EFFECT OF DIET METHIONINE RATE ON PERFORMANCES AND BLOOD PROTEIN LEVELS OF FATTENING RABBITS

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ABSTRACT

Nutrition is known to have an effect on immune system. Amino acids, such as methionine, must be used by animals to build their antibodies or gamma globulins. It was suggested that more methionine is needed to provide the greatest immune protection than is needed for maximum growth in broilers. This trial aimed at defining more precisely the effect of methionine addition in fattening rabbit diet on both production performances and on blood immune system indicators. The trial compared four diets (9,65 MJ/kg) with different methionine incorporation rates: 0.26%, 0.36%, 0.46% and 0.56%. The trial was made on 120 Hyplus rabbits from weaning (35 days of age) to 70 days of age. Live weight (LW) and feed intake (FI) were registered at 49 and 70 days of age. Mortality and morbidity were controlled to determine the Sanitary Risk Index (SRI). In order to measure the effect of the methionine rate in feed on immunity, blood protein concentrations (total protein, albumin, alpha-1, alpha-2, beta-1, beta-2 and gamma globulins) were measured by electrophoresis in six animals per treatment. The ratio between each protein and the total protein concentration was compared between diets, as well as the total protein concentration. No significant effect of methionine rate was observed, neither on mortality, morbidity and SRI, nor on zootechnical performances. Total blood protein concentration did not change significantly from a diet to another one. The gamma globulin rate in blood protein tended to change with methionine rate in feed (P=0.0528). The diet containing 0.56% of methionine led to the highest Gamma/Total Protein ratio. The Gamma/Total Protein ratio increased linearly with methionine rate in feed. Since blood was taken from animals showing no sign of morbidity and no growth problem, this trial brings to the conclusion that immune defences could be improved by a methionine rate of 0.56% in feed.

Key words: Rabbit, Methionine, Immune system, Blood protein, Gamma globulin.

INTRODUCTION

Nutrition is known to have an effect on immune system. Nevertheless, effects of the different nutrients are contradictory (Fortun-Lamothe *et al.*, 2001). Nutritional immunology is considered as a new scientific discipline and aims at defining the role played by nutrients on immune system (Peng *et al.*, 2007). Antibodies or gamma globulins must be synthesized from amino acid pool. As a consequence, immune system can be affected by amino acids in diet (Sunde, 1988). More precisely, a lack or a non balanced amino acid profile depresses more humoral than cellular immunity (Fortun-Lamothe *et al*., 2001). Studies were conducted in broilers suggesting that more methionine is needed to provide the greatest immune protection than is needed for maximum growth (Sunde, 1988). Our trial aimed at defining more precisely the effect of addition of methionine in fattening rabbit diet on both production performances and on blood immune system indicators.

MATERIALS AND METHODS

The trial was conducted in CRZA, the animal research centre of INZO in France from the 31st of July to the 4th of September 2007. 120 Hyplus breed rabbits weaned at 35 days of age were blocked on weight and mother origin, in 30 replicates, in individual cages. Four experimental diets were given from 35 to 70 days of age. Individual cages were 0.153 m² big. The animals received light 16 hours/day and the room temperature was 21±3°C. Feed and water were available *ad libitum*. Methionine level was the only variation factor. The methionine rates in diets MetI, MetII, MetIII and MetIV were respectively 0.26%, 0.36%, 0.46% and 0.56%.

Feed composition was analysed. We recorded live weight (LW) at 35, 49 and 70 d of age and feed intake (FI) (49 and 70 day-old). A morbidity control was made at 49 and 70 days of age. Mortality was controlled everyday. From these data, live weight gain (LWG) and feed conversion index (FCI) were calculated. The Sanitary Risk Index (SRI) was calculated from morbidity and mortality at 49 and 70 days of age. Blood was collected from the heart of 6 healthy animals per diet at 70 days of age. Some immunity indicators were measured in blood by electrophoresis: total amount of blood proteins, albumin, alpha-1, alpha-2, beta-1, beta-2 and gamma globulins. In order to go through a potential problem of blood dilution, the ratio Protein/Total Protein is compared between results.

The SAS 8.02 software was used for the statistical analysis. The General Linear Model procedure (Proc GLM) was used to make an Analysis of Variance (ANOVA) with two factors (diet effect and block effect). Means were compared by Student test. A linear and a quadratic test (contrast method) were done to test the response of live weight, feed intake, live weight gain, feed conversion index, total blood protein and Protein/Total Protein ratios to an increase of 0.10 point of methionine in diet. Protein ratios were transformed by using Arcsinus function before statistical analysis to change them into continued data. Mortality, morbidity and Sanitary Risk Index were compared by a Chi² test.

RESULTS AND DISCUSSION

The measured methionine incorporation rate is included in the analytical error range (12%) (Table 1).

Table 1: Ingredients and chemical composition of the diets

	Diets			
	MetI	MetII	MetIII	MetIV
Ingredients (%)				
Wheat	6.5	6.5	6.5	6.5
Straw	3.4	3.4	3.4	3.4
Soya bean	2.5	2.5	2.5	2.5
alfalfa	26.45	26.35	26.25	26.3
Wheat bran	20	20	20	19.85
Soya oil	1	1	1	1
Sunflower meal	12	12	12	12
Beet pulp	16.4	16.4	16.4	16.4
Molasse	4.9	4.9	4.9	4.9
Grape seed meal	5	5	5	5
Carbonate	0.19	0.19	0.19	0.19
Phosphate	0.45	0.45	0.45	0.45
Salt	0.5	0.5	0.5	0.5
L-Lysine	0.18	0.18	0.18	0.18
DL-Méthionine	0.03	0.13	0.235	0.335
Mineral and vitamine premix[1]	0.5	0.5	0.5	0.5
Chemical composition (%)				
Moisture	11.18	10.42	11.50	11.21
Crude Protein	15.80	14.6	14.58	14.73
Crude Fiber	20.5	20.04	19.65	18.3
Starch	9.75	8.3	9.65	9.85
ADF	23.3	24.5	23.62	23.36
NDF	38.31	37.47	38.38	39.19
ADL	7.16	7.84	7.47	6.95
Lysine	0.708	0.709	0.708	0.707
Methionine (%)	0.26	0.36	0.46	0.56
Methionine + Cystine (%)	0,49	0,59	0,69	0,79
Estimated digestible energy (MJ/kg)	9.63	9.65	9.67	9.68

[1]5g of premix provided per kg of feed: vitamin A, 8,000 IU; vitamin D_3, 600 IU; vitamin E acetate, 5 mg; Cu, 11 mg; Mn, 2 mg; Co,0.2 mg; I, 0.2 mg; Zn, 30 mg; Se, 0.05 mg

No significant difference in mortality and morbidity between diets was put forward (Table 2). Nevertheless, 47% of rabbits dead between 49 and 70 d of age were eating diet MetIV and the global mortality rate in this diet reached 40% against 20%, 30%, and 23% respectively in diets MetI, MetII and MetIII. About morbidity symptoms, at age 49 days, diet MetII showed significantly more net diarrhoeas than other diets (P<0.05) but significantly less dry diarrhoeas than others (P<0.05). No difference was observed in morbidity symptoms at age 70 days.

Table 2: Morbidity, mortality and sanitary risk

	Diets				
	MetI	MetII	MetIII	MetIV	Prob.
Morbid animals at 49 days of age	6	9	5	5	NS
Morbid animals at 70 days of age	4	3	5	2	NS
Mortality from 42 to 49 days of age (%)	13.33	13.33	20.00	16.67	NS
Mortality from 63 to 70 days of age (%)	7.69	19.23	4.17	28.00	NS
Cumulated mortality at 70 days of age (%)	20.00	30.00	23.33	40.00	NS
SRI 15 days after weaning (% of starting animals)	33.33	43.33	36.67	33.33	NS
SRI 35 days after weaning (% of starting animals)	33.33	40.00	40.00	46.67	NS
Net diarrhoeas at 49 days of age (% of morbid animals)	0 b	20,0 a	0 b	0 b	<0.05
Dry diarrhoeas at 49 days of age (% of morbid animals)	20.0 a	16.0 b	20.0 a	20.0 a	<0.05

Means with different letters on the same row differ significantly (Bonferroni test)

No significant difference was observed on live weight at the beginning of the trial (Table 3). Data from dead or morbid animals were removed from the analysis. 20; 18; 20 and 17 animals were left respectively from diet MetI, MetII, MetIII and MetIV for statistical analysis. No significant effect of the diet was observed on any performance criteria. The response of feed intake to an increase of 0.10 point was significantly quadratic (P<0.05). The lowest feed intake was observed with MetIII.

Table 3: Growth performance

	Methionine level (%)				Prob. Model	Prob. Diet	RMSE	Contrast	
	MetI 0.26%	MetII 0.36%	MetIII 0.46%	MetIV 0.56%				Linear	Quadratic
LW at 35 d (g)	1072	1054	1054	1060	0.82	NS	69	NS	NS
LW at 49 d (g)	1850	1847	1832	1886	0.63	NS	213	NS	NS
LW at 70 d (g)	2842	2864	2752	2867	0.04	NS	177	NS	NS
Daily gain 35-49 d (g/d)	51.8	52.9	51.9	55.1	0.83	NS	14.0	NS	NS
Daily gain 49-70 d (g/d)	49.6	50.9	46.0	49.1	0.57	NS	7.7	NS	NS
Daily gain 35-70 d (g/d)	50.6	51.7	48.5	51.6	0.14	NS	4.8	NS	NS
Feed intake 35-49 d (g/d)	136.4	135.0	140.1	142.0	0.90	NS	29.7	NS	NS
Feed intake 49-70 d (g/d)	200.3	193.4	187.5	197.5	<0.0001	NS	12.3	NS	0.047
Feed intake 35-70 d (g/d)	174.0	169.3	168.0	174.6	0.04	NS	14.5	NS	0.092
Feed conversion 35-49 d	2.71	2.68	2.78	2.67	0.26	NS	0.43	NS	NS
Feed conversion 49-70 d	4.09	3.86	4.13	4.07	0.86	NS	0.49	NS	NS
Feed conversion 35-70 d	3.44	3.28	3.47	3.39	0.19	NS	0.19	NS	NS

The total blood protein level remains consistent from a diet to another one (Table 4). The only significant difference observed between diets is the Gamma/Total Protein ratio. Diet MetIV induced a significant higher gamma rate in blood than diets MetI and MetII.

Table 4: Blood proteins

	Methionine level (%)				Prob. Diet	RMSE	Contrast	
	MetI 0.26%	MetII 0.36%	MetIII 0.46%	MetIV 0.56%			Linear	Quadratic
Total protein (g/l)	54.2	52.3	55.5	54.2	9.3	NS	NS	NS
Albumine/Total protein	0.684	0.684	0.676	0.675	2.6	NS	NS	NS
Alpha 1/ Total protein	0.021	0.020	0.021	0.021	15.7	NS	NS	NS
Alpha 2/ Total protein	0.042	0.041	0.043	0.039	18.8	NS	NS	NS
Beta 1/ Total protein	0.078	0.076	0.076	0.067	11.4	NS	0.044	NS
Beta 2/ Total protein	0.075	0.076	0.072	0.070	8.4	NS	NS	NS
Gamma/ Total protein	0.101a	0.103a	0.111ab	0.128b	15.6	0.0528	0.01	NS

Means with different letters on the same row differ significantly (Bonferroni test)

Moreover, the trial underlined a linear increase of gamma protein rate (Figure 1a) and a linear decrease of Beta-2 protein rate in blood (Figure 1b) when methionine rate in feed increased by 0.10 point. Relations are: Gamma/Total Protein = 0.08847+0.0899*methionine rate and Beta-1/Tot Prot = 0.08252-0.0334*methionine rate (SAS).

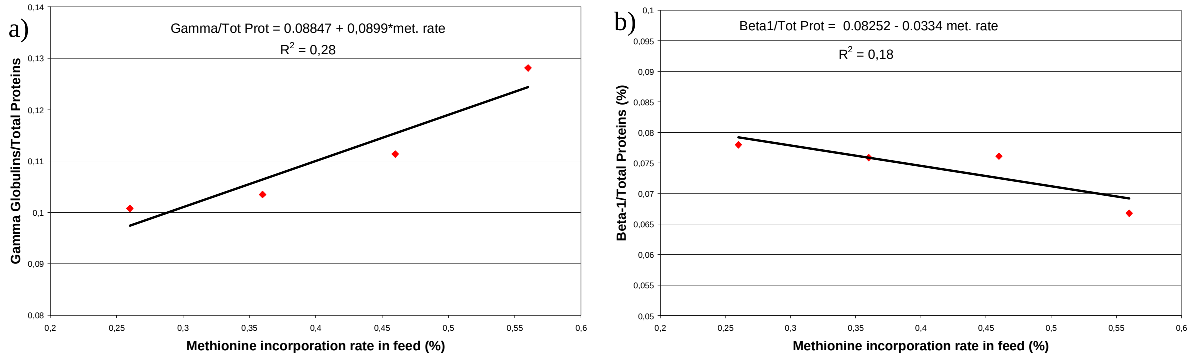

Figure 1: a) Linear increasing relation between Gamma/Total Protein and methionine rate in diet; b) Linear decreasing relation between Beta-1/Total Protein and methionine rate in diet

Performances

Berchiche and Lebas (1994) showed that the optimum growth was recorded when sulphur amino acids (SAA) in feed reached 0.62% for a diet of 11.18 MJ/kg (0.53% for 9.63 MJ/kg) and that performances decreased over this incorporation rate. Nevertheless, taking into consideration the fact that needs in methionine for growing performances are lower than needs for immunity, it is interesting to study higher methionine rates. The absence of significant observations may be due to the lack of data and the high mortality rate. Second, a non balanced amino acid profile can avoid the expression of a supplementation in methionine. A minimal requirement for digestible SAA was established at 66.7% of the digestible lysine requirement (Taboada *et al*., 1994 cited in Taboada *et al*., 1996). In our trial, the ratios SAA/lysine were 69, 83, 98 and 112% respectively in diet MetI, MetII, MetIII and MetIV. Increasing methionine only did not bring to any improvement of performances in our trial.

Other reasons of the absence of response to methionine rate can be suggested. Firstly, a trial conducted by Jensen (1996) showed that the benefit of a SAA supplementation in fattening diet depends on the SAA rate of the diet fed to litters before weaning. An improvement in growth performances during fattening was observed with a 0.8% SAA fattening diet, in comparison with 0.6%, only if the diet fed before weaning also contained 0.8% of AAS. No benefit was observed if the diet before weaning contained 0.6%. It would mean that the benefit of % methionine in the fattening diet can be detected only if the diet fed before weaning contains at least the same methionine %. In our trial, all kits had access to the same diet containing 0.36% of methionine before weaning, from age 28 to age 35 days. In that case, the lack of benefit to diets III and IV (% > 0.36) could be explained. Nevertheless, no decreasing in animal performance was observed on diet MetI compared to the other ones. Secondly, an export of the methionine to rabbits' fur could also explain the lack of increasing performances. Coan *et al*. (1988), in a trial comparing feed supplemented or not in methionine, brought the hypothesis that methionine in excess was used to make the fur thicker and denser.

Blood proteins

The determination of the composition of gamma globulin in rabbits showed it is first composed by threonine, glutamine and valine and far less by methionine (Tenenhouse and Deutsch, 1966 cited in Sunde, 1988). Jose and Good (1973 cited in Fortun-Lamothe, 2001) also showed leucine, tryptophane, valine and SAA as regulators of immune response in mice. According to Gascón *et al*. (1988), blood protein profile changes in the first 51 days of a rabbit life. For instance, gamma globulin concentration increases in blood whereas albumin one decreases. In their trial, rabbits reached higher total blood protein concentration (67.7±0.41 g/l) and gamma globulin rate (19.16±1.91%) at 51 days of age than in ours. The increase in gamma-globulin rate observed with MetIV could lead to the conclusion that animals were ill or morbid. However, the significant increase in gamma globulin rate only, the absence of significant loss of growth and the fact that blood was taken from animals presenting no morbidity signs at 70 days of age showed that the increase in gamma globulins was not due to a dysfunction but could be considered as a real benefit for immunity. Indeed, Rodriguez *et al*. (2007) showed that the proportion of gamma-globulins in rabbits who survive a diarrhea outbreak was much lower ($P<0.0001$) than free-disease health rabbits (8.1±1.0 vs. 14.0± 1.0). The usual increase of gamma-globulins in rabbits described by Gascón has been greater with high methionine rate in diet. Taking into consideration the hypothesis that needs in amino acids are greater for the immune system than for performances, we can think that a rate of 0.56% of methionine in fattening rabbit diet improves humoral immunity of animals whereas no improvement can be noticed on performances. It confirms the hypothesis of Fortun-Lamothe *et al*. (2001). According to the composition of gamma globulin in amino acid, the effect of other amino acids on immune system can be investigated.

CONCLUSIONS

This study did not show any effect of diet methionine concentration (from 0.26 to 0.56%) on fattening rabbit performances. A rate of 0.56% of methionine in diet induced an increase in gamma globulins (blood proteins involved in immunity) of healthy animals. Higher methionine rates could be studied to control blood protein concentrations response. The measure of fur thickness and softness could also be part of such a study to measure the export of sulphur amino acid to fur.

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