# 2A-113: ENHANCING THE EFFICACY OF VACCINATION THROUGH ZINC SUPPLEMENTATION

**Report prepared for the**
**Co-operative Research Centre for High Integrity Australian Pork**

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# Executive Summary

Vaccines are widely used in production animals to prevent the rise and spread of disease and the associated costs to the animal and the business. Vaccines prevent the outbreak, or the severity, of many diseases whilst the introduction of vaccines like Improvac allows the producer to control production issues such as boar taint. Vaccines are an integral part of the herd health plan but do not guarantee full protection from these diseases.

The cause of vaccine failure in production animals can be numerous and when a failure occurs the loss in performance or increase in mortalities can be devastating. Vaccines may fail due to issues associated with the animal itself whilst there is also natural variation in the response of animals to immune challenge. Failure can also occur because of issues with the vaccine itself or from handling and administration.

The inclusion of zinc methionine in the diet of feedlot cattle resulted in an improved rate of disease recovery when challenged, similarly, its role in enhancing immune response in poultry is understood. Zinc methionine's role in pigs has been less well investigated.

This study will investigate the efficacy of a zinc amino acid complex and a chromium methionine complex to enhance the adaptive immune response in grower pigs when challenged with a novel antigen, tetanus toxoid. Pigs were randomly allocated to four treatment groups based on diets with differing levels and sources of zinc and/or chromium. Pigs were vaccinated with tetanus toxoid 3 weeks after feeding commenced and received a second dose 4 weeks after first vaccination. Pigs were serially bled with samples tested for TT-specific IgG antibody concentrations (SP ratio) via ELISA to show difference in humoral immune response. Differences in immune response were statistically analysed.

As expected, there were no significant differences between treatments throughout the growth period, with no effect on weight gain. There was a similar lack of difference between treatments for carcass characteristics. Vaccination of pigs resulted in a significant rise in serum TT IgG antibody concentration across all treatment groups. The antibody titres observed followed the expected pattern of protection, with the peak reached two to six weeks after vaccination before gradually falling. There was no difference between treatments in the baseline levels prior to vaccination; however, two weeks after the first dose of TT the treatment group receiving the additional Zn from ZnAAC had a significantly lower SP ratio than other treatment groups. A similar numerical difference occurred in subsequent weeks with a lower SP ratio observed in the ZnAAC group.

Despite its use as a recall antigen in studies it appears that tetanus toxoid may be directly influence by zinc. Tetanus toxin has a zinc-binding sequence; saturation of this site with zinc will lead to decreased recognition by the immune system. Therefore, the results observed may in fact be a result of the enhanced availability of zinc from the zinc amino acid complex. The lack of observed difference when chromium methionine was combined with the zinc amino acid complex is likely explained by its immunomodulatory activity. Therefore, it appears that the inclusion of chromium in this treatment was potentially offsetting any saturation effects from the enhanced availability of zinc.

Despite evidence in cattle and poultry of enhanced vaccination response from the supplementation of diets with zinc amino acid complex, this study, was not able to improve the immune response. There is some evidence within this study that chromium may be of specific interest in elevating antibody titres, where it appeared to have a restorative effect on the zinc amino acid complex treatment.

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# 1. Introduction

Vaccines are widely used in production animals to prevent the rise and spread of disease and the associated costs to the animal and the business. Amongst others, vaccines are used to prevent the outbreak, or the severity, of diseases ranging from *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*, *Porcine Parvovirus*, Leptospirosis, Erysipelas, *Clostridium spp.*, and *Escherichia coli*. Whilst the introduction of vaccines like Improvac (Zoetis, Rhodes, NSW), allows the producer to control production issues such as boar taint. Vaccines are an integral part of the herd health plan but do not guarantee full protection from these diseases.

The cause of vaccine failure in production animals can be numerous and when a failure occurs, the loss in performance or increase in mortalities can be devastating. Vaccines may fail due to issues associated with the animal itself. The age of the animal and issues associated with generalised stress such as poor level of nutrition, over-crowding, sub-clinical disease have all been linked to vaccine failure, whilst there is also natural variation in the response of animals to immune challenge (Rice et al., 1986). Failure can also occur due to issues with the vaccine itself or handling and administration.

Zinc is essential for all highly proliferating cells in the body (Ibs and Rink, 2003), and in particular those of the immune system. The response to vaccination can be low in humans that are zinc-deficient (Sandstaed et al., 1982). The antibody response is not enhanced by the inclusion of zinc as an adjuvant (Provinciali et al., 1998), however, low-dose supplementation of zinc was shown to yield improvements in the humoral response post-vaccination (Girodon et al., 1999). Why these improvements occur is not completely understood but it is speculated that it may be due to restored B-cell function with zinc supplementation, increases in interferon production and/or the restoration of impaired T-cell function (Ibs and Rink, 2003).

The supplementation of zinc in the diet of production animals has shown similar responses to those in humans. The inclusion of zinc methionine in the diet of feedlot cattle resulted in an improved rate of disease recovery when challenged with infectious bovine rhinotracheitis virus (BHV-1, Chirase et al., 1991). Similarly, stressed steers showed a response in antibody titers to BHV-1 vaccination when zinc methionine was included in the diet, but not when zinc oxide was included (Spears et al., 1991). Kidd et al. (1993) showed the inclusion of zinc methionine in the diets of dams and progeny enhanced the primary antibody titres in response to challenge with Salmonella pullorum antigens and higher antibody titres were observed in broiler breeders when higher levels of zinc methionine were included in the diet (Khajarem et al., 2002). Whilst the role of zinc methionine in enhancing immune response in poultry (e.g. Turkeys – Ferket and Qureshi, 1992) is known, the potential role in pigs is less investigated. A comparison of zinc sources and concentrations in weaner pigs (van Heugten et al., 2003) showed little difference in growth performance or immune response between treatments, although pigs receiving zinc methionine had a significantly greater response against pokeweed mitogen, a T-cell dependent B-cell mitogen.

This study will investigate the efficacy of a zinc amino acid complex and a chromium methionine complex to enhance the adaptive immune response in grower pigs when challenged with a novel antigen, tetanus toxoid (TT, Equivac® T Vaccine, Zoetis, Rhodes, NSW). We chose tetanus toxoid as a novel vaccine as it is a registered vaccine for pigs, has no withholding period, not routinely used and has a history of use in experimental investigations (Miller et al., 2013). The use of Improvac® (Zoetis, Rhodes, NSW) was considered as a potential novel vaccine; however, discussions (D'Souza pers.comm.) have indicated variation in immune response is not large.

# 2. Methodology

*Summary*

Pigs were randomly allocated to four treatment groups based on diets with differing levels and sources of zinc and/or chromium. Pigs were vaccinated with tetanus toxoid 3 weeks after feeding commenced and received a second dose 4 weeks after first vaccination. Pigs were serially bled with samples tested for TT-specific IgG antibody concentrations via ELISA to show difference in humoral immune response. Differences in immune response were statistically analysed.

*Animals and treatments*

One-hundred and thirty-two (132) male pigs were randomly allocated to one of twelve pens (n=11) in a conventional naturally ventilated finisher shed at seven weeks of age. Pigs were weighed within their pen groups and then pens were allocated to one of four treatment groups (n=3, Table 1):

- Control, 100 ppm zinc (Zn) from Zinc sulfate ($ZnSO_4$) in vitamin/mineral premix.
- $ZnSO_4$, 150 ppm Zn, 100 ppm from $ZnSO_4$ in premix plus 50 ppm Zn from $ZnSO_4$.
- ZnAAC, 150 ppm Zn, 100 ppm from $ZnSO_4$ in premix plus 50 ppm Zn from Zn amino acid complex (ZnAAC).
- Zn/Cr, 150 ppm Zn and 0.4 ppm chromium (Cr), 100 ppm from $ZnSO_4$ in premix plus 50 ppm Zn from ZnAAC plus 0.4 ppm Cr from chromium methionine (CrMet).

Treatments were applied as additions to four base diets fed serially as the weight of the animal increased; weaner - fed from the start of the experiment to approximately 25 kg, grower – 25 to 50 kg, porker – 50 to 70 kg, and finisher – from 70 kg to slaughter (Table 2).

*Table 1. Summary of inclusions for treatment diets.*

| | **Base premix ($ZnSO_4$)** | **$ZnSO_4$** | **ZnAAC (AvailaZn 120)** | **CrMet (AvailaCr 1000)** |
|---|---|---|---|---|
| Control | 275 ppm (100 ppm Zn) | | | |
| $ZnSO_4$ | 275 ppm (100 ppm Zn) | 137 ppm (50 ppm Zn) | | |
| ZnAAC | 275 ppm (100 ppm Zn) | | 420 ppm (50 ppm Zn) | |
| Zn/Cr | 275 ppm (100 ppm Zn) | | 420 ppm (50 ppm Zn) | 400 ppm (0.4 ppm Cr) |

*Table 2. Ingredients and nutritional analysis of diets offered to progeny.*

| *Ingredients (g/kg)…* | **Weaner (15-25 kg)** | **Grower (25-50 kg)** | **Porker (50-70 kg)** | **Finisher (70+ kg)** |
|---|---|---|---|---|
| Barley | 66.7 | | | 173.3 |
| Sorghum | | 200.0 | 403.3 | 501.1 |
| Wheat | 622.0 | 601.8 | 390.0 | 100.0 |
| Millrun | | | | 70.0 |
| Canola meal | | 26.7 | 100.0 | 93.3 |
| Soybean meal | 80.0 | 76.0 | 36.7 | |
| Full-fat soybean | 40.0 | | | |
| Blood meal | 25.3 | 22.0 | 27.3 | 15.3 |
| Meat and bone meal | 50.7 | 50.0 | | |
| Fishmeal | 20.7 | | | |
| Chocolate milk powder | 50.0 | | | |
| Whey powder | 25.3 | | | |
| Vegetable oil | 10.0 | 10.0 | 10.0 | 5.3 |
| Limestone | | 2.67 | 10.7 | 12.7 |
| Dicalphos | | | 11.3 | 8.0 |
| Salt | 2.00 | 2.00 | 2.33 | 2.40 |
| Choline chloride | 0.40 | 0.53 | 0.60 | 0.27 |
| MHA calcium | 0.80 | 0.73 | 0.53 | 0.40 |
| Lysine HCl | 3.00 | 4.00 | 4.00 | 4.00 |
| L-threonine | 0.53 | 0.47 | 0.13 | 0.13 |
| L-tryptophan | | 0.07 | | |
| Xylanase[1] | 0.50 | | | |
| Phytase[2] | 0.075 | 0.075 | 0.075 | 0.075 |
| Bentonite | | | | 10.7 |
| De-Oderase[3] | | 1.0 | 1.0 | 1.0 |
| Vit/min premix[4] | 2.0 | 2.0 | 2.0 | 2.0 |
| *Analysis* | | | | |
| Energy (MJ DE/kg) | 14.5 | 14.0 | 13.8 | 13.2 |
| Available lysine (g/MJ DE) | 0.80 | 0.70 | 0.64 | 0.55 |
| Crude protein (%) | 21.5 | 19.0 | 16.8 | 13.9 |
| Crude fibre (%) | 2.6 | 2.6 | 3.0 | 4.0 |

[1]Xylanase, Rovabio Excel, Adisseo; [2]Phytase, RONOZYME HiPhos, DSM; [3]Deodoarase, yucca extract, Alltech; [4]Vitamin/mineral premix delivers (per tonne of feed) 8 MIU vitamin A, 1.5 g vitamin B1, 4 g vitamin B2, 17 g vitamin B3, 13 g vitamin B5, 1 g vitamin B6, 100 mg vitamin B7, 0.5 g vitamin B9, 10 g vitamin B12, 1.5 MIU vitamin D3, 35 g vitamin E, 1.5 g vitamin K, 60 g iron, 100 g zinc, 40 g manganese, 20 g copper, 0.3 g selenium, 0.4 g chromium, 0.5 g cobalt, 1.5 g iodine.

Animals were housed within a conventionally ventilated finisher shed. Pens consisted of open steel paneling with concrete flooring, two-thirds solid, one-third slatted. Pens measured 2.65 m x 3.3 m, with a space allowance of 0.80 $m^2$ per pig, in excess of minimum space requirements (0.70 m2 for a 110 kg pig, PISC (2008)). Water was supplied *ad libitum* from two nipple drinkers per pen and feed was supplied *ad libitum* from a single penguin-style self-feeder in each pen. Pigs were weighed individually upon entry, at a midpoint and at the end of the experiment

primarily for monitoring purposes. The CHM Alliance Animal Care and Ethics Committee (CHM PP 76/15) approved the experimental design and procedures.

*Tetanus Toxoid*

Equivac T Vaccine (Zoetis, West Ryde) was the source of tetanus toxoid (TT). Equivac T is a purified, adjuvanted vaccine containing the formalinized toxin of *Clostridium tetani* (20 Lf/mL). The adjuvant is an aluminium salt and thiomersal (0.1 mg/ml) is added as a preservative in a saline base.

*Experimental design*

Diets were fed for three weeks prior to vaccination, upon allocation to pens/treatments, and continued through to slaughter. The TT was administered as a two dose program as per label requirements. The first dose (1 ml) was administered during week 10, 21 days after diets first offered and the second dose (1 ml) during week 14(28 days after the first vaccination). TT was administered as a subcutaneous injection as per label requirements (Table 3).

Blood samples were collected from all pigs via jugular venipuncture (SST (Serum Separator Tubes) vacutainer, BD, North Ryde) at week 9, prior to vaccination to determine baseline, and at weeks 12, 15, 17 and 19 to establish antibody response. After each collection SST, vacutainers were centrifuged (3,000 rpm, 10 minutes) and serum was separated into duplicates and frozen for subsequent analysis.

*Table 3. Project plan for the investigation of enhanced efficacy of vaccination by supplementation of diets with zinc amino acid complex and/or chromium methionine (AvailaZn and AvailaCr, Zinpro Corp, Eden Prairie, MN).*

| Week | Event |
|---|---|
| 7 | Weigh pigs, allocate to pens/treatments, commence diets |
| 8 | |
| 9 | Baseline blood sampling |
| 10 | Vaccinate initial dose |
| 11 | |
| 12 | Blood sampling |
| 13 | |
| 14 | Weigh pigs, vaccinate booster dose |
| 15 | Blood sampling |
| 16 | |
| 17 | Blood sampling |
| 18 | |
| 19 | Blood sampling |
| 20 | Weigh pigs, market pigs (first cut) |
| 21 | Market pigs (second cut) |

*TT antibody titres*

Frozen serum samples were shipped to the Elizabeth Macarthur Agricultural Institute (NSW Trade and Investment, Menangle, NSW), where samples were processed. A commercial ELISA kit was investigated, however, after some work, the

kit detected antibodies to *Clostridium tetani*, rather than the toxoid produced. As a result, serum samples were tested for TT-specific IgG antibody concentrations using an indirect TT enzyme-linked immunosorbent assay (ELISA) as per Miller *et al.* (2013).

The concentration of TT antibodies was determined by measuring the optical density (OD) of each reaction. Plates contained standard positive and negative controls. To take account of plate differences, results were expressed as a ratio of the sample to positive (SP ratio) OD, which takes account of the optical densities of both the negative and positive controls, which can differ between plates due to the kinetics of antigen-antibody binding (Bassey and Collins, 1997).

$$\text{SP ratio} = (OD_{\text{sample}} - OD_{\text{negative control}}) / (OD_{\text{positive control}} - OD_{\text{negative control}})$$

*Statistical analyses*

All statistical analyses were performed using GenStat 18th Edition (VSN International, Hemel Hempstead, UK). Individual pig was the experimental unit for the SP ratio, with pen as a blocking factor. Whilst not the aim of this study, weight, growth rate and carcass characteristics were analysed with the pen as the replicate.

# 3. Outcomes

As expected, there were no significant differences between treatments at the start of the experiment or throughout the growth period (Table 4), with no effect on weight gain.

*Table 4. Weights and growth rate (ADG) of pigs receiving control diets or those supplemented with additional 50 ppm Zn from $ZnSO_4$, 50 ppm Zn from zinc amino acid complex (ZnAAC), and 50 ppm from ZnAAC and 0.4 ppm chromium from chromium methionine (Zn/Cr).*

| | **Control** | **$ZnSO_4$** | **ZnAAC** | **Zn/Cr** | **SED** | ***P* value** |
|---|---|---|---|---|---|---|
| Start wt. (kg) | 14.9 | 14.0 | 15.6 | 14.9 | 1.55 | 0.79 |
| Midpoint wt. (kg) | 44.4 | 42.1 | 46.6 | 43.9 | 3.17 | 0.60 |
| End wt. (kg) | 95.2 | 92.3 | 98.4 | 96.6 | 4.76 | 0.65 |
| ADG (kg/d) | 0.923 | 0.900 | 0.951 | 0.939 | 0.041 | 0.63 |

SED, standard error of difference of the means.

There was a similar lack of difference between treatments for carcass characteristics (Table 5).

*Table 5. Carcass characteristics and age at slaughter of pigs receiving control diets or those supplemented with additional 50 ppm Zn from $ZnSO_4$, 50 ppm Zn from zinc amino acid complex (ZnAAC), and 50 ppm from ZnAAC and 0.4-ppm chromium from chromium methionine (Zn/Cr).*

| | **Control** | **$ZnSO_4$** | **ZnAAC** | **Zn/Cr** | **SED** | ***P* value** |
|---|---|---|---|---|---|---|
| HSCW (kg) | 82.3 | 81.5 | 80.8 | 81.7 | 0.94 | 0.35 |
| P2 fat (mm) | 10.9 | 11.0 | 11.1 | 10.5 | 0.60 | 0.75 |
| P2 muscle (mm) | 54.4 | 53.2 | 55.5 | 55.6 | 1.50 | 0.39 |
| Age at slaughter (d) | 149.6 | 151.6 | 147.5 | 148.5 | 2.30 | 0.35 |

HSCW, hot standard carcass weight (Aus-Meat Trim 1); P2, depth of tissue (fat or muscle) 65 mm from the midline at the head of the last rib; SED, standard error of difference of the means.

Vaccination of pigs with TT resulted in a significant rise in average serum TT IgG antibody concentration (Figure 1) across all treatment groups. After vaccination at week 10, SP ratio rose significantly (P<0.001) from the baseline (week 12 sample), with a similar significant increase (P<0.001, week 15 sample) after the booster dose was given at week 14. Two weeks (week 17 sample) later the SP ratio had fallen, but remained significantly higher (P<0.001) than after the first dose was given. Although the fall in SP ratio began to plateau, by week 19 the SP ratio was not significantly different than that obtained after the first administration of the vaccine in all treatments except the Zn/Cr treatment, where levels were still higher than other treatments.

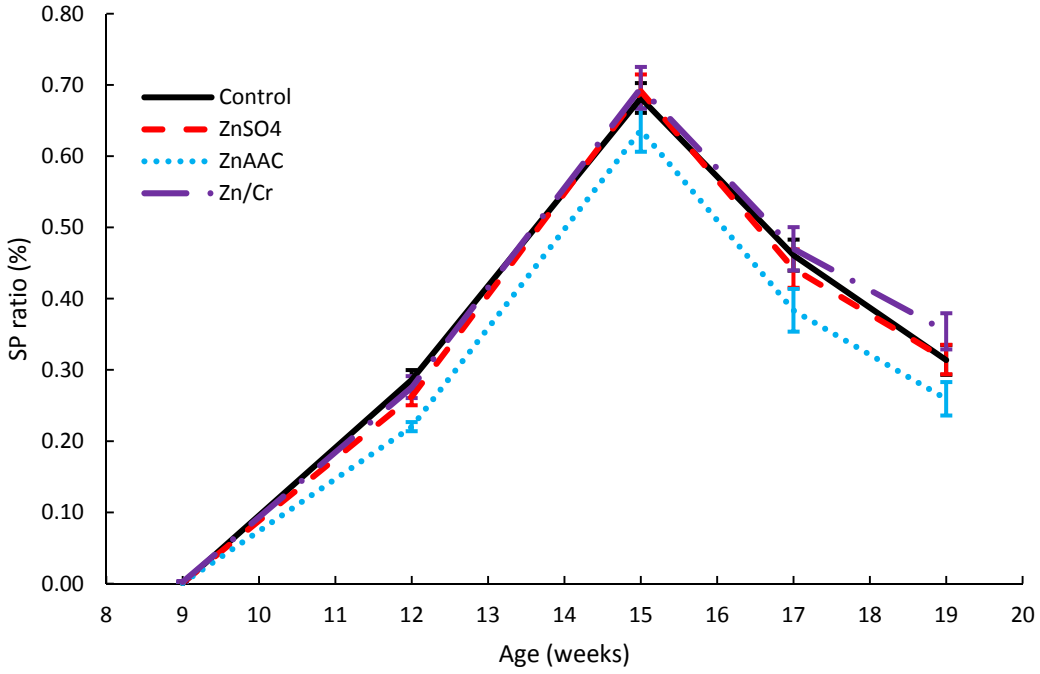


*Figure 1. The tetanus toxoid specific IgG concentration (SP ratio) in serum at 9, 12, 15, 17 and 19 weeks of age of pigs vaccinated with tetanus toxoid at week 10 and 14 receiving control diets or those supplemented with additional 50 ppm Zn from $ZnSO_4$, 50 ppm Zn from zinc amino acid complex (ZnAAC), and 50 ppm from ZnAAC and 0.4 ppm chromium from chromium methionine (Zn/Cr). Vertical bars indicate standard errors of the means.*

When looking at treatments within serum sampling periods (Table 6) there were mixed responses. There was no difference between treatments in the baseline levels prior to vaccination, however, two weeks after the first dose of TT was administered (week 12) the treatment group receiving the additional Zn from ZnAAC had a significantly lower ($P<0.01$) SP ratio than other treatment groups. A similar numerical difference occured in subsequent weeks (week 15 and 17) with a lower SP ratio observed in the ZnAAC group. In the final sample period (week 19), the Zn/Cr treatment group was significantly higher ($P<0.05$) than the ZnAAC group, with the control and $ZnSO_4$ groups being intermediate. This consistently lower SP ratio is obvious in Figure 1.

*Table 6. The tetanus toxoid specific IgG concentration (SP ratio) in serum at 9, 12, 15, 17 and 19 weeks of age of pigs vaccinated with tetanus toxoid at week 10 and 14 receiving control diets or those supplemented with additional 50 ppm Zn from $ZnSO_4$, 50 ppm Zn from zinc amino acid complex (ZnAAC), and 50 ppm from ZnAAC and 0.4 ppm chromium from chromium methionine (Zn/Cr).*

| SP ratio | Control | $ZnSO_4$ | ZnAAC | Zn/Cr | SED | *P* value |
|---|---|---|---|---|---|---|
| Week 09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.002 | 0.79 |
| Week 12 | $0.29^{b}$ | $0.26^{b}$ | $0.22^{a}$ | $0.28^{b}$ | 0.020 | 0.005 |
| Week 15 | 0.68 | 0.69 | 0.64 | 0.70 | 0.037 | 0.37 |
| Week 17 | 0.46 | 0.44 | 0.38 | 0.47 | 0.039 | 0.11 |
| Week 19 | $0.31^{ab}$ | $0.31^{ab}$ | $0.26^{a}$ | $0.35^{b}$ | 0.032 | 0.037 |

$^{ab}$Means in a row with different superscripts differ significantly; SED, standard error of difference of the means; SP ratio = (ODsample – ODnegative control)/(ODpositive control – ODnegative control).

# 4. Application of Research

Supplementation of diets with amino acid complexes of zinc has been found to have many benefits across multiple species. Increases in lactation performance and improved udder health have been seen in dairy cows (Kellogg et al., 2004). Layer hens have been shown to maintain egg production through nutritional stress events (Kienholz et al., 1992) and improvements in small intestinal integrity during severe heat stress in pigs (Sanz Fernandez et al., 2014) have all resulted when zinc amino acid complexes are included in diets.

Zinc is an essential trace element for the immune system (Rink and Gabriel, 2000). The effects of zinc are multifaceted, with both innate and specific parts of the immune being influenced by its presence. Oral zinc supplementation of the elderly resulted in significant improvements in the number of circulating lymphocytes, reduced skin hypersensitivity and increased the IgG response to tetanus vaccine (Duchateau et al., 1981) and humoral immune response was improved in lactating dairy cows as a result of supplementation with a zinc amino acid chelate (Wang et al., 2013).

This study saw the inclusion of super nutritional levels of zinc in both sulfate and amino acid complex form, in a bid to increase the response to vaccination. Improvements in the rate of disease recovery to challenge with bovine rhinotracheitis virus (BHV-1, Chirase et al., 1991) and a response in antibody titres

to BHV-1 vaccination (Spears et al., 1991) have been observed as a response to supplementation in cattle. Similar responses have also been observed in poultry with enhanced primary antibody titres to challenge (Kidd et al., 1993; Khajarem et al., 2002) and a similarly designed study to this, in guinea pigs, also saw a significantly improved immune response to zinc supplementation which was more prominent in the zinc amino acid complex treatment (Shinde et al., 2006). Results from this study did not lead to comparable enhancements in immune response from vaccination with tetanus toxoid. Similar to van Heugten et al. (2003) there was little difference in growth performance between treatments resulting from feeding zinc above nutritional requirements.

The antibody titres observed followed the expected pattern of protection, with the peak being reached two to six weeks after vaccination before gradually falling. However, the addition of 50 ppm of zinc from the zinc amino acid complex saw a reduction in the antibody titre as measured by SP ratio. This response was not expected, nor readily explained. It should also be remembered that the highest antibody titre does not necessarily protect the animal any better – in the case of tetanus (in humans), there is a ten-fold window of titres that is effective against infection (Plotkin, 2010).

Despite its use as a recall antigen in many studies (Duchateau et al., 1981; Faldyna et al., 2003; Faber et al., 2004) it appears that tetanus toxoid is influenced by zinc. Tetanus toxin has a zinc-binding sequence in a region frequently used as a B- and T-cell binding site; saturation of this site with zinc will lead to decreased recognition by the immune system (Rink and Gabriel, 2000). Therefore, the results observed may in fact be a result of the enhanced availability of zinc from the zinc amino acid complex.

The lack of observed difference when chromium methionine was used in conjunction with the zinc amino acid complex is likely explained by its immunomodulatory activity (Burton et al., 1996). In lactating dairy cows, Faldyna et al. (2003) found significant elevation in tetanus toxoid antibody titres as a result of supplementing with 5 mg/head/day of an amino acid and lactate-bound chromium (Agrobac, Dubec, Czech Republic). Therefore, it appears that the inclusion of chromium in this treatment was potentially offsetting any saturation effects from the enhanced availability of zinc.

# 5. Conclusion

Despite evidence in cattle and poultry of enhanced response to vaccination with BHV-1 and *Salmonella pullorum* vaccines respectively from the supplementation of diets with zinc amino acid complex, this study, like van Heugten et al. (2003) was not able to improve the immune response. There is some evidence within this study that chromium may be of specific interest in elevating antibody titres, where it appeared to have a restorative effect on the zinc amino acid complex treatment.

# 6. Limitations/Risks

Despite its widespread use as a recall antigen, the specific interactions that are apparent between zinc and tetanus toxoid may have influenced the outcomes of this study.

# 7. Recommendations

Because of the outcomes in this study, the following recommendations are:

- Despite its common use as a recall antigen, and its novelty of use in pigs, tetanus toxoid appears to not be the best vaccine to test immune response when manipulating the diet.
- The offsetting of the impact of zinc amino acid complex on immune response by the inclusion of chromium warrants further investigation.

# 8. References


Burton, J.L., Nonnecke, B.J., Dubeski, P.L., Elsasser, T.H. and Mallard, B.A. (1996) Effects of supplemental chromium on production of cytokines by mitogen-stimulated bovine peripheral blood mononuclear cells. *Journal of Dairy Science* 79, 2237-2246.

Chirase, N.K., Hutcheson, D.P. and Thompson, G.B. (1991) Feed intake, rectal temperature, and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. *Journal of Animal Science* 69, 4137-4145.

Duchateau, J., Delepesse, G., Vrijens, R. and Collet, H. (1981) Beneficial effects of oral zinc supplementation on the immune response of old people. *The American Journal of Medicine* 70, 1001-1004.

Faber, C., Gabriel, P., Ibs, K-H. and Rink, L. (2004) Zinc in pharmacological doses suppresses allogenic reaction without affecting the antigenic response. *Bone Marrow Transplantation* 33, 1241-1246.

Faldyna, M., Pechova, A. and Krejci, J. (2003) Chromium supplementation enhances antibody response to vaccination with tetanus toxoid in cattle. *Journal of Veterinary Medicine, Series B* 50, 326-331.

Ferket, P.R. and Qureshi, M.A. (1992) Effect of level of inorganic and organic zinc and manganese on the immune function of turkey toms. *Poultry Science* 71(Suppl. 1), 60(Abstr).

Girodon, F., Galan, P., Monget, A.L., Boutron-Ruault, M.C., Brunet-Lecomte, P., Preziosi, P., Arnaud, J., Manguerra, J.C. and Herchberg, S. (1999) Effects of trace elements and vitamin on immunity and infections in institutionalized elderly patients: a randomized controlled trial. MIN. VIT. AOX. geriatric network. *Archives of Internal Medicine* 159, 748-754.

Ibs, K-H. and Rink, L. (2003) Zinc-Altered Immune Function. *Journal of Nutrition* 133, 1452S-1456S.

Kellogg, D.W., Tomlinson, D.J., Socha, M.T. and Johnson, A.B. (2004) Effects of zinc methionine complex on milk production and somatic cell count of dairy cows: twelve-trial summary. *The Professional Animal Scientist* 20, 295-301.

Khajarem, J., Ratanasethalul, C., Kharajarem, S., Ward, T.L., Fakler, T.M. and Johnson, A.B. (2002) Effect of zinc and manganese amino acid complexes (Availa Z/M) on broiler breeder production and immunity. *Poultry Science* 81(Suppl. 1), 40(Abstr).

Kidd, M.T., Anthony, N.B. and Lee, S.R. (1992) Progeny performance when dams and chicks are fed supplemental zinc. *Poultry Science* 71, 1201-1206.

Kienholz, E.W., Moreng, R.E. and Flinchum, J.D. (1992) Zinc methionine for stressed laying hens. *Poultry Science* 71, 829-832.

Miller, Y.J., Collins, A.M., Emery, D., Begg, D.J., Smits, R.J. and Holyoake, P.K. (2013) Piglet performance and immunity is determined by the parity of both the birth dam and the rearing dam. *Animal Production Science* 53, 46-51.

PISC (2008) Model Code of Practice for the Welfare of Animals – Pigs (3rd edition). PISC Report No. 92. CSIRO Publishing, Collingwood.

Plotkin, S.A. (2010) Correlates of protection induced by vaccination. *Clinical and vaccine immunology.* 17, 1055-1065.

Provinciali, M., Montenovo, A., Di-Stefano, G., Colombo, M., Daghetta, L., Cairati, M., Veroni, C., Cassino, R., Della-Torre, F. and Fabris, N. (1998) Effect of zinc or zinc plus arginine supplementation on antibody titre and lymphocyte subsets after influenza vaccination in elderly subjects: a randomized controlled trial. *Age and Ageing* 27, 715-722.

Rice, D., Erickson, E.D. and Grotelueschen, D. (1986) "G86-797 Causes of Vaccination-Immunization Failures in Livestock" Historical Materials from University of Nebraska-Lincoln Extension. Paper 229.
http://digitalcommons.unl.edu/extensionhist/229

Rink, L. and Gabriel, P. (2000) Zinc and the immune system. *Proceedings of the Nutrition Society* 59, 541-552.

Sandstaed, H.H., Henriksen, L.K., Greger, J.L., Prasad, A.S. and Good, R.A. (1982) Zinc nutriture in the elderly in relation to taste acuity, immune response, and wound healing. *American Journal of Clinical Nutrition* 36, 1046-1059.

Sanz Fernandez, M.V., Pearce, S.C., Gabler, N.K., Patience, J.F., Wilson, M.E., Socha, M.T., Torrison, J.L., Rhoads, R.P. and Baumgard, L.H. (2014) Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. *Animal* 8, 43-50.

Shinde, P., Dass, R.S., Garg, A.K., Chaturvedi, V.K. and Kumar, R. (2006) Effect of zinc supplementation from different sources on growth, nutrient digestibility, blood metabolic profile, and immune response of male Guinea pigs. *Biological Trace Element Research* 112, 247-262.

Spears, J.W., Harvey, R.W. and Brown, T.T. (1991) Effects of zinc methionine and zinc oxide on performance, blood characteristics, and antibody titer response to viral vaccination in stressed feeder calves. *Journal of the American Veterinary Medicine Association* 199, 1731.

van Heugten, E., Spears, J.W., Kegley, E.B., Ward, J.D. and Qureshi, M.A. (2003) Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response of weanling pigs. *Journal of Animal Science* 81, 2063-2071.

Wang, R.L., Liang, J.G., Lu, L., Zhang, L.Y., Li, S.F. and Luo, X.G. (2013) Effect of zinc source on performance, zinc status, immune response, and rumen fermentation of lactating cows. *Biological Trace Element Research* 152, 16-24.