.59, 0.68 and 0.77 g per kg of feed. The body weight and feed intake of the birds were controlled weekly. All birds were immunized by live, freeze dried duck plague virus (*Duck herpesvirus 1*) at 16 and 20 weeks of age. Blood samples for virus neutralization (VN) tests were taken from 14 birds per treatment just before the first immunization (week 16), 2 weeks after (week 18) and 2 weeks after the 2nd immunization (week 22).

Our data show that the specific humoral immune response to duck plague virus tended to be higher in birds fed surplus 15% threonine (P = 0.083). Higher supply, however, resulted in no difference in VN test compared to the group fed with the recommended dietary threonine level. Therefore it can be concluded that elevated dietary threonine supply boosts the immune defense of rearing ducks, however, the supplementation of surplus dietary threonine needs some caution.

Key words: ducks / immunology / immune response / duck plague virus / animal nutrition / threonine

1 INTRODUCTION

Threonine is required primary for maintenance purposes (Fuller *et al.*, 1989). Epithelial cells and mucus protein contain relative high amount of threonine (28–35%, according to Nichols and Bertolo, 2008), as well as it is one of the main components of IgG (Smith and Greene, 1947). It has been reported in pigs that at sufficient threonine supply, 40–50% of ingested threonine is directly used in the gut by epithelial cells; however, this value can be even 90% if dietary threonine content is limited (reviewed by Stoll, 2006). As reviewed by Wang *et al.* (2009) under inflammatory condition threonine availability may become limited for the synthesis of in-

testinal mucins. However, according to results of Faure *et al.* (2007) from rat study, increased threonine supply can promote mucin synthesis and equilibrate the gut micro-biota to support intestinal barrier functions.

Some data from broiler studies suggest that under low hygiene condition chickens need higher Thr to Lys ratio in their feed for the maximal growth performance (Kidd *et al.*, 2003; Corzo *et al.*, 2007). Moreover, Bikker *et al.* (2006) found that the average daily gain of growing and fattening pigs (25–110 kg) improved when the NRC (1998) recommended Thr to Lys ratio increased from 56% to 70%, and pigs fed antibiotic free diet with 70% of Thr to Lys ratio did grow with the same rate as pigs received diets supplemented with antibiotic growth pro-

1 Corresponding author. Kaposvár Univ., Fac. of Animal Science, Dept. of Animal Nutrition, P.O.Box 16, 7401 Kaposvár, Hungary, e-mail: halas.veronika@ke.hu
2 Bács-Tak kft., Mélykút, Malom u. 2, Hungary
3 Somogy County Government Office, Food Chain Security and Animal Health Directorate, Kaposvár, Cseri major, Hungary

moter (30 ppm Salinomycin). Therefore, it can be concluded that under antibiotic free feeding the threonine requirement of farm animals should be reconsidered (Halas *et al.*, 2006).

Li *et al.* (1999) showed that increasing dietary threonine level above the requirement for the maximal growth enhanced the humoral immune response, particularly the IgG production in young swine. It has been reported that threonine supply determines the quality of immune response in challenged animals. Furthermore, increasing dietary threonine supply enhanced antibody production, serum IgG levels in young pigs challenged with *Escherichia coli* (Wang *et al.*, 2006).

Although threonine is an essential amino acid in ducks like in other species, the World wide used NRC (1994) for poultry species does not give data on threonine requirement of ducks. Therefore, the aim of the present field trial was to study the plague virus specific humoral immune response of ducks fed surplus threonine (+15 or +30%) above to the recommendation of the breeding company.

2   MATERIAL AND METHODS

2.1   ANIMALS AND DIETARY TREATMENTS

A total of 1050 Cherry Valley female ducks were assigned to be used in the experiment. The birds arrived at 1-day-of age and were settled into 3 groups. Each group was identical with an experimental treatment. The trail started at 7 weeks of age and took 15 weeks until 22 weeks of age. Until 7 weeks of age ducks were fed with commercial starter diet and during the trail with growing duck breeder diet supplemented with different doses of threonine. The basal diet was formulated in corn (48.7–48.9%) wheat (25.0%), sunflower meal (6.0%) and soybean meal

Table 1: Nutrient content of the experimental diets

	Thr-100	Thr-115	Thr-130
ME (MJ/kg)	11.95	11.95	11.95
Crude protein (g/kg)*	148	151	149
Total lysine (g/kg)*	6.6	7.0	6.6
Total methionine (g/kg)*	2.2	2.3	2.3
Total Met + Cys (g/kg)*	4.5	4.7	4.6
Total threonine (g/kg)*	5.8	6.6	7.8
Total tryptophan (g/kg)	1.9	1.9	1.9
Ca (g/kg)	10.0	10.0	10.0
Digestible P (g/kg)	4.0	4.0	4.0

* analyzed values

(12.7%). Dietary threonine levels were 0.59, 0.68 and 0.77 g per kg of feed in Thr-100, Thr-115 and Thr-130 treatments, respectively. The nutrient content of the experimental feeds are shown in Table 1.

2.2   HOUSING AND FEEDING OF DUCKS

The birds were kept in large groups (350 birds/group). The stock density and lightning (17 hours light/day) was in accordance with Cherry Valley recommendation. Restricted feeding was used and free access to water was allowed during the trial. The daily feed allowance was controlled weekly based on the body weight of the ducks compared to technological standard.

2.3   EXPERIMENTAL PROCEDURE

The body weight and feed intake of the birds were controlled weekly. The body weight was monitored by randomly sampling at least 8% of each stock. Homogeneity of the stock was defined as the rate of the ducks (in %) being within a range of ±10% from the average. Mortality was also recorded. All birds were immunized by live, freeze dried duck plague virus (Vaxiduk vaccine, Merial, Hungary) *subcutan* on the neck (0.5 ml/bird) at 16 and 20 weeks of age. For blood sampling 14 birds per treatment were assigned and the same birds were used in all blood sampling. Blood samples for virus neutralization (VN) tests were obtained from the *vena cutanea ulnaris* in Eppendorf tubes without anticoagulant just before the first immunization (week 16), and 2 weeks after 1st (week 18) and 2nd immunization (week 22).

2.4.   ANALYTICAL PROCEDURE

2.4.1   IMMUNOLOGICAL TESTS

The systemic humoral immune responsiveness of the ducks was measured through the specific antibody response to the duck herpesvirus by virus neutralization (VN) test according to standard procedure. Briefly, the virus was titered in continuous chicken embyoblast cell cultures (CECC) in a 96-well tissue culture plate. For the VN assays 100 TCID50 (tissue culture infectious dose) of the virus was used for each of the twofold serially diluted serum samples. During the analysis 50 µl diluted serum was incubated against 50 µl 100 TCID50 titre diluted virus for 60 minutes at 37 Co and then 100 µl CECC cell suspension was added and incubated for 96 hours at 37 Co. The plate was evaluated with Zeiss invert microscope.

2.4.2 CHEMICAL AND STATISTICAL ANALYSIS

The chemical composition of the feeds, i.e. dry matter, crude protein, crude fat, crude fiber, crude ash and Ca and P was determined according to AOAC procedures (1989), dietary amino acid content was measured with method developed by Bech-Anderson *et al.* (1992).

Pair wise Student's *t*-test was used to check the experimental contrast in immune response.

3 RESULTS AND DISCUSSION

The growth performance parameters of the ducks in each dietary treatment were in accordance with the technological standards. Figure 1 shows that the mean body weight of the experimental groups were within the range of ±5% of the technological recommendation. The approximate 5% difference between the feed consumption of ducks in treatment Thr-100 and Thr-130 can be considered by chance (Table 2). In working practice the dustiness and feed waste has big influence on the apparent feed allowance of the birds. It is important to note that the homogeneity of the stocks were quite high. Worst data were obtained at the beginning of the trail (73–84%); however, it increased and reached 90% at the end of the experimental period. The very low mortality reflects to excellent condition in the farm; data derived primarily from the culling of the weak birds during the weekly weighing.

Indeed the specific antibody titre was not detectable in blood samples taken just before the immunization with duck plague virus. The second blood sampling was obtained 2 weeks after the first immunization. The mean virus neutralizing antibody titre in those sam-
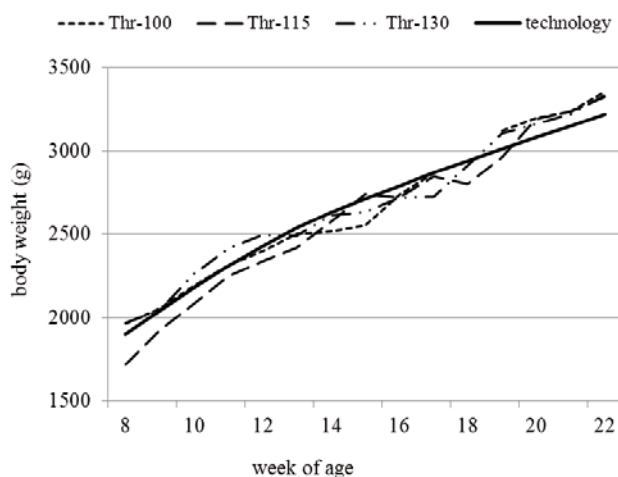
Table 2: The feed allowance, homogeneity of the stock and culling of the ducks in dietary treatments during the trial

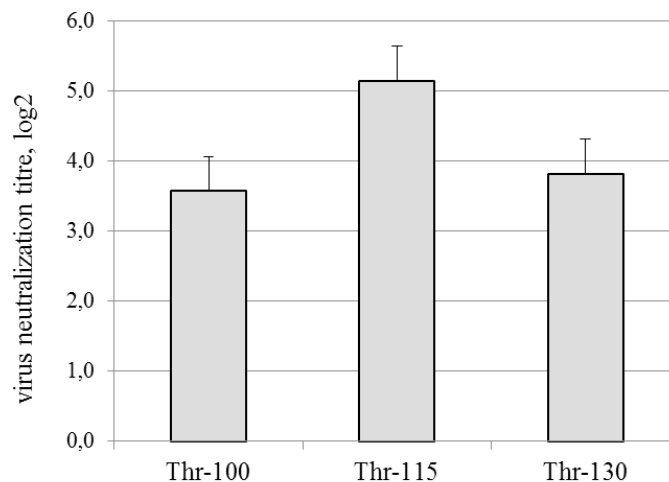|	Thr-100	Thr-115	Thr-130
Total feed allowance (kg/duck)	20.23	20.65	21.28
Homogeneity (%)	83.7	83.6	83.1
Mortality, culling (%)	1.55	1.63	1.85

Table 3: Statistical reliance (P-value) of virus neutralization tests in different dietary treatments according to two samples Student's t -Test

	Thr-100	Thr-115
Thr-115	0.083	
Thr-130	0.388	0.075

ples were similar in all treatments being 1:4 (VN titre log2 = 2). The blood samples obtained 2 weeks after the repeated immunization showed differences in virus neutralization titres (Table 3). The VN titres tended to be higher in ducks fed 15% surplus threonine compared to the control birds (Fig. 2; P < 0.10). Considering that the trial was conducted in a commercial farm and the immune response was checked with relatively low number of ducks the results are promising. The nearly 1.5 times higher values in Thr-115 group compared to the control treatment is wondrous since the duck plague belongs to *herpesviriade* family considered as a weak antigen. Other studies have also proved that the increasing dietary threonine supply boost the humoral immune responsiveness of livestock. Most of the publications reported elevated serum IgG level (Li *et al.*, 1999; Kidd *et al.*, 1999; Wang *et al.*, 2006). However, the immune response was provoked usually by non-viral challenge in different studies. In ac-

Figure 1: The body weight of ducks in different treatments and the technological standard during the experiment

Figure 2: *The effect of dietary threonine level on the specific humoral immune response of ducks two weeks after the repeated immunization (duck plague virus neutralization titre log2; mean, s.e.)*

cordance with our results in the study of Bhargava *et al.* (1971) serum antibody titres increased with increasing dietary intake of threonine in virus infected chickens. In the referred trial the challenge was the Newcastle disease virus (Bhargava *et al.* 1971).

Important message of our results is also that dietary threonine should not increase above a certain level. There was no difference between specific humoral immune response of ducks belonged to Thr-100 and Thr-130 (P > 0.10; Table 3). The reason why 15% surplus threonine improved, but 30% surplus threonine had no effect on responsiveness of humoral immune defense is not clear. However, the known phenomenon is that an amino acid, for example methionine in a lower level of supplementation is beneficial but in a higher dose has no or even immunosuppressive effect (Cook, 1991).

4 CONCLUSION

In conclusion, elevated dietary threonine supply boosts the immune defense of rearing ducks; however, supplementation of surplus threonine in the feed needs some caution. For breeding ducks during the growing period 6.6 g threonine per kg of feed can be recommended to fulfill the higher requirement of specific humoral immune functions.

5 ACKNOWLEDGEMENT

6 REFERENCES

AOAC. 1989. Official methods of analysis. Washington, D.C., Association of Official Analytical Chemist 13th edition.

Bech-Anderson S., Mason V.C., Dhanoa M.S. 1990. Hydrolysata preparation for amino acid determination in feed constinuens modification to oxidation and hidtrolysis conditions for streamlined procedures. Journal of Animal Physiology and Animal Nutrition, 63: 188–197

Bhargava K.K., Hanson R.P., Sunde M.L. 1971. Effects of threonine on growth and antibody production in chicks infected with Newcastle disease virus. Poultry Science, 50: 710–713

Cook M.E. 1991. Nutriton and the immune response of the domestic fowl. Crit-Rev. Poult. Biol., 3: 167–189

Corzo A., Kidd M.T., Dozier W.A., Pharr G.T., Koutsos E.A. 2007. Dietary threonine needs for growth and immunity of broilers raised under different litter conditions. Journal of Applied Poultry Research, 16: 574–582

Faure M., Choné F., Mettraux C., Godin J.P., Béchereau F., Vuichoud J., Papet I., Breuillé D., Obled C.. 2007.Threonine utilization for synthesis of acute phase proteins, intestinal proteins, and mucins is increased during Sepsis in rats. Journal of Nutrition, 137: 1802–1807

Fuller M.F., McWilliam R., Wang T.C., Giles L.R.. 1989. The optimum dietary amino acid pattern for growing pigs. Journal of Nutrition, 62: 225–267

Halas V., Kovács M., Babinszky L. 2006. The impact of nutrient supply on the immune functions in pig: a review [A táplálóanyag ellátás immunmoduláló hatása sertésben: irodalmi feldolgozás]. Hungarian Veterinary Journal, 128: 533–543

Kidd M.T., Barber S.J., Virden W.S., Dozier W.A., Chamblee D. W., and Wiernuz C. 2003. Threonine responses of cobb male finishing broilers in differing environmental conditions. Journal of Applied Poultry Research 12: 115–123

Kidd M.T., Lerner S.P., Allard J.P., Rao S.K., Halley J.T.. 1999. Threonine needs of finishing broilers: an evaluation of

growth, carcass, and economic responses. Journal of Applied Poultry Research, 8: 160–169

Li D., Changting X., Shiyan, Q., Jinhui Z., Johnson E.W., Thacker P.A. 1999. Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. Animal Feed Science and Technology, 78: 179–188

Nichols N.L., Bertolo R.F. 2008. Luminal Threonine Concentration Acutely Affects Intestinal Mucosal Protein and Mucin Synthesis in Piglets. Journal of Nutrition, 138: 1298–1303

NRC (National Research Council). Nutrient requirements of poultry. 1994. Washington, National Academy Press

NRC (National Reseaech Council). Nutrient requirements of swine. 1998. Washington, National Academy Press Smith

E.L., Greene R.D. 1947. Furter studies on the amino acid composition of immune proteins. Journal of Nutrition. http://www.jbc.org/content/171/1/355.full.pdf

Stoll B. 2006. Intestinal Uptake and Metabolism of Threonine: Nutritional Impact. Advances in Pork Production, 17: 257–263

Wang X., Qiao S.Y., Liu M., Ma Y.X. 2006. Effects of graded levels of true ileal digestible threonine on performance, serum parameters and immune function of 10–25 kg pigs. Animal Feed Science and Technology, 129: 264–278

Wang W.W., Oiao S.Y., Li D.F. 2009. Amino acids and gut functions. Amino acids, 37, 1: 105–110