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A Nutritional Approach to the Use of Anticoccidial Vaccines in Broilers: Glutamine Utilization in Critical Stages of Immunity Acquisition

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Abstract: The utilization of vaccines has proven to be a good strategy to prevent coccidiosis but the process of immunity acquisition needs to be approached from a nutritional point of view as well if complete success in broiler performance is to be achieved. It has been reported that Glutamine (GLU) plays a key role both in the gastrointestinal tract and the immune system and its utilization could be beneficial to cocci-vaccinated broilers. In this study, twelve hundred one-day-old male chicks were vaccinated at a commercial hatchery with a coccidiosis vaccine and randomly allocated to four treatments, each of which had six replications with 50 birds per pen. Birds were maintained in pens with built up wood shavings litter. Each treatment consisted of the same basal diet that met average nutrient levels in the U.S. poultry industry with four different inclusion rates of GLU (0, 0.5, 0.75 and 1%). Birds were fed the experimental diets from 1 to 28 days of age and a common unsupplemented diet to 42 d. Body weights were significantly improved at 21 and 28 days for all the treatments where the GLU was included. Feed conversion was not significantly affected by the inclusion of GLU. There were no significant differences in body weight and feed conversion at 42 days but the numerical difference in weight between the control and the treatments with GLU observed earlier were maintained. At 43 days, eight birds per pen were processed in a pilot processing plant. Breast meat yield was not significantly different among treatments. Glutamine proved to be beneficial during the process of immunity acquisition improving broiler performance significantly until 28 days and maintaining the body weight difference until the end of the experiment.

Key words: Glutamine, coccidial vaccine, immunity acquisition, broilers

INTRODUCTION

Coccidiosis is considered one of the most important poultry diseases worldwide due to the economic losses it produces in performance and due to medication usage (Williams, 2002; Dalloul and Lillehoj, 2005). The use of vaccines to prevent coccidiosis has proven to be successful (Lee *et al*., 2009; Danforth, 1998; Williams and Gobbi, 2002) and is widely used today in the U.S poultry industry (Agri-Stats, Fort Wayne IN). However, in the process of developing immunity, Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) could be negatively affected due to the coccidia replication in the enterocytes at early stages of the bird life (Williams, 2002; Lee *et al*., 2009). At the same time, it has been reported that compensatory growth takes place after the immunity is acquired, with immunity development taking place within the first 21 to 28 days of age (Giraldo and Southern, 1988; Williams, 2002; Lee *et al*., 2009). Therefore, a nutritional approach to the temporary intestinal damage should be considered and a nutrient that could alleviate or prevent this transitory loss of performance could be used. Due to its multiple effects, Glutamine (GLU) could be used. GLU functions by stimulating intestinal cell proliferation and activating mitogen-active protein kinases (Rhoads *et al*., 1997). It accounts for 35% of the energy consumed in the small intestine and is used for purine nucleotide and glutathione synthesis. In rats these account for 30% of glutamine utilization for the whole body (Windmueller, 1982). Moreover, broilers fed with 1% Glutamine increased microvilli width, villi density and the surface area of the tip of enterocytes compared to broilers fed a basal diet (da Silva *et al*., 2007). Similar results were reported by Bartell and Batal (2007) and Sakamoto (2009). On a proteomics level, glutamine per se prevents enterocytes apoptosis (Ban and Kozar, 2010) and increases the level of Heat Shock Proteins (Eliasen *et al*., 2006). At the immune system level, GLU increases phagocytic capacity of neutrophils and monocytes as well as lymphocytes proliferation. It also regulates antigen presentation by macrophages and it is the number one energy source for the immune system cells (Calder and Yaqoob, 1999; Newsholme, 2001). Skeletal muscle contains 90 % of the whole body glutamine pool (Darmaun *et al*., 1986). According to Nieto and Lobley (1999), as a response to a gut infection or parasitic infestation in an animal, the demand for GLU increases and the net efflux is increased to provide both the GIT and the immune system with this amino acid. As a consequence, a state of lowered anabolism or net

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catabolism in the muscle results due to decreased protein synthesis or even breakdown of muscle protein to provide amino acids for GLU production. If the GLU needed by the GIT or immune system is provided in the feed then the bird would not have to use the muscle reserves of amino acids to supply the higher demands for GLU. This study evaluated the response of coccidiosis-vaccinated broilers to different inclusion levels of GLU in the diet during the stages of immunity acquisition on performance and processing yield.

MATERIALS AND METHODS

The University of Arkansas Institutional Animal Health and Use Committee approved all procedures. In this study, twelve hundred one-day-old male birds (Cobb 500) were vaccinated (Coccivac B, Intervet Inc., Merck Animal Health, Summit NJ) in a commercial hatchery and randomly allocated to four treatments. Each treatment had six replications of fifty birds each. Birds were housed in floor pens with built up wood shavings litter. Bird density was 0.09 m^2/bird. Each pen was equipped with one hanging feeder and one bell drinker. Feed and water was provided for *ad libitum* consumption. Lighting was continuous 24 hr during the experiment. Management practices followed recommended guidelines (FASS, 2010).

Basal starter, grower and finisher diets were prepared that met average nutrient levels in the U.S. poultry industry (Agri-stats, Fort Wayne IN). Composition and calculated analysis of the diets is shown in Table 1. Aliquots of the basal starter and grower were mixed with four different levels of GLU (0, 0.5, 0.75 and 1%) and fed to the birds. Broilers were fed the experimental starter until 21 days of age. Following that, experimental grower diets were fed until 28 days followed by a common basal grower to 35 days of age. At that point, the same finisher was fed to all the treatments until 42 days of age. The starter diets were presented as mash, with the grower and finisher diets as pellets. Pen weights and feed intake were measured and FCR calculated at 14, 21, 28, 35 and 42 days of age. At 43 days, eight birds from each pen were tagged, weighed and processed at a pilot processing plant as described by Fritts and Waldroup (2006). Carcass, breast meat, legs and wings were weighed. These weights were expressed as a proportion of 43-d live weight to calculate yield data.

Statistical analysis of data was performed using the JMP Pro (SAS Institute Inc., Cary, NC, USA). Pen means served as the experimental unit for live performance data while individual means served as the unit for processing data as birds were processed in random order. Data were analyzed by a one-way Analysis of Variance (ANOVA) and comparison of means was done using the students t test ($\alpha = 0.05$). Mortality data were converted to the square root of n+1; data are shown as natural numbers. Statements of probability are based on $p \leq 0.05$.

Table 1: Composition (g/kg) and calculated nutrient content of basal diets. Values in bold italic are at minimum specified level. Values in Italic were not included in formulation specifications

Ingredient	Starter	Grower	Finisher
Yellow corn	563.97	591.85	657.65
Soybean meal	314.43	272.15	202.48
Pro-Plus 54[1]	46.70	40.40	31.40
Poultry oil	11.43	19.50	19.86
DDGS	43.30	56.90	67.30
Ground limestone	3.47	4.31	5.70
Dicalcium phosphate	5.67	4.77	5.00
Feed grade salt	4.00	4.09	4.05
MHA-84[2]	3.07	2.53	2.23
L-Lysine HCl	1.78	1.57	2.16
L-Threonine	0.43	0.18	0.42
2 X Broiler premix[3]	0.25	0.25	0.25
Mintrex P_Se[4]	0.50	0.50	0.50
Choline Cl 60%	1.00	1.00	1.00
	1000.00	1000.00	1000.00
ME kcal/lb	***1377.00***	***1412.00***	***1440.00***
Crude protein %	22.87	21.13	18.31
Calcium %	***0.90***	***0.83***	***0.77***
Total P %	0.75	0.70	0.63
Nonphytate P %	***0.46***	***0.41***	***0.37***
Methionine %	0.62	0.56	0.50
Lysine %	***1.32***	***1.19***	***1.04***
Threonine %	***0.89***	***0.80***	***0.71***
Arginine %	1.50	***1.36***	1.12
Tryptophan %	***0.26***	0.23	***0.19***
TSAA %	***1.00***	***0.91***	***0.82***

[1]Blended animal protein. H.J. Baker & Bro., Stamford CT.

[2]Methionine hydroxy analogue calcium salt. Novus International, St. Louis MO.

[3]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.

[4]Provides per kg of diet: Mn (as manganese methionine hydroxy analogue complex) 20 mg; Zn (as zinc methionine hydroxy analogue complex) 20 mg; Cu (as copper methionine hydroxy analogue complex) 10 mg; Se (as selenium yeast) 0.15 mg

RESULTS AND DISCUSSION

Results for performance are shown in Table 2. The BW and FCR were not affected by GLU at 14 days. At 21 days the three groups of birds fed diets with GLU had a significantly heavier weight than those fed the control. The FCR was not significantly influenced by the GLU. At 28 days the birds fed the GLU treatments continued the significant difference from the control group. The FCR was numerically but not significantly improved by the GLU addition. At 35 and 42 days there was no significant difference between the treatments for BW but the numerical difference observed earlier was maintained. The FCR continued to show the same positive improvement but was not significantly different. No significant differences were detected for mortality throughout the trial.

Table 2: Body weight, feed conversion ratio and mortality for different glutamine inclusion rates

Glutamine inclusion	14 d BW (kg)	0-14 d FCR (kg/kg)	21 d BW (kg)	0-21 d FCR (kg/kg)	28 d BW (kg)	0-28 d FCR (kg/kg)	35 d BW (kg)	0-35 d FCR (kg/kg)	42 d BW (kg)	0-42 d FCR (kg/kg)	Mortality 0-42 d (%)
0.00%	0.348	1.22	0.749[b]	1.316	1.318[b]	1.425	2.031	1.48	2.606	1.609	0.333
0.50%	0.344	1.209	0.774[a]	1.316	1.362[a]	1.408	2.058	1.469	2.623	1.603	0.667
0.75%	0.364	1.204	0.776[a]	1.318	1.379[a]	1.404	2.085	1.467	2.66	1.602	1.00
1.00%	0.357	1.19	0.775[a]	1.306	1.365[a]	1.405	2.08	1.466	2.658	1.602	1.00
Prob > F	0.0917	0.624	0.0343	0.8013	0.0304	0.3051	0.3929	0.2217	0.7728	0.9097	0.6832
SEM	0.0033	0.008	0.004	0.004	0.008	0.004	0.012	0.002	0.02	0.004	0.2928

[1]Means of six replications of 50 male broiler chicks.

[ab]Means in same column with common superscripts do not differ significantly ($p \leq 0.05$)

Table 3: Processing results at 43 days of age for diets varying in Glutamine inclusion rates[1]

Glutamine inclusion	Dressing percentage	Wing yield (% of carcass)	Leg yield (% of carcass)	Breast yield (% of carcass)	Rack yield (% of carcass)
0.00%	73.27	7.79	23.29	22.73	19.300
0.50%	73.82	7.78	23.00	22.52	19.900
0.75%	73.90	7.82	23.05	22.90	19.690
1.00%	73.86	7.70	22.58	23.42	19.650
Prob > F	0.4276	0.4039	0.0681	0.0739	0.271
SEM	0.150	0.026	0.097	0.126	0.103

[1]Means of six replicate groups of eight birds per treatment

As long as the Glutamine was fed to the birds there was a significant positive effect in BW. This is in agreement with Sakamoto (2009), who fed 1.5% GLU to vaccinated birds and got a significant response as long as the amino acid was fed. Bartell and Batal (2007) reported a significant improvement in BW when feeding 1% GLU to chicks in agreement with Soltan (2009), found similar responses at 42 days. At 28 days all the birds were fed the same basal diet without GLU until the end of the trial. Since there was no longer a GLU effect, all the treatments had a similar growth but the difference acquired at 28 days remained until the end of the trial. It seems that the GLU effect reported by Bartell and Batal (2007); da Silva *et al*. (2007) and Sakamoto (2009) at villi level compensate for the reduction of nutrient absorption caused by the replicating coccidia a and the increased demand for GLU by the GIT supplied by the GLU present in the feed. Mortality was not affected by the treatments (data not shown).

Results for processing are shown in Table 3. There were improvements ($p = 0.07$) in breast yield with concomitant reductions in leg quarter yield ($p = 0.07$) as the level of GLU fed from 0 to 38 d. There was no treatment effect for dressing percentage, wing yield, or rack yield. The effect of Glutamine on breast meat yield could be explained by the theory of Nieto and Lobley (1999) that the GLU provided in the feed prevented a lowered catabolism state in the broiler resulting in a higher breast meat yield for birds fed diets with GLU while the birds in the control treatment (0% GLU) could have had the need to use GLU from the muscle amino acid pool to supply the GIT and immune system. When using cocci vaccines in broilers, GLU could help alleviate transitory loss of performance during immunity acquisition at the early stages of the bird life and improve BW, FCR and breast meat Yield. Further studies are required to determine properly when GLU needs to be included in the feed to maximize benefits of cocci vaccination.

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REFERENCES

Ban, K. and R.A. Kozar, 2010. Glutamine protects against apoptosis via down regulation of Sp3 in intestinal epithelial cells. Am. J. Physiol.-Gastrointestinal Liver Physiol., 299: G1344.

Bartell, S. and A. Batal, 2007. The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract and humoral immune response of broilers. Poult. Sci., 86: 1940.

Calder, P.C. and P. Yaqoob, 1999. Glutamine and the immune system. Amino Acids, 17: 227-241.

da Silva, A.V.F., A. Maiorka, S.A. Borges, E. Santin, I.C. Boleli and M. Macari, 2007. Surface area of the tip of the enterocytes in small intestine mucosa of broilers submitted to early feed restriction and supplemented with glutamine. Int. J. Poult. Sci., 6: 31-35.

Dalloul, R.A. and H.S. Lillehoj, 2005. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis., 49: 1-8.

Danforth, H., 1998. Use of live oocyst vaccines in the control of avian coccidiosis: Experimental studies and field trials. Int. J. Parasitol., 28: 1099-1109.

Darmaun, D., D.E. Matthews and D.M. Bier, 1986. Glutamine and glutamate kinetics in humans. Am. J. Physiol.-Endocrinol. Metab., 251: E117.

Eliasen, M.M., M. Brabec, C. Gerner, J. Pollheimer, H. Auer, M. Zellner, G. Weingartmann, F. Garo, E. Roth and R. Oehler, 2006. Reduced stress tolerance of glutamine-deprived human monocytic cells is associated with selective down-regulation of Hsp70 by decreased mRNA stability. J. Mol. Med., 84: 147-158.

FASS, 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd Edn., Federation of Animal Science Societies, Savoy IL.

Fritts, C.A. and P.W. Waldroup, 2006. Modified phosphorus program for broilers based on commercial feeding intervals to sustain live performance and reduce total and water-soluble phosphorus in litter. J. App. Poult. Res., 15: 207-218.

Giraldo, C. and L.L. Southern, 1988. Role of compensatory gain in *Eimeria acervulina*--induced liver copper accumulation in chicks. J. Nutr., 118: 871.

Lee, J.T., C. Broussard, S. Fitz-Coy, P. Burke, N.H. Eckert, S.M. Stevens, P.N. Anderson, S.M. Anderson and D.J. Caldwell, 2009. Evaluation of live oocyst vaccination or salinomycin for control of field-strain Eimeria challenge in broilers on two different feeding programs. The J. Appl. Poult. Res., 18: 458.

Newsholme, P., 2001. Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? J. Nutr., 131: 2515S.

Nieto, R. and G.E. Lobley, 1999. Integration of protein metabolism within the whole body and between organs. In Protein and metabolism. EAAP Publ. No. 96. pp: 69-99.

Rhoads, J.M., R.A. Argenzio, W. Chen, R.A. Rippe, J.K. Westwick, A.D. Cox, H.M. Berschneider and D.A. Brenner, 1997. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. Am. J. Physiol.-Gastrointestinal Liver Physiol., 272: 943.

Sakamoto, M.I., 2009. Desempenho, desenvolvimento e atividade enzimática da mucosa intestinal de frangos de corte alimentados com dietas suplementadas com glutamina e nucleotídeos. Performance, development and enzymatic activity of the intestinal mucosa of broiler chickens fed with diets supplemented with glutamine and nucleotides. Doctoral dissertation. Universidad de Sau Paulo. 2009.

Soltan, M., 2009. Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and some blood parameters of broiler chickens. Int. J. Poult. Sci., 8: 60-68.

Williams, R.B., 2002. Anticoccidial vaccines for broiler chickens: Pathways to success. Avian Pathol., 31: 317-353.

Williams, R. and L. Gobbi, 2002. Comparison of an attenuated anticoccidial vaccine and an anticoccidial drug programme in commercial broiler chickens in Italy. Avian Pathol., 31: 253-265.

Windmueller, H.G., 1982. Glutamine utilization by the small intestine. Adv. Enzymol. Relat. Areas Mol. Biol., 53: 201-237.