POULTRY CRC LTD

FINAL REPORT

Sub-Project No:

2.1.15

SUB-PROJECT LEADER:

Dr Reza Barekatain

Sub-Project Title:

Zinc and glutamine to enhance intestinal function and performance of broilers

DATE OF COMPLETION: 30 November 2016

ISBN 1 921010 75 4

Title: Zinc and glutamine to enhance intestinal function and performance of broilers
Sub-Project No. 2.1.15

Researcher Contact Details
Dr Reza Barekatain
SARDI, JS Davies Building
Roseworthy Campus, Roseworthy
5371 SA, Australia

In submitting this report, the researcher has agreed to the Poultry CRC publishing this material in its edited form.

Poultry CRC Ltd Contact Details
PO Box U242
University of New England
ARMIDALE NSW 2351

Phone: 02 6773 3767
Fax: 02 6773 3050
Email: admin@poultrycrc.com.au
Website: http://www.poultrycrc.com.au

Published in 2016

Executive Summary

Sub-project 2.1.15 comprised two experiments to investigate 1) the effect of supplemental L-glutamine (L-Gln) and higher concentration of zinc (Zn) on excreta moisture under nutritionally induced wet droppings (decreased water reabsorption) and 2) impact of L-Gln on broiler performance under necrotic enteritis (NE) challenge. In the first experiment, a 2×2×2 factorial arrangement of treatments was used to investigate three dietary factors of L-Gln supplementation (0 or 10 g/kg), added Zn concentration (80 and 160 mg/kg) with or without magnesium chloride (MgCl) (2 g/kg – only in grower diets). There was no significant effect of any of the three main treatments on body weight gain and feed intake of birds during the experiment. Birds fed higher Zn (160 mg/kg) tended (P = 0.09) to have higher weight gain only in the first 9 days of age. Feeding 10 g/kg L-Gln increased the feed conversion ratio (FCR) of the birds only from hatch until day (d) 9 after which there was no significant effect. No effect of experimental treatments was found on digesta or excreta moisture except a reduction in ileal moisture at starter phase resulted from higher Zn concentration in the diets. MgCl was not effective in inducing wet droppings in birds. In the second experiment, the main effect of L-Gln supplementation (un-supplemented or supplemented at 10 g/kg on top of either all starter, grower and finisher or only in starter and grower diets) and NE challenge was investigated. Supplementation of L-Gln independently increased body weight gain, feed consumption and improved FCR of the birds throughout the experiment. Withdrawing L-Gln from the finisher diets from d 25 to 35 of age diminished the effect on weight gain. Birds challenged with NE showed a reduction in weight gain and a higher FCR. In challenged group of birds, feeding L-Gln reduced the lesion scores associated with NE in jejunum and ileum of the birds suggesting that L-Gln could alleviate the adverse effects of NE through impacting intestinal function. In conclusion, the results of this sub-project showed that the response or broilers to supplemental L-Gln may possibly vary depending on diet formulation, health status of the birds (stressors) and various experimental conditions. Under the condition of the first study, no conclusive response was observed in terms of excreta moisture when birds fed diets containing 10 g/kg L-Gln or higher concentration of Zn.

Table of Contents

Chapter 1. General Introduction and Objectives

Amino acids are usually categorised into non-essential or essential amino acids depending on whether they can be synthesised by the body at the sufficient rate to support normal protein synthesis and ultimately growth. However, in some case of accelerated growth or other conditions like disease or malnutrition, some non-essential amino acid are not adequately synthesised to the animal requirements when they are so called “conditionally essential amino acids” (Soltan 2009). Glutamine (Gln) is one of these amino acids that plays a critical role for maintaining the mucosal structure through mucin synthesis and also affects gut permeability. Gln which is used for treatment of diarrhoea also stimulates water and electrolyte absorption in the human and rabbit ileum. In addition, several studies have shown that Gln may be a conditionally essential amino acid to maintain gut integrity and reduce inflammation in animals suffering from stress, injury or malnutrition (Soares, *et al.* 2014). Gln-enriched diets have been linked with favourable intestinal effects including maintenance of gut barrier function and enterocyte differentiation. There is evidence for the beneficial effect of Gln in mono-gastric animals (Bartell and Batal 2007). However, its potential for enhancement in barrier function and water excretion in poultry are still not understood. Furthermore, there are no data regarding the interaction between Gln with other minerals, such as Zn, in particular for broilers.

Zinc (Zn) is also essential for normal intestinal barrier function and the regeneration of damaged gut epithelium and it effectively prevents or attenuates the loss of intestinal integrity during malnutrition (Fernandez *et al.* 2014). The effect of Zn in reducing diarrhoea and growth improvement in piglets and humans is also established. For water reabsorption, the proposed mechanism is through inhibition of Cl secretion and increased absorption of Na from the lumen preventing water excretion caused by osmotic differences (Tran *et al.* 2014). However, little is known about the effectiveness of dietary Zn and its potential synergy with Gln, performance of broiler chickens and, reducing excreta moisture through enhancement of intestinal barrier function.

Research in humans and rats indicates that a combination of Gln and minerals such as Zn improves intestinal barrier function suggesting the potential for improving performance, gut health and reabsorption of water (Ladd *et al.* 2010). Furthermore, Zn

and Gln are considered critical gut-trophic nutrients, which have protective roles, as demonstrated in various animal models of intestinal injury and have been proved beneficial in clinical studies in humans afflicted with malnutrition and diarrhoea (Ladd *et al.* 2010). In poultry, there are no investigations reported of the potential synergy between these two nutrients for enhancement of bird performance, increasing gut barrier function and the possible reduction of water excreted. Excess excreted water commonly occurs in the poultry industry and preventative treatment measures are required. It is hypothesized that a synergy between Gln and Zn will also counteract a nutritionally induced water imbalance.

Thus, the current project aimed to determine whether a positive response in broiler chickens can be observed with the inclusion of Gln at low or high level of Zn. A second experiment later added to the project after obtaining the results of the originally designed experiment. The second experiment aimed to investigate the effect of Gln on broilers under NE challenge.

Objectives of this sub-project were as follows:

1. Investigate the effect of Gln and higher inclusion of Zn for gut development and function as well as digesta and excreta moisture
2. Study the counteracting effect of Zn, Gln and their combination in broilers exposed to a water imbalanced induced by reduction of water reabsorption in the gut
3. Investigate the effect of Gln under NE challenge
4. Investigate the underlying mechanism of any effect of Gln on gut function

Chapter 2. Evaluation of high dietary Zn and Gln for intestinal function, digesta and excreta moisture with and without Mg supplementation to reduce intestinal water reabsorption

Introduction

Gln is an amino acid that has several important metabolic functions. Several studies have confirmed that Gln is vital in maintaining the functional integrity of the gut as it plays a nourishing role for rapidly dividing intestinal epithelial cells in particular enterocytes and lymphocytes (Soares *et al.* 2014). This maintenance role is directly related to tight junctions, mucosal cell proliferation and differentiation (Soares, et al., 2014). Gln is involved in mucin synthesis. N-acetylglucosamine is a glycoprotein and a component of the mucin that protects mucosal surfaces and its formation is fully dependent on Gln (Coster *et al.* 2004). Gln may be considered conditionally essential amino acid when animal suffers from stress, injury or malnutrition. Under such circumstances the requirement may exceed the capacity for endogenous synthesis required to maintain gut integrity and reduce inflammation because during an immune response a marked increase in the uptake of plasma Gln by immunocytes occurs (Coster, et al., 2004). Beneficial effects of Gln on growth performance and gut development in broiler chickens have been previously shown (Bartell and Batal 2007; Murakami *et al.* 2007) with inclusion levels between 5 to 10 g/kg of the diets. Nevertheless, its effectiveness in regards to enhancement of gut barrier function, water reabsorption and more importantly its interaction with other nutrients with similar regulatory effects are still largely unknown for poultry. Gln is also widely known as a treatment for diarrhea in humans (Yalçin *et al.* 2004). It has been revealed that Glu is superior to glucose alone in promoting net absorption of water, sodium and potassium in rabbit ileum (Islam *et al.* 1997). For poultry, Gln potential for reabsorption of water and thereby reducing excreta moisture has been overlooked and needs to be investigated.

Zn is regarded as an essential trace mineral for normal intestinal barrier function and reconstitution of damaged gut epithelium. During malnutrition, Zn effectively prevents

or attenuates the loss of intestinal integrity (Fernandez *et al.* 2014). Given that Zn plays an important role in multiple aspects of the immune system, the requirement of Zn may increase for optimum immune response compared with growth (Klasing, 1992). Also depending on the form of Zn, its biological function may differ. For example, ZnO appears to be more effective than $ZnSO_4$ in healing skin damage in pigs or enhancement of reepithelialisation (Ågren *et al.* 1991). Research in piglets demonstrated that Zn reduces gut permeability during weaning and thereby is widely used as a treatment for controlling postweaning diarrhoea (Fernandez, et al., 2014). Positive effect of Zn-fortified oral rehydration solution on intestinal permeability, mucosal recovery and treatment of diarrhea in human children also has recently been demonstrated (Tran *et al.* 2014). In poultry, however, there is very little information about the effect of Zn on water reabsorption and excreta moisture. Using a biological based model, van der Hoeven-Hangoor (2013a) found that there was a negative correlation between Zn content and moisture in excreta of broiler chickens (a total of 131 samples) suggesting that further investigation is warranted to understand the effect of Zn on for excreta moisture. Although dietary Zn and its different forms for poultry has been investigated (Burrell *et al.* 2004; Hu *et al.* 2013), its role for intestinal barrier function and it interaction with other nutrients are not fully understood.

Interaction of Zn and Gln

As discussed above, Zn and Gln are both known as gut-trophic nutrients affect gut barrier function and thereby have effect on bird performance. To the best of this researcher's knowledge, there are no data regarding interaction or additivity between Gln and Zn in poultry. In a study on humans, Lima et al., (2014) found that Gln alone or in combination with Zn and vitamin A improved intestinal barrier function in children. In an animal study, Zn and Gln also improved behaviour and growth in undernourished mice during suckling (Ladd *et al.* 2010). There is therefore justification to investigate synergy between these two nutrients for gut function/development, excreta moisture and growth performance.

Water reabsorption and wet dropping

The occurrence of wet litter is regarded as a multifactorial issue influenced by key factors such as management, housing, disease, gut health and diet. This phenomenon

is a major concern to the chicken meat industry and has an impact on production; the environment and bird welfare. Poultry excreta contains approximately 80% moisture substantially contributing to litter moisture content. Diet composition and individual nutrients can affect excreta moisture through nutrient digestibility, electrolyte balance or gut integrity which may contribute to the prevalence or alleviation of wet droppings (van der Hoeven-Hangoor, 2013b). Therefore, in addition to investigating the effects and interaction of Zn and Gln, it is of interest to study the potential of supplementation of Zn and Gln on water reabsorption and therefore reduction of excreta moisture.

The logic for the use of MgCl to assess the water reabsorption regulatory effects of Zn and Gln

Different individual minerals have diverse contribution to digesta osmolality (Etheridge *et al.* 1984). The use of Mg laxative in human (Vu *et al.* 2000) and different animal species (Antonisamy *et al.* 2009; Ikarashi *et al.* 2011) is widely established. Recently, van der Hoeven-Hangoor *et al.* (2013b) investigated the use of Mg sources to establish a model for wet litter studies suitable for nutritional and management intervention. It was found that MgCl was more effective than any other source of Mg to increase the excreta moisture through reduced water reabsorption. The role of Mg and its preference to be used as a model is quoted here from this publication: ***"****Besides the nutritional effects of Mg, it may also serve as a wet litter model in broilers, increasing excreta moisture by reduced water reabsorption in contrast to increased water consumption****.*** *This model could facilitate the development of dietary or management intervention studies to reduce wet litter problems in practice****"*** (van der Hoeven-Hangoor et al., 2013b).

Although, MgCl is not used commercially in broiler diets, its role to reduce water reabsorption in contrast to water consumption (i.e. through use of NaCl) makes it an attractive candidate to use as model to test the counteracting effect of nutrients that have potential to increase water reabsorption in the intestine. In other word, in contrast to Mg, Na affects more on blood osmotic pressure resulting in increased water consumption and excreta moisture. However, Cl alone does not seem to have such effect (Pesti *et al.* 1999). Therefore, this experiment aimed to investigate the effects of

Zn and Gln in addition to the use of MgCl on excreta moisture and intestinal function and performance of broiler chickens.

Material and methods

The Animal Ethics Committees of the University of Adelaide and Primary Industries and Regions South Australia approved all the experimental procedures.

Experimental design and diets

A 2 × 2 × 2 factorial arrangement of treatments was used to investigate three main factors of L-Gln inclusion (0 vs 10 g/kg), Zn supplementation (80 and 160 mg/kg) and MgCl (0 or 2 g/kg). To be able to accurately control the added Zn to the diets, a no added Zn premix was formulated and supplied (DSM, Wagga Wagga, NSW). Zn was added in form of ZnO (80% Zn) at the levels of 100 and 200 mg/kg to obtain the desired Zn concentration of 80 and 160 mg/kg. L-Gln was added to the diets in lieu of L-Alanine (12.2 g/kg) in control diets to make all diets isonitrogenous. MgCl was used in half of grower treatments at 2 g/kg. In starter and finisher diets, no MgCl was included.

The composition of all experimental diets is shown in Table 1.

Housing and bird management

A total of 576 male day-old Ross 308 broiler chickens were purchased from a local hatchery (Baiada hatchery, Willaston, South Australia). On arrival, chicks were assigned to 48 rearing pens on wood shavings in two temperature-controlled rooms. Each treatment was replicated 6 times with 12 birds per replicate. Birds were fed starter (d 0-9), grower (d 10-23) and finisher (d 24-35) experimental diets. Temperature was set at 33-34ºC on the first day of the experiment and then gradually decreased by 1ºC every second day until a stable temperature of 24ºC was reached by d 21. In the first week of age, birds had 23 h light and 1 h dark. Subsequently, birds received 16 h light and 8 h dark.

Feed and water were provided *ad libitum* throughout the experiment. At the end of each phase of feeding, birds were weighed and feed consumed measured. FCR was calculated and adjusted for mortality if occurred for any treatment.

Excreta and digesta moisture

On d 10 and 25, three birds per replicate were randomly chosen and euthanized in order to obtain digesta from ileum, jejunum, caeca and colon. The contents of all sections were separately collected into plastic containers and subsequently placed in -20 freezer until analysed for moisture content. The moisture content was measured by differences of wet and dry samples after being freeze-dried.

Fresh excreta samples were collected on d 9 (on plastic sheets), and d 22 and 35 by placing two birds per pens in metabolism cages. Excreta samples were subsequently collected from trays placed under each metabolism cages. Moisture content was determined by differences of wet and dry samples after being oven-dried at 85 °C.

Statistical analysis

Data of the experiment were subjected to statistical analysis using 3-way ANOVA of GLM procedure of SAS (2003) according to a 2×2×2 factorial arrangement of treatments to assess the main effects of Zn inclusion, L-Gln, MgCl and their 2- or 3-way interactions. Data were checked for normal distribution. One cage constituted an experimental unit and the values presented in the tables are means with pooled standard error of mean (SEM) (n=48). If a significant effect was found, differences between treatments or main effects were separated by Duncan multiple range test. All statements of significance were considered on a *P*-value less than 0.05 and tendencies were specified for $0.05 < P < 0.10$.

Table 1 Composition of experimental diets (normal Zn 80 mg/kg)

Ingredients (%)	Starter		Grower		Finisher	
	Control	**Gln**	**Control**	**Gln**	**Control**	**Gln**
Wheat	32.539	32.539	36.649	36.649	41.903	41.903
Soybean meal	31.720	31.720	26.714	26.714	22.991	22.991
Sorghum	20.000	20.000	20.000	20.000	20.000	20.000
Canola meal expeller	5.000	5.000	5.000	5.000	4.781	4.781
Canola oil	4.968	4.968	6.026	6.026	5.345	5.345
Di-calcium phosphate	1.785	1.785	1.552	1.552	1.351	1.351
Limestone	1.171	1.171	1.093	1.093	1.013	1.013
L-Alanine	1.220	0.000	1.220	0.000	1.218	0.000
L-Glutamine	0.000	1.000	0.000	1.000	0.000	1.000
Sodium bicarbonate	0.391	0.391	0.389	0.389	0.367	0.367
DL-Methionine	0.357	0.357	0.313	0.313	0.261	0.261
L-Lysine	0.299	0.299	0.278	0.278	0.224	0.224
L-Threonine	0.173	0.173	0.170	0.170	0.127	0.127
Premix[1]	0.150	0.150	0.150	0.150	0.150	0.150
Salt	0.142	0.142	0.145	0.145	0.160	0.160
Xylanase	0.005	0.005	0.005	0.005	0.005	0.005
ZnO[2]	0.010	0.010	0.010	0.010	0.010	0.010
Choline Chloride (60%)	0.062	0.062	0.076	0.076	0.082	0.082
Sand	0.010	0.230	0.210	0.427	0.010	0.228
Nutrients %						
Dry matter	88.76		88.71		88.40	
ME MJ/kg	12.61		13.04		13.03	
Crude protein	22.56		20.72		19.50	
Crude fat	6.83		7.87		7.22	
Crude fibre	2.63		2.57		2.57	
Digestible lysine	1.28		1.15		1.03	
Digestible methionine	0.65		0.58		0.52	
Digestible methionine + cysteine	0.95		0.87		0.80	
Digestible threonine	0.83		0.77		0.69	
Digestible tryptophan	0.26		0.24		0.22	
Calcium	0.96		0.87		0.79	
Phosphorus available	0.48		0.43		0.39	

[1] Composition of the premix per kg of diet; vit. A 14000 IU, vit. D_3 5000 IU, vit. E 75 mg, vit. K_3 3.75mg, vit. B_1 3 mg, vit. B_2 9 mg, vit. B_6 5mg, vit. B_{12} 0.03 mg, Biotin 0.2 mg, Pantothenic acid 15 mg, Folic acid 2.5 mg, Niacin 55 mg, Copper 20 mg, Co 0.25 mg, Iodine 1.25 mg, Fe 40 mg, Mn 120 mg, Mo 2 mg, Se 0.3 mg, Phytase 100 mg , Ethoxyquin 100mg.

[2] ZnO was added at the expense of sand to make up the high Zn diets. Therefore high Zn diets contained 200 mg/kg of added ZnO.

Results

The growth performance results are presented in Table 2. There was no significant effect of any of the three main treatments on body weight gain and feed intake of birds at any stage of the experiment. No interaction was found between Zn, Gln or MgCl supplementation. Birds fed higher Zn (160 mg/kg) tended (P = 0.09) to have higher weight gain only in the first 9 days of age. Feeding 10 g/kg L-Gln increased (P<0.001) FCR of the birds only from hatch until d 9 after which there was no effect. Feeding higher concentration of Zn or MgCl showed no effect on FCR at any stage of the experiment.

Intestinal and excreta moisture contents of broilers are shown in Table 3. Excreta moisture on average was above 81.2% for starter, grower and finisher phases of feeding. Similar to performance results, there were almost no effect of experimental treatments on intestinal digesta or excreta moisture content except for a significantly reduced moisture content of the ileal samples taken at d 10 of age as a result of higher Zn concentration.

MgCl only tended (P = 0.08) to increase caecal moisture when assessed on d 23 of age.

Table 2. Growth performance of broiler chickens fed L-Gln, two Zn concentration and MgCl

	Body weight gain (g/bird)				Feed intake (g/bird)				FCR			
	d 0-9	d 10-23	d 24-35	d 0-35	d 0-9	d 10-23	d 24-35	d 0-35	d 0-9	d 10-23	d 24-35	d 0-35
Main effects[1]												
L-Gln												
0	248	1128	1478	2854	247	1435	2306	3988	0.995[b]	1.271	1.561	1.397
10 g/kg	245	1135	1456	2836	250	1445	2294	3989	1.019[a]	1.274	1.576	1.406
Zn[2]												
80 mg/kg	245	1130	1457	2831	247	1438	2299	3985	1.012	1.272	1.579	1.407
160 mg/kg	249	1133	1477	2859	249	1442	2301	3992	1.002	1.273	1.558	1.396
MgCl[3]												
0	-	1132	1459	2838	-	1448	2292	3987	-	1.278	1.571	1.405
2 g/kg	-	1131	1475	2853	-	1433	2308	3990	-	1.267	1.566	1.398
SEM	1.1	5.5	11.0	13.7	1.2	8.9	18.0	23.2	0.0032	0.0056	0.0071	0.0043
Source of variation (P values)[4]												
L-Gln	0.257	0.570	0.318	0.503	0.185	0.561	0.733	0.975	0.001	0.811	0.299	0.274
Zn level	0.091	0.765	0.353	0.314	0.498	0.812	0.976	0.876	0.153	0.974	0.136	0.204
Magnesium	-	0.893	0.486	0.588	-	0.398	0.657	0.948	-	0.309	0.738	0.485

[1] Each value for the main effects represent mean of 24 replicates

[2] Added as ZnO (80% Zn) as 100 and 200 mg/kg to obtain the desired added Zn levels of 80 and 160 mg/kg, respectively.

[3] MgCl was only added to the grower diets fed from d 10-23.

[4] No significant two- or three-way interactions were detected (*P* values not shown).

Table 3. Intestinal and excreta moisture content (%) of broiler chickens fed L-Gln, Zn and MgCl

	Starter phase (d 10)				Grower phase (d 23)				Excreta moisture		
	Jejunum	Ileum	Caeca	Colon	Jejunum	Ileum	Caeca	Colon	Starter (d 9)	Grower (d 22)	Finisher (d 34)
Main effects[1]											
L-Gln											
0	82.67	81.70	82.79	80.44	80.32	80.77	81.43	80.41	81.26	82.95	81.75
10 g/kg	82.67	81.94	83.26	80.92	81.11	81.01	81.90	80.19	81.24	83.11	82.15
Zn[2]											
80 mg/kg	82.73	82.21[a]	82.74	80.22	80.59	80.45	81.17	80.01	81.20	83.21	82.28
160 mg/kg	82.61	81.43[b]	83.32	80.81	80.83	81.29	82.16	80.61	81.30	82.85	81.62
MgCl[3]											
0	-	-	-	-	80.86	80.85	80.93	80.23	-	82.53	81.38
2 g/kg	-	-	-	-	80.60	80.90	82.39	80.38	-	83.53	82.53
SEM	0.194	0.166	0.371	0.291	0.247	0.212	0.417	0.275	0.130	0.339	0.384
Source of variation (P values)[4]											
L-Gln	0.999	0.473	0.527	0.794	0.117	0.587	0.582	0.686	0.938	0.813	0.601
Zn level	0.745	0.023	0.435	0.319	0.622	0.067	0.244	0.282	0.702	0.595	0.394
Magnesium	-	-	-	-	0.561	0.841	0.088	0.799	-	0.147	0.142

[1] Each value for the main effects represent mean of 24 replicates

[2] Added as ZnO (80% Zn) as 100 and 200 mg/kg to obtain the desired added Zn levels of 80 and 160 mg/kg, respectively.

[3] MgCl was only added to the grower diets fed from d 10-23.

[4] No significant two- or three-way interactions were detected (*P* values not shown).

Discussion

Birds in this study performed well above the expected standards of Ross 308 broilers (2014). There was no sign of disease or any abnormal behaviour throughout the experiment. However, this experiment culminated into no significant results for almost all the performance related parameters as well as intestinal digesta and excreta moisture content. Therefore, there was no justification to investigate the underpinning mechanisms in relation to water reabsorption or intestinal function associated with experimental treatments.

In the literature, the effect of Gln on broiler performance is contradictory. While there is evidence for positive response (Bartell and Batal 2007), in some studies no significant effect was found (Sakamoto *et al.* 2006; Shakeri *et al.* 2014). The lack of the effect of Gln could be explained through several possibilities. First, the diets used in this study met all the specifications recommended for Ross 308 (2014) and contained adequate amount of protein and all essential, including conditionally-essential and most likely non-essential amino acids. Therefore, as there is no determined requirement for Gln in broilers, the Gln may have been more than adequately supplied and therefore not limiting under the conditions of this experiment. It is shown that supplementation of Gln in addition to other amino acids, in particular glycine may help to improve performance of broilers fed low-protein diets (Awad *et al.* 2015). Second, in present study, birds did not respond to the MgCl as a potential stress/challenge under which Gln could have possibly been beneficial. As previously explained, under certain physiological and disease challenges, *de novo* synthesis of Gln may not be sufficient to support optimum growth and function of the intestine and whole body. In this study, it was hypothesized that under increased concentration of MgCl, a reduced water reabsorption may cause a water imbalance at intestinal level (van der Hoeven-Hangoor *et al.* 2013b) for which Gln could have been a possible effective treatment (see Introduction). Failure of MgCl to induce such effect may therefore have contributed to non-significant impact of Gln. Third, L-Gln was supplemented to the diets at 10 g/kg compared with 12.2 g/kg (equivalent nitrogen) of L-Alanine to ensure that all diets were isonitrogenous. That way, any possible differences could be explained and related to Gln supplementation and not the nitrogen or balance/ratio of amino acids as previously documented and explained in the literature (Wang *et al.* 2008). It may be possible that under normal conditions as tested in this study, Gln supplementation may not be necessary.

While Gln and Zn are known as treatments of diarrhoea and water imbalance, no particular effect of these two nutrients was observed in this study for intestinal digesta and excreta moisture. No apparent reason can be given for this lack of effect rather than perhaps general high moisture content of the all intestinal content in the study which may have possibly limited the effect of treatments.

As indicated before, different sources of Mg have been proposed as a model for inducing water imbalance through reduction of water reabsorption rather than increasing water intake (van der Hoeven-Hangoor *et al.* 2013b). While van der Hoven-Hangour (2013b) reported that 2 g/kg of MgCl had a significant effect on excreta moisture, the result of current experiment does not support such observation. It appears that the required amount of MgCl should have been substantially greater to cause any significant effect. The suitability of Mg for experimental purposes involving live animals therefore can be questioned.

Similar to MgCl and L-Gln, higher concentration of Zn had no effect on bird performance with subtle effect only on ileal moisture content. The lack of effect of higher Zn on bird performance was expected, similar to other research (Huang *et al.* 2007). In fact, Zn was included as a factor mainly for its known effect on water reabsorption and intestinal function. van der Hoeven-Hangoor *et al.* (2013a) found that Zn content of excreta was negatively correlated with moisture in a data set of 351 observations obtained from 8 different broiler feeding trials. Results of the current study did not give any conclusive response in terms of excreta moisture in response to dietary Zn concentration where only two levels of 80 and 160 mg/kg were supplemented. To be able to objectively investigate the effect of Zn on intestinal digesta moisture and/or water reabsorption it appears that Zn may need to be fed both under and close to the requirement.

As there was no significant effect associated with experimental factors, there was no justification to further investigate the underlying mechanisms associated with the role of both Zn and Gln in this study. As a result, the trial was concluded with all the performance and intestinal digesta moisture content data. Nevertheless, investigation of the role of Zn and Gln under challenge conditions particularly intestinal disorders still warrants research. As indicated, there is clear lack of data in poultry in regard to the role of Gln for intestinal barrier function and regeneration of the epithelial cells.

In conclusion, under the condition of this study, birds were not responsive to the tested levels of Gln, Zn and MgCl. No clear effect on digesta and excreta moisture was found. Despite previous studies, the result of the current experiment questions the suitability of Mg to use as nutritional model for inducing wet dropping or reducing water reabsorption at intestinal level. While the current industry practice of 80-100 mg/kg added Zn appears more than adequate to support the optimum growth of birds further research into the role of Zn in relation to water reabsorption and intestinal function is of great scientific interest.

Chapter 3. Effects of glutamine supplement on growth performance of broilers challenged with necrotic enteritis

Guangda (Danny) Xue – University of New England

Introduction

Necrotic enteritis (NE) is one of the most devastating acute and subclinical diseases caused by *Clostridium perfringens* in boiler chickens. It is estimated that NE outbreaks could impose a cost of 5 cents per broiler, or about $2.5b each year globally. Thus, this disease attracts more attention in the poultry industry since the ban on in-feed antibiotics in some countries has led to an elevation of the incidence of NE in broiler flocks. Subclinical NE, considered to be the most economically important form, increases FCR without causing high mortality and obvious disease symptoms and pathology. To our best knowledge, the mechanism as to how this disease development results in the productivity loss still remains largely unknown. To accomplish this, a better and comprehensive understanding of metabolic cascade involving immunological and physiological perspectives is essential. Enteric inflammation can be augmented by various factors including dietary stress, poor management, dysbacteriosis and pathogen infection. For example NE infection given by microscopic examination of early stages of NE shows strong inflammatory reactions to *C. perfringens*; The lamina propria is hyperaemic and infiltrated with numerous inflammatory cells, mainly heterophilic granulocytes with most significant early changes seen at the interface of the basal domain of enterocytes and lamina propria (Olkowski *et al.* 2006). Researches have shown dramatically increased pro-inflammatory cytokines and chemokines in NE challenged chickens (Lee *et al.* 2011).

It has also been well accepted that intestinal inflammation is an energy-consuming process. The gastrointestinal tract of most animals has been estimated to consume 25% of total body energy needs (Cant *et al.* 1996). The immune system has been estimated to account for approximately 1 to 3% of the basal metabolic rate in healthy vertebrates under normal circumstances (Romanyukha *et al.* 2006), whereas immunologically challenged vertebrates can have resting metabolic rates that are increased from 8 to 27% (Martin *et al.* 2003), showing the increased maintenance energy cost of immune challenge. Having additional

evidence, isolated mitochondria of laboratory rats stimulated *in vivo* with TNF- a or IL-1 can undergo a 30% increase in respiration rate (Jin *et al.*1995). To fuel this up-regulation, immune cells require glucose and Gln at high levels, which leads to the rapid breakdown of the body's reserves of protein (to provide Gln), carbohydrates, and lipids (Michie 1996). Moderate infections can easily lead to 150–200% increase in rates of gluconeogenesis in the host, often leading to severe wasting of lean tissue (Lochmiller and Deerenberg 2000). This strongly explains the impaired growth performance of broilers suffering from subclinical NE which is often overlooked as there are no clear clinical signs. Gln-enriched diets have been linked with favourable intestinal effects including maintenance of gut barrier function and enterocyte differentiation (Murakami *et al.* 2007). Gln is responsible for mucosa structure maintenance, through mucin synthesis and the maintenance of a barrier against bacteria attacks. Because Gln is the main enterocyte energy source, effect of Gln supplementation on reconstitution of the intestinal mucosa, after some damage, has been investigated (Blikslager *et al.* 2001). Thus, it is logical to have a hypothesis that additional Gln in diet may provide protection against the NE challenge by maintaining intestinal integrity and compensating for metabolic loss due to the enteric inflammation from NE challenge. Therefore this study was designed to examine the potential protective effects of L-Gln supplement on broiler growth performance during and after NE challenge.

Materials and methods

The experiment was conducted at Kirby Smart Farm, University of New England, Armidale, Australia. All the experimental procedures were approved by the Animal Ethic Committee of the University of New England.

A total 714 male Ross 308 broilers were allocated to the experimental treatments in a 2×3 factorial arrangement of treatments to test the main effect of L-Gln supplementation (un-supplemented or supplemented at 10 g/kg on top of either in all starter, grower and finisher or in starter and grower diets only) without or with NE challenge.

Treatments were therefore as follow:

1) No challenge – no additive in starter, grower and finisher
2) No challenge – 10 g/kg Gln addition in starter, grower and finisher
3) No challenge – 10 g/kg Gln addition in in starter and grower, 0% in finisher
4) NE challenge – no additive in starter, grower and finisher

5) NE challenge – 10 g/kg Gln addition in starter, grower and finisher
6) NE challenge – 10 g/kg Gln addition in in starter and grower, 0% in finisher

Each treatment was replicated 6 times in a total of 36 pens accommodating 15 birds per pen/replicate. The study was conducted from 0 to 35 d of age. And birds were fed starter, grower and finisher diets. The basal diets were wheat, barley, SBM, MBM based meeting the Ross 308 broiler specification (Table 4). Starter diets were fed from hatch to d 10 of age followed by grower diet fed until d 24 of age. Finisher diets were then fed from d 24 to d 35. All grower and finisher diets were fed in pelleted form. Starter diets were fed as crumbles.

Birds were reared in floor pens that had dimensions of 80 cm x 30 cm or 0.24 m^2 (taking into account hanging feeders). Wood shavings were provided as bedding material. Temperature were held at 33-34 °C initially and gradually decreased by 3°C every week until 22-24 °C was reached by d 21 of age. Chicks were subjected to artificial fluorescent illumination of 23 hours on d0 to 7 (30 lux), then 18 h from d 8 to 35 (15 lux). Each pen was equipped with a tube feeder and 2 drinkers. Water and feed were provided *ad libitum*.

NE challenge model

A well-established NE challenge model was used to include NE in birds according to the descriptions of Wu et al. (2010). As described by Wu *et al.* (2010), on d 9, birds in appropriate groups were orally gavaged with a suspension of 5000 sporulated oocysts of *Eimeria acervulina*, Eimeria *maxima*, and *Eimeria tenella* (5000 of total three species) (Bioproperties Pty., Glenorie, NSW, Australia) in 1 ml sterile phosphate-buffered saline (PBS). Birds in other groups were given sterile PBS in lieu of *Eimeria*. On d 14, birds were challenged *per os* with 2 ml of *C. perfringens* suspension at a concentration of 10^8 –10^9 CFUs/ml. A primary poultry isolate of *C. perfringens* type A strain EHE-NE18 (CSIRO Livestock Industries, Geelong, Australia) was incubated overnight at 39 degree in 100 ml of thioglycollate broth (USP alternative; Oxoid) followed by subsequent overnight incubations of 1 ml of the previous culture in 200 ml of cooked meat medium (Oxoid), and then in 1000 ml of thioglycollate broth (USP alternative; Oxoid) containing starch (10 g/L) and casitone (5 g/L) to obtain the challenge inoculum. Birds in unchallenged groups received 2 ml of sterile thioglycollate broth (USP alternative; Oxoid).

Measurements

- Live weight d 0,d 10,d 24,d 35
- Feed intake and FCR
- Mortality
- Lesion scores on d16. The pathologic diagnosis of NE was performed applying the methods previously used by Wu *et al.* (2010). The NE lesions of duodenum, jejunum, and ileum of all sampled birds were assessed with lesions scored as 0, 1, 2, 3, or 4 (depending on severity) when present in the tissues.

Statistical analyses

Performance data were analysed using the statistical package IBM® SPSS® Statistics package version 22 (IBM Corporation). The main effects of dietary supplementation, NE challenge and their interactions were examined by two-way analysis of variance using the General Mixed Model. Mortality and lesion score data were analysed by the nonparametric Kruskal-Wallis test as they were not normally distributed. Treatment means were separated using Tukey HSD post hoc test where appropriate. Statistical significance was declared at $P < 0.05$.

Table 4. Ingredient and nutrient composition (as-fed) of experimental basal diets

Ingredients (%)	Starter	Grower	Finisher
Wheat	38.1	42.3	46.4
Barley	20.0	20.0	20.0
Soybean meal	30.9	25.9	22.7
Meat and bone meal	5.6	6.0	4.0
Cottonseed oil	3.4	3.8	4.9
Limestone	0.51	0.18	0.57
Sodium chloride	0.09	0.09	0.12
Na bicarbonate	0.20	0.18	0.20
Vitamin mineral premix[1]	0.2	0.2	0.2
Choline Cl (60%)	0.08	0.08	0.07
L-lysine HCl	0.30	0.28	0.24
DL-methionine	0.41	0.36	0.33
L-threonine	0.22	0.19	0.15
TiO_2	0	0.5	0
Nutrient composition, percent unless otherwise noted			
ME, MJ/kg	12.34	12.55	12.97
Crude protein	24.1	22.4	20.3
Crude fat	5.7	6.1	7.0
Crude fibre	3.0	2.9	2.9
Calcium	0.96	0.87	0.78
Available phosphorus	0.48	0.50	0.39
Sodium	0.18	0.16	0.16
Chloride	0.25	0.22	0.20
Digestible Arg	1.370	1.230	1.090
Digestible Lys	1.280	1.150	1.020
Digestible Met	0.694	0.631	0.573
Digestible Cys	0.258	0.241	0.228
Digestible M+C	0.950	0.870	0.800
Digestible Trp	0.271	0.245	0.227
Digestible Leu	1.418	1.298	1.168
Digestible Ile	0.876	0.795	0.727
Digestible Thr	0.860	0.770	0.680
Digestible Val	0.994	0.908	0.825
Choline, mg/kg	1700	1600	1500
Linoleic acid	2.47	2.65	3.20

[1]Vitamin and mineral concentrate supplied per kilogram diet: vit A, 12,000 IU; vit D, 5000 IU; vit K, 3 mg; vit E, 75 mg, vit B1, 3 mg; vit. B2, 8 mg; niacin, 55 mg; pantothenic acid, 13 mg; vit. B6, 5 mg; folic acid, 2 mg; vit. B16, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg. Copper, 16 mg; Fe , 40 mg; Iodine, 1.25 mg; Se, 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg;

Results

All the performance data and NE intestinal lesion scores are given in Tables 5 to 10. Results showed a successful NE challenge with retardation of weight gain and FCR observed in challenged birds independent of dietary inclusion of L-Gln. There was no interaction between NE challenge and L-Gln supplementation for bird performance at any stage of growth. Inclusion of 10 g/kg L-Gln in the diet improved ($P < 0.01$) weight gain and FCR of the birds in starter phase of feeding (d 0-10).

For grower phase from d 10 to 25, supplementation of L-Gln increased feed consumption ($P < 0.01$), weight gain ($P < 0.001$) and improved FCR ($P < 0.05$). Withdrawing L-Gln from the finisher diets fed to bird from d 25 to 35 diminished the effect on weight gain. However, at the same time, the birds that received L-Gln higher a higher weight gain compared to control birds ($P < 0.01$).

When data were assessed for the first 25 days of age (Table 8), 10 g/kg L-Gln supplementation led to higher feed consumption and weight gain and improved FCR independent of NE challenge. Conversely, NE challenge reduced ($P < 0.001$) both feed intake and weight gain and increased FCR. For d 1 to 35 (Table 9), while there was no effect of either NE challenge or L-Gln on feed intake, birds fed L-Gln throughout the experiment gained more weight and had lower FCR than birds on non-supplemented diets.

There was a significant interaction between diets and NE challenge for lesion scores in duodenum, jejunum and ileum ($P < 0.01$). For jejunal tissues, inclusion of L-Gln decreased the lesion score caused by NE. Supplementation of L-Gln in starter and grower only ameliorated lesions caused by NE challenge in ileal tissues. Regardless of the diets, there was a distinct effect of NE challenge on lesion scores for all the three intestinal sections. The effect of NE challenge or L-Gln addition on bird mortality was not significant at any stage of this experiment.

Table 5. Performance of broilers from 1 to 10 days

Treatment means		Intake g/bird	Weight gain g/bird	FCR	Mortality
Interactions					
Challenge	**L-Gln**				
No	G0	200	169	1.190	0.00%
No	G1	200	179	1.119	0.00%
Yes	G0	201	166	1.212	0.00%
Yes	G1	206	180	1.147	0.00%
SEM		2.1	2.2	0.011	0.00%
Main Effects*					
Challenge					
No		200	174	1.154	0.00%
Yes		204	173	1.179	0.00%
10 g/kg L-Gln addition					
G0		201	168b	1.201a	0.00%
G1		203	180a	1.133b	0.00%
***P* value**					
Challenge		0.473	0.754	0.247	1.000
L-Gln		0.571	0.009	0.003	1.000
Challenge * L-Gln		0.590	0.606	0.886	1.000

G0 (control) = (no L-Gln addition in starter),
G1= (10 g/kg L-Gln in starter)

Table 6. Performance of broilers from 10 to 25 days

Treatment means		Intake g/bird	Weight gain g/bird	FCR	Mortality
Interactions					
Challenge	**L-Gln**				
No	G0	1455	1158	1.256	1.11%
No	G1	1507	1234	1.222	0.00%
Yes	G0	1307	982	1.331	1.11%
Yes	G1	1360	1045	1.301	1.67%
SEM		17.3	18.5	0.008	0.39%
Main Effects*					
Challenge					
No		1481a	1196a	1.239b	0.56%
Yes		1334b	1013b	1.316a	1.39%
10 g/kg L-Gln addition					
G0		1381b	1070b	1.294a	1.11%
G1		1433a	1139a	1.261b	0.83%
***P* value**					
Challenge		<.0001	<.0001	<.0001	0.154
L-Gln		0.043	0.001	<.0001	0.737
Challenge * L-Gln		0.999	0.742	0.772	0.367

G0 (control) = (no L-Gln addition in starter, grower),
G1= (10 g/kg L-Gln in starter, grower)

Table 7. Performance of broilers from 25 to 35 days

Treatment means		Intake g/bird	Weight gain g/bird	FCR	Mortality
Main Effects*					
Challenge	**L-Gln**				
No	G0	1853	1151	1.608	0.00%
No	G1	1909	1175	1.624	1.11%
No	G2	1788	1140	1.568	0.00%
Yes	G0	1812	1203	1.506	0.00%
Yes	G1	1997	1275	1.566	1.11%
Yes	G2	1915	1213	1.576	0.00%
SEM		33.0	10.5	0.022	0.27%
Interactions					
Challenge					
No		1850	1155b	1.600	0.37%
Yes		1908	1230a	1.549	0.37%
10 g/kg L-Gln addition					
G0		1832	1177b	1.557	0.00%
G1		1953	1225a	1.595	1.11%
G2		1851	1176b	1.572	0.00%
***P* value**					
Challenge		0.398	<.0001	0.291	0.552
L-Gln		0.286	0.022	0.796	0.346
Challenge * L-Gln		0.570	0.424	0.652	0.672

G0(control)=(no L-Gln addition in starter, grower and finisher),
G1= (10 g/kg L-Gln in starter, grower and finisher),
G2= (10 g/kg L-Gln in starter, grower, no L-Gln in finisher)

Table 8. Performance of broilers from 1 to 25 days

Treatment means		Intake g/bird	Weight gain g/bird	FCR	Mortality
Interactions					
Challenge	**L-Gln**				
No	G0	1588	1335	1.190	1.17%
No	G1	1671	1419	1.178	0.00%
Yes	G0	1406	1154	1.217	1.17%
Yes	G1	1490	1231	1.210	1.75%
SEM		20.4	19.2	0.003	0.41%
Main Effects*					
Challenge					
No		1643a	1391a	1.184b	0.00%
Yes		1462b	1205b	1.214a	2.00%
10 g/kg L-Gln addition					
G0		1497b	1244b	1.203a	1.00%
G1		1581a	1325a	1.194b	1.00%
***P* value**					
Challenge		<.0001	<.0001	<.0001	0.154
L-Gln		0.003	<.001	0.033	0.737
Challenge * L-Gln		0.989	0.854	0.581	0.367

G0 (control)=(no L-Gln addition in starter, grower),
G1= (10 g/kg L-Gln in starter, grower)

Table 9. Performance of broilers from 1 to 35 days

Treatment means		Intake g/bird	Weight gain g/bird	FCR	Mortality
Main Effects*					
Challenge	**L-Gln**				
No	G0	3353	2476	1.354	1.4%
No	G1	3452	2584	1.336	1.2%
No	G2	3335	2523	1.321	1.2%
Yes	G0	3284	2357	1.393	1.2%
Yes	G1	3440	2509	1.371	2.3%
Yes	G2	3351	2441	1.373	2.3%
SEM		25.2	19.2	0.005	0.5%
Interactions					
Challenge					
No		3380	2528a	1.337b	1.2%
Yes		3359	2436b	1.379a	1.9%
10 g/kg L-Gln addition					
G0		3319	2417b	1.374a	1.3%
G1		3446	2547a	1.353b	1.8%
G2		3343	2482ab	1.347b	1.8%
***P* value**					
Challenge		0.668	0.007	<.0001	0.429
L-Gln		0.102	0.010	0.001	0.855
Challenge * L-Gln		0.784	0.842	0.375	0.940

G0(control) = (no L-Gln addition in starter, grower and finisher),
G1= (10 g/kg L-Gln in starter, grower and finisher),
G2= (10 g/kg L-Gln in starter, grower, no L-Gln in finisher)

Table 10. Duodenum, jejunum and ileum NE lesion scores

		NE lesion scores (d16)		
		Duodenum	**Jejunum**	**Ileum**
Interactions				
Challenge	**L-Gln**			
No	G0	0.3b	0.2b	0.1b
No	G1	0.3b	0.0b	0.0b
Yes	G0	0.5ab	1.3a	0.8a
Yes	G1	0.9a	0.3b	0.2b
SEM		0.09	0.10	0.06
Main effect				
Challenge				
No		0.3b	0.1b	0.0b
Yes		0.7a	0.8a	0.5a
10 g/kg L-Gln addition				
G0		0.4	0.7a	0.4a
G1		0.6	0.2b	0.1b
***P* value**				
Challenge		0.002	0.015	0.003
L-Gln		0.310	0.020	0.028
Challenge * L-Gln		0.009	0.005	0.002

G0 (control) = (no L-Gln addition in starter, grower),
G1 = (10 g/kg L-Gln in starter, grower),

Discussion

Reduction in broiler growth performance resulted from subclinical NE (SNE) has been well documented (Skinner *et al.* 2010). There was a considerable retarded performance of broilers exposed to SNE challenge in the current study. However, mortality of the birds was not significant which further confirm that the NE challenge applied to the birds was subclinical. Broiler weight gain, feed intake and FCR were largely influenced by dietary L-Gln during the experimental period which may suggest that L-Gln was playing a role in improving growth performance. Previous reports of broiler performance subjected to 10 g/kg L-Gln supplementation have been inconsistent. Sakamoto *et al.* (2006) reported no

differences in performance responses when 14-d-old broilers were fed corn-soy diets supplemented with 10 g/kg L-Gln whereas Bartell and Batal (2007) observed significant improvements in body weight gain fed same amount of L-Gln. However, effects of L-Gln are likely to be more obvious in presence of stressors as the findings of Dai *et al.* (2011), Hu *et al.* (2015) and Olubodun *et al.* (2015) showed that 10 g/kg L-Gln significantly improved the birds performance under the heat stress suggesting that L-Gln may become conditionally essential for broilers health and productivity under such critical conditions (Novak *et al.* 2002). For the first time, the current study confirmed that the impacts of SNE on broiler growth performance can be diminished by including 10 g/kg L-Gln in the diet. The mode of action can be speculated upon in two main ways. Firstly, SNE, mediating by inflammatory response, results in gluconeogenesis maintaining glucose level, especially during the anorexia as one of the consequences of SNE (Fischer *et al.* 1995; Scanes 2009; Shojadoost *et al.* 2012). Gln, on the other hand, will be largely used as the key substrate of gluconeogenesis and up taken in skeletal muscle where it is mainly stored (Lacey and Wilmore 1990; Wu *et al.* 1991). Supplementation with L-Gln in diet may therefore compensate this effect and prohibit the lean muscle from exceeding loss. Secondly, Gln provides metabolic fuel to enterocytes and promotes the gut morphology and mucosa forming a healthier gut (Lacey and Wilmore 1990). Bartell and Batal (2007) confirmed that birds fed diets with L-Gln had significantly longer intestinal villi than those fed a control diet. An enhanced gut barrier from Gln can potentially prevent the invasion and colonization of pathogens (Choct 2009) and increase in intestinal villi height in the chicks' early life may allow a more efficient utilization of nutrients and consequently improved growth performance (Lilburn and Loeffler 2015), which can partially explain the observation of L-Gln affected growth performance positively in both NE challenge and unchallenged groups. The protective effect of L-Gln on alleviating intestinal lesion scores can also be associated with enhanced development of the intestinal mucosa (Yi *et al.* 2005; Bartell and Batal 2007). Gln is responsible for retaining the mucosal structure (Khan *et al.* 1999) and reconstruction after damage (Newsholme 2001). Souba *et al.* (1990) suggested Gln is an important amino acid for maintenance of gut metabolism, structure and function especially during critical illness when the gut mucosal barrier compromised based on human research.

It can be concluded that 10 g/kg L-Gln supplementation can significantly alleviate the negative impacts of SNE on broiler chicken by improving growth performance and gut health. The exact mode of action of L-Gln as a functional amino acid affecting on poultry under critical conditions is pending on further investigation.

Chapter 4. General conclusion and future research

In general, the current project showed that the response of broiler chickens to supplemental L-Gln may vary possibly depending on experimental condition, disease challenge, dietary formulation and composition and health status of the birds. While a positive response was observed in the study conducted at UNE (Chapter 3) both in unchallenged and challenged group of birds, in the first trial conducted at SARDI (Chapter 2) no significant effect was observed when diets were made isonitrogenous by addition of equivalent amount of nitrogen from L-Alanine. These inconsistences highlight that further research is required.

In the first experiment, it was originally aimed to investigate some underlying aspects of intestinal function in response to L-Gln through expression of genes associated with intestinal barrier function and water absorption channels (aquaporin). The lack of any significant effect of L-Gln on bird performance left that aspect of the project unjustified and subsequently replaced by the experiment described in Chapter 3. The positive response of broilers under NE challenge and fed L-Gln further strengthens the possibility that L-Gln may have a fundamental role in reconstruction of a damaged epithelium, a possible topic for future research from both applied and scientific aspects. Gln is metabolic fuel for the rapid growing enterocytes through which it supports intestinal growth and function as well as mucosal structure. The intestinal barrier function response to L-Gln is a relevant topic for investigation, an area of research that is well stablished across different species except poultry.

Although Zn and L-Gln are known to affect water reabsorption, under the condition of this project birds were not responsive to the treatments (Chapter 2). Not many studies have taken a holistic approach on excreta moisture and the mechanisms to influence that through normal dietary formulation. While the main nutritional causes of wet droppings and wet litter have been previously reviewed (Collett 2012), there is very little known about the mechanism of water reabsorption and water balance in relation to nutritional intervention and various nutrients in poultry. This topic deserves further investigation.

Acknowledgements

The project funding and support of the Poultry CRC is gratefully acknowledged. The researchers are thankful of the CRC management in particular Professor Mingan Choct and Dr Tim Walker.

This one-year project was initiated by the project leader, Dr Reza Barekatain at SARDI involving the first trial described in Chapter 2. For the first experiment conducted at SARDI, the project leader is grateful to have the assistance of SARDI technical staff, Mr Derek Schultz and Ms Kylee Swanson as well as PhD student Mr Saad Gilani from the University of Adelaide.

The second experiment (Chapter 3) which was later added to the project after consultation by the Poultry CRC, was designed and conducted at the University of New England (UNE) as part of the PhD program of Mr Guangda (Danny) Xue under supervision of Professor Bob Swick and Dr Shubiao Wu. All the data of the experiment described in Chapter 3 were obtained by Mr Xue and will be part of his PhD dissertation to be submitted at UNE.

List of References

Ågren, MS, Chvapil, M, Franzén, L (1991) Enhancement of re-epithelialization with topical zinc oxide in porcine partial-thickness wounds. *Journal of Surgical Research* **50**, 101-105.

Antonisamy, P, Kannan, P, Ignacimuthu, S (2009) Anti-diarrhoeal and ulcer-protective effects of violacein isolated from Chromobacterium violaceum in Wistar rats. *Fundamental & clinical pharmacology* **23**, 483-490.

Awad, E, Zulkifli, I, Soleimani, A, Loh, T (2015) Individual non-essential amino acids fortification of a low-protein diet for broilers under the hot and humid tropical climate. *Poultry Science* **94**, 2772-2777.

Bartell, S, Batal, A (2007) The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract, and humoral immune response of broilers. *Poultry Science* **86**, 1940-1947.

Blikslager, A, Hunt, E, Guerrant, R, Rhoads, M, Argenzio, R (2001) Glutamine transporter in crypts compensates for loss of villus absorption in bovine cryptosporidiosis. *Am J Physiol Gastrointest Liver Physiol* **281**, G645-53.

Burrell, A, Dozier, W, Davis, A, Compton, M, Freeman, M, Vendrell, P, Ward, T (2004) Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. *British poultry science* **45**, 225-263.

Cant, JP, McBride, BW, Croom, WJ (1996) The regulation of intestinal metabolism and its impact on whole animal energetics. *J Anim Sci* **74**, 2541-2553.

Choct, M (2009) Managing gut health through nutrition. *British Poultry Science* **50**, 9-15.

Collett, SR (2012) Nutrition and wet litter problems in poultry. *Animal feed science and technology* **173**, 65-75.

Coster, J, McCauley, R, Hall, J (2004) Glutamine: metabolism and application in nutrition support. *Asia Pacific journal of clinical nutrition* **13**, 25-31.

Dai, S, Gao, F, Zhang, W, Song, S, Xu, X, Zhou, G (2011) Effects of dietary glutamine and gamma-aminobutyric acid on performance, carcass characteristics and serum parameters in broilers under circular heat stress. *Animal Feed Science and Technology* **168**, 51-60.

Etheridge, R, Seerley, R, Huber, T (1984) The effect of diet on fecal moisture, osmolarity of fecal extracts, products of bacterial fermentation and loss of minerals in feces of weaned pigs. *Journal of Animal Science* **58**, 1403-1411.

Fernandez, S, Pearce, S, Gabler, N, Patience, J, Wilson, M, Socha, M, Torrison, J, Rhoads, R, Baumgard, L (2014) Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. *animal* **8**, 43-50.

Fischer, C, Bode, B, Abcouwer, S, Lukaszewicz, G, Souba, W (1995) Hepatic uptake of glutamine and other amino acids during infection and inflammation. *Shock (Augusta, Ga.)* **3**, 315-322.

Hu, C, Qian, Z, Song, J, Luan, Z, Zuo, A (2013) Effects of zinc oxide-montmorillonite hybrid on growth performance, intestinal structure, and function of broiler chicken. *Poultry Science* **92**, 143-150.

Hu, H, Bai, X, Shah, A, Wen, A, Hua, J, Che, C, He, S, Jiang, J, Cai, Z, Dai, S (2015) Dietary supplementation with glutamine and γ-aminobutyric acid improves growth performance and serum parameters in 22-to 35-day-old broilers exposed to hot environment. *Journal of Animal Physiology and Animal Nutrition*

Huang, Y, Lu, L, Luo, X, Liu, B (2007) An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. *Poultry Science* **86**, 2582-2589.

Ikarashi, N, Ushiki, T, Mochizuki, T, Toda, T, Kudo, T, Baba, K, Ishii, M, Ito, K, Ochiai, W, Sugiyama, K (2011) Effects of magnesium sulphate administration on aquaporin 3 in rat gastrointestinal tract. *Biological and Pharmaceutical Bulletin* **34**, 238-242.

Islam, S, Mahalanabis, D, Chowdhury, A, Wahed, M, Rahman, A (1997) Glutamine is superior to glucose in stimulating water and electrolyte absorption across rabbit ileum. *Digestive diseases and sciences* **42**, 420-423.

Khan, J, Iiboshi, Y, Cui, L, Wasa, M, Sando, K, Takagi, Y, Okada, A (1999) Alanyl-glutamine-supplemented parenteral nutrition increases luminal mucus gel and decreases permeability in the rat small intestine. *Journal of Parenteral and Enteral Nutrition* **23**, 24-31.

Lacey, JM, Wilmore, DW (1990) Is glutamine a conditionally essential amino acid? *Nutrition Reviews* **48**, 297-309.

Ladd, FVL, Ladd, AABL, Ribeiro, AACM, Costa, SBC, Coutinho, BP, Feitosa, GAS, de Andrade, GM, Maurício de Castro-Costa, C, Magalhães, CEC, Castro, IC, Oliveira, BB, Guerrant, RL, Lima, AÂM, Oriá, RB (2010) Zinc and glutamine improve brain development in suckling mice subjected to early postnatal malnutrition. *Nutrition* **26**, 662-670.

Lee, KW, Lillehoj, HS, Jeong, W, Jeoung, HY, An, DJ (2011) Avian necrotic enteritis: Experimental models, host immunity, pathogenesis, risk factors, and vaccine development. *Poultry Science* **90**, 1381-1390.

Lilburn, MS, Loeffler, S (2015) Early intestinal growth and development in poultry. *Poult Sci* pev104.

Lima, AA, Anstead, GM, Zhang, Q, Figueiredo, ÍL, Soares, AM, Mota, R, Lima, NL, Guerrant, RL, Oriá, RB (2014) Effects of glutamine alone or in combination with zinc and vitamin A on growth, intestinal barrier function, stress and satiety-related hormones in Brazilian shantytown children. *Clinics* **69**, 225-233.

Lochmiller, RL, Deerenberg, C (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87-98.

Martin, LB, Scheuerlein, A, Wikelski, M (2003) Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society B-Biological Sciences* **270**, 153-158.

Murakami, A, Sakamoto, M, Natali, M, Souza, L, Franco, J (2007) Supplementation of glutamine and vitamin E on the morphometry of the intestinal mucosa in broiler chickens. *Poultry Science* **86**, 488-495.

Newsholme, P (2001) Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? *The Journal of nutrition* **131**, 2515S-2522S.

Novak, F, Heyland, DK, Avenell, A, Drover, JW, Su, X (2002) Glutamine supplementation in serious illness: a systematic review of the evidence. *Critical Care Medicine* **30**, 2022-2029.

Olkowski, A, Wojnarowicz, C, Chirino-Trejo, C, Laarveld, B (2006) Natural history of lesions development in chickens succumbing to necrotic enteritis. *Poultry science* **85**, 40-41.

Olubodun, JO, Zulkifli, I, Farjam, AS, Hair-Bejo, M, Kasim, A (2015) Glutamine and glutamic acid supplementation enhances performance of broiler chickens under the hot and humid tropical condition. *Italian Journal of Animal Science* **14**, 3263.

Pesti, GM, Cervantes, H, Bakalli, RI, Bafundo, K, Garcia, M (1999) Studies on Semduramicin and nutritional responses: 3. electrolyte balance. *Poultry Science* **78**, 1552-1560.

Romanyukha, AA, Rudnev, SG, Sidorov, IA (2006) Energy cost of infection burden: An approach to understanding the dynamics of host-pathogen interactions. *Journal of Theoretical Biology* **241**, 1-13.
Sakamoto, M, Murakami, A, Silveira, T, Fernandes, J, de Oliveira, C (2006) Influence of glutamine and vitamin E on the performance and the immune responses of broiler chickens. *Revista Brasileira de Ciência Avícola* **8**, 243-249.
SAS (2003) SAS Institute, 2003. SAS® Qualification Tools User's Guide. Version 9.1.2. SAS Institute Inc., Cary, NC.
Scanes, CG (2009) Perspectives on the endocrinology of poultry growth and metabolism. *General and comparative endocrinology* **163**, 24-32.
Shakeri, M, Zulkifli, I, Soleimani, A, o'Reilly, E, Eckersall, PD, Anna, A, Kumari, S, Abdullah, FFJ (2014) Response to dietary supplementation of l-glutamine and l-glutamate in broiler chickens reared at different stocking densities under hot, humid tropical conditions. *Poultry Science* **93**, 2700-2708.
Shojadoost, B, Vince, AR, Prescott, JF (2012) The successful experimental induction of necrotic enteritis in chickens by Clostridium perfringens: a critical review. *Veterinary Research* **43**,
Skinner, JT, Bauer, S, Young, V, Pauling, G, Wilson, J (2010) An Economic Analysis of the Impact of Subclinical (Mild) Necrotic Enteritis in Broiler Chickens. *Avian Diseases* **54**, 1237-1240.
Soares, AD, Costa, KA, Wanner, SP, Santos, RG, Fernandes, SO, Martins, FS, Nicoli, JR, Coimbra, CC, Cardoso, VN (2014) Dietary glutamine prevents the loss of intestinal barrier function and attenuates the increase in core body temperature induced by acute heat exposure. *British Journal of Nutrition* **112**, 1601-1610.
Soltan, M (2009) Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and some blood parameters of broiler chickens. *International Journal of Poultry Science* **8**, 60-68.
Souba, WW, Klimberg, VS, Plumley, DA, Salloum, RM, Flynn, TC, Bland, KI, Copeland, EM (1990) The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. *Journal of Surgical Research* **48**, 383-391.
Tran, CD, Hawkes, J, Graham, RD, Kitchen, JL, Symonds, EL, Davidson, GP, Butler, RN (2014) Zinc-Fortified Oral Rehydration Solution Improved Intestinal Permeability and Small Intestinal Mucosal Recovery. *Clinical pediatrics* 0009922814562665.
van der Hoeven-Hangoor, E, Paton, N, van de Linde, I, Verstegen, M, Hendriks, W (2013a) Moisture content in broiler excreta is influenced by excreta nutrient contents. *Journal of Animal Science* **91**, 5705-5713.
van der Hoeven-Hangoor, E, Van de Linde, I, Paton, N, Verstegen, M, Hendriks, W (2013b) Effect of different magnesium sources on digesta and excreta moisture content and production performance in broiler chickens. *Poultry Science* **92**, 382-391.
Vu, M, Nouwens, M, Biemond, I, Lamers, C, Masclee, A (2000) The osmotic laxative magnesium sulphate activates the ileal brake. *Alimentary Pharmacology and Therapeutics* **14**, 587-596.
Wang, J, Chen, L, Li, P, Li, X, Zhou, H, Wang, F, Li, D, Yin, Y, Wu, G (2008) Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. *The Journal of nutrition* **138**, 1025-1032.
Wu, G, Thompson, JR, Baracos, VE (1991) Glutamine metabolism in skeletal muscles from the broiler chick (Gallus domesticus) and the laboratory rat (Rattus norvegicus). *Biochemical Journal* **274**, 769-774.

Wu, SB, Rodgers, N, Choct, M (2010) Optimized necrotic enteritis model producing clinical and subclinical infection of Clostridium perfringens in broiler chickens. *Avian diseases* **54**, 1058-1065.

Yalçin, SS, Yurdakök, K, Tezcan, I, Öner, L (2004) Effect of glutamine supplementation on diarrhea, interleukin-8 and secretory immunoglobulin A in children with acute diarrhea. *Journal of pediatric gastroenterology and nutrition* **38**, 494-501.

Yi, G, Allee, G, Knight, C, Dibner, J (2005) Impact of glutamine and oasis hatchling supplement on growth performance, small intestinal morphology, and immune response of broilers vaccinated and challenged with Eimeria maxima. *Poultry Science* **84**, 283-293.

POULTRY CRC

Plain English Compendium Summary

Sub-Project Title:	Zinc and glutamine to enhance intestinal function and performance of broilers
Poultry CRC Sub-Project No.:	2.1.15
Researcher:	Dr Reza Barekatain, Mr Guangda Xue
Organisation:	SARDI and UNE
Phone:	08 83137720
Fax:	
Email:	Reza.Barekatain@sa.gov.au
Sub-Project Overview	Sub-project 2.1.15 comprised two experiments to investigate 1) the effect of supplemental L-glutamine (L-Gln) and higher concentration of zinc (Zn) on excreta moisture under a nutritionally induced wet dropping and 2) impact of L-Gln on broiler performance under necrotic enteritis (NE) challenge.
Background	Zn and Gln are considered critical gut-trophic nutrients, which have protective roles, as demonstrated in various animal models of intestinal injury and have been proved beneficial in clinical studies in humans afflicted with malnutrition and diarrhoea. In poultry, there are no investigations reported of the potential synergy between these two nutrients for enhancement of bird performance, increasing gut barrier function and the possible reduction of water excreted.
Research	The response of broiler chickens to supplemental L-Gln may vary possibly depending on experimental condition, disease challenge, dietary formulation and composition and health status of the birds. Ten g/kg L-Gln supplementation can significantly alleviate the negative impacts of SNE on broiler chicken by improving growth performance and gut health. Under the condition of this project, Zn and L-Gln showed no conclusive effect on intestinal digesta and excreta moisture.
Sub-Project Progress	Completed
Implications	Inclusion of L-Gln in broiler diets may be more justified when broiler are under certain stressors or physiological challenge or a suboptimal nutrition. The mechanism of gut-trophic nutrients such as Zn and Gln needs further investigation.
Publications	In progress