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Building Confidence in Kangaroo Meat for Pet Nutrition

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by Duncan Hall

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Foreword

This study was commissioned by RIRDC as a meta-analysis and review of a series of RIRDC funded studies titled: “Kangaroo sulphur dioxide and thiamine relationship study”, exploring observed thiamine levels in the presence of sulphite in chilled kangaroo meat for pet consumption. This project collates the data of this series of controlled studies, which explore the relationship between the addition of sulphites and thiamine in kangaroo meat.

The addition of sulphites assists the preservation of kangaroo meat used for chilled kangaroo pet meat. The addition of exogenous thiamine is important to ensure these products meet the nutritional requirements of dogs and cats, as defined by the American Association of Feed Control Officials (AAFCO) nutrient profiles. This information should be of interest to kangaroo pet meat products supply chain operators, veterinarians, key industry stakeholders and pet owners.

This project found that under controlled laboratory conditions, supplementation of kangaroo meat with considerable levels of exogenous thiamine resulted in samples which met the AAFCO 2011 pet food nutrient profiles for thiamine in the diet of dogs. Most, but not all, samples met the AAFCO 2011 nutrient profiles for the diet of cats. These samples were measured over a 28 day period, and the target level of sulphur dioxide (SO_2) in the meat was limited to levels at or below 200ppm.

This project was funded by RIRDC through the Kangaroo R&D Program, which is financed through kangaroo industry levies. This funding was in addition to cash and in-kind contributions from kangaroo pet meat processors.

This report is an addition to RIRDC’s diverse range of over 2000 research publications and it forms part of our New Animal Products R&D program, which aims to accelerate the development of viable new animal industries.

Most of RIRDC’s publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
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About the Author

Dr Duncan Hall (BVSc) is the Director of Biologic Pty Ltd. Duncan is an experienced pet food industry consultant who designed and supervised this study project, including reviewing the scientific literature, developing the project methodology and supervising the meat chemistry trials.

Acknowledgments

Laboratory studies were conducted in association with Dr Yasmina Sultanbawa (PhD), Senior Research Fellow at Qld Alliance for Agriculture & Food Innovation (QAAFI), Department of Agriculture, Fisheries and Forestry (DAFF), Kessels Road, Cooper Plains , QLD 4108.

Chemistry and dry matter testing was undertaken at Symbio Alliance, 52 Brandl St. Eight Mile Plains Queensland, 4113.

Abbreviations

AS: Australian standard

AAFCO: The Association of American Feed Control Officials

D.M: Dry matter

Kcals: kilocalories

M.E: metabolisable energy

NRC: National Research Council.

ppm: parts per million

SMBS: sodium metabisulphite

SO2: sulphur dioxide

w/w: weight for weight

Contents

Executive Summary

What the report is about

The pet meat sector processes a large portion of kangaroo meat harvested in Australia. Most kangaroo pet meat is sold domestically. Preservatives that liberate sulphur dioxide are added to fresh kangaroo pet meat to extend shelf life by inhibiting bacterial growth, diminishing the odour produced by bacteria that multiply in food, and delaying the reduction of myoglobin (which results in the meat appearing brown rather than red). Sulphur dioxide is antagonistic to thiamine, and can result in substantial depletion of thiamine in meat. Veterinary reports of illness in cats and dogs due to thiamine deficiency have undermined confidence in the safety of kangaroo pet meat.

This report collates findings from a series of RIRDC funded projects undertaken in 2011 and 2012 (PRJ-007258, PRJ-008154, PRJ-008402 and PRJ-008460). These projects investigate the extent of thiamine degradation in the presence of sulphur dioxide in fresh, chilled kangaroo meat over a period broadly equivalent to the shelf life of kangaroo pet meat products. The research also explored the potential level of thiamine supplementation required to offset the degradation by sulphur dioxide, in order to provide adequate thiamine levels to meet the nutritional needs of cats and dogs over a period of 28 days. The levels of thiamine required are outlined in the nutrient profiles for dog and cat foods, published by the Association of American Feed Control Officials (AAFCO). The laboratory studies in this project provide results with the potential to enhance the quality, safety, and reputation, of kangaroo meat as a pet food, and to assist operators in the pet food industry to comply with relevant industry standards in providing fresh, ground, kangaroo pet meat.

Who is the report targeted at?

This information should be of interest to kangaroo pet meat supply chain operators, veterinarians, key influencers and pet owners, in regards to the safety of feeding fresh kangaroo meat to companion animals, and reducing the risk of adverse impacts to the health of pets due to thiamine deficiency.

Where are the relevant industries located in Australia?

The kangaroo industry provides significant economic, social and environmental benefits to Australia and operates in all States except Victoria and the Northern Territory.

Aims/objectives

The aims/objectives of this project are to:

- Assess the impact of varied concentrations of sulphur dioxide (SO_2) on thiamine levels (endogenous and exogenous) in kangaroo meat over a period of time, reflecting the normal product shelf life for chilled “fresh” pet foods

- Investigate the thiamine supplementation necessary to counter the depletion of thiamine in kangaroo meats in the presence of SO_2, so that the minimum nutrient level for thiamine in pet meat is met. This level is specified by the widely recognised nutrient guidelines for dogs and cats on a dry matter (DM) basis (AAFCO 2011b; AAFCO 2011a).

Methods used

The project design throughout these studies was broadly similar, involving preparation of 300g or 600g samples of ground kangaroo meat, with varying levels of added thiamine and sodium metabisulphite (SMBS). Samples were chilled and sent for analysis on Day 1 and Day 28 in each trial.

The trials extended for a minimum of 28 days, a duration which broadly reflects the likely shelf life, or best before dates, for chilled pet foods made from fresh kangaroo meat.

Results/key findings

Results of this series of trials reflect that a large proportion of thiamine present in chilled kangaroo meat, including both existing (endogenous) and additional added (exogenous) thiamine, is depleted within one day in the presence of sulphur dioxide at levels at or above 200 ppm inclusion rate of SO_2.

The results of the final trial in this series (PRJ-008460) indicate that supplementation of kangaroo meat with levels of exogenous thiamine of either 900ppm or 1000ppm resulted in thiamine levels that comfortably exceed the published AAFCO nutrient guidelines requirements of both dogs and cats for thiamine on a dry matter basis. The length of the trial was 28 days, and these results were obtained in the presence of sodium metabisulphite included at levels targeting a concentration of sulphur dioxide in meat of 150 ppm or below.

Where targeted sulphur dioxide concentration was 200ppm (mg/kg), supplementation with 1000ppm thiamine resulted in a thiamine concentration of 1.6ppm on an “as is” basis. This is equivalent to 6.7ppm (mg/kg) thiamine on a dry matter basis, which exceeds the AAFCO published requirement for both dogs and cats.

At a level of SMBS targeting an SO_2 concentration of 200ppm, addition of 900ppm exogenous thiamine resulted in a measured concentration of 0.7ppm on an ‘as is’ basis. This is equivalent to 3.2ppm on a dry matter basis. This exceeds the AAFCO published requirement for dogs (1.0 mg/kg), but is below the published requirement for cats (5.0 mg/kg).

Implications for relevant stakeholders for:

It is envisaged that this examination of the interaction of SO_2-liberating preservatives and thiamine supplementation will assist the kangaroo industry by providing objective data on thiamine degradation in the presence of sulphites to the kangaroo pet meat supply chain. This data provides a basis for the kangaroo industry to assess and, if required, modify practices through the supply chain to enhance confidence in the feeding of fresh kangaroo meat products to companion animals.

Recommendations

The results of this study were obtained under controlled laboratory conditions. Therefore, in the author’s opinion, it would be prudent and appropriate to implement studies within commercial kangaroo meat processing facilities. This is necessary to assess the relevance, repeatability and practicality of these findings within a complex manufacturing environment. Extending this applied research through the chilled supply chain nationally would validate the results and findings of this series of trials.

Introduction

Kangaroo meat has been fed to Australian cats and dogs for many years. It is recognised to be low in fat, an excellent source of protein, iron and zinc, and is a rich source of conjugated linoleic acid (CLA) (Wright,2004).

The pet meat sector processes a large portion by volume of kangaroo meat (Kelly 2005), and most kangaroo pet meat is sold domestically. The pet meat sector operates within an independent supply chain, with dedicated pet meat field chillers and processing at premises registered to only process pet meat. Hygiene and operational standards are high, but not as stringent as within the human consumption processing chain. Preservatives that liberate SO_2 (220 - sulphur dioxide, 221 - sodium sulphite, 222 - sodium bisulphite, 223 - sodium metabisulphite, 224 - potassium metabisulphite, 225 - potassium sulphite, 228 - potassium bisulphite) are frequently added to fresh kangaroo pet meat to extend shelf life by inhibiting bacterial growth, diminishing the odour produced by bacteria that multiply in food, and delaying the reduction of myoglobin (which results in the meat appearing brown rather than red). The effect of sulphites and sulphur dioxide on thiamine in stored food has been recognised by some veterinarians and consumers to be a major issue for pet food manufacturers and suppliers using kangaroo meat as a raw ingredient.

Thiamine is a heat-labile, water-soluble vitamin, which plays an essential role in energy metabolism, and nerve transmission. Thiamine is not stored in the body to any extent, and therefore must be continually ingested in food. It is relatively easy to develop deficiency in a short time, even in adult animals (McDowell 2000). Cats are more susceptible to thiamine deficiency than dogs because they require about four times as much thiamine in their diet (NRC, 2003). Thiamine deficiency causes severe neurological and cardiovascular symptoms and can be rapidly fatal.

Expressed per kg of food (dry matter), the dietary requirement for thiamine for cats is 5mg/kg (AAFCO Handbook 2011, NRC 1986). For dogs, it is 1mg/kg (AAFCO Handbook 2011, NRC 1985). Cooking and processing may cause a loss of 40 to 50% of thiamine present in raw materials (McDowell 1989). AAFCO states that processing may destroy up to 90% of thiamine present in the diet. Reputable prepared pet food manufacturers and chilled pet meat producers adjust for this loss by supplementation with exogenous thiamine.

Sulphur dioxide has been shown to deplete thiamine in meat. The sulphite ion cleaves thiamine at the methylene bridge between the pyrimidine and thiazole rings, acting as a thiaminase (McDowell 2000). Sulphur dioxide (SO_2) rapidly inactivates thiamine present normally in meat and meat by-products. Early research on the destruction of thiamine in sulphited meat found a linear relationship with increasing SO_2 content: a level of 400 mg SO_2/kg depleted thiamine by 55%, while 1000 mg SO2/kg depleted it by 95% (Hermus 1969, cited by Studdert & Labuc 1991).

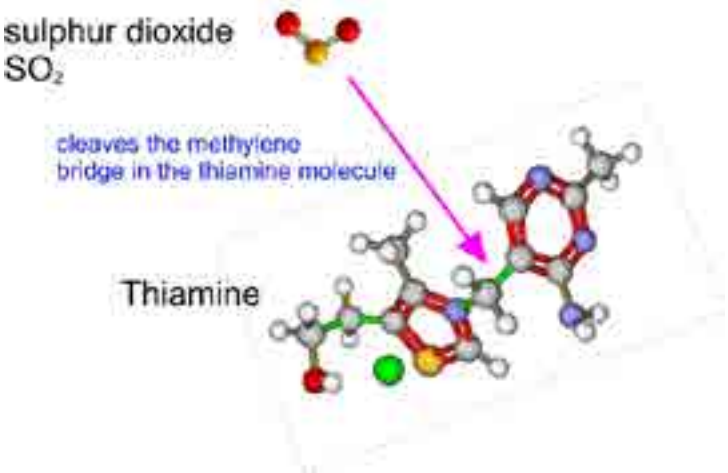

Figure 1 Sulphite ion cleaves the methylene bridge of thiamine molecule

Thiamine deficiency has been diagnosed in a number of cats and dogs being fed fresh minced kangaroo meat containing sulphur dioxide as a preservative. In one case, Studdert and Labuc tested the thiamine content of the pet meat at less than 0.5mg/kg thiamine, and concluded that thiamine in the meat and in added dietary ingredients, including a supplementary vitamin mixture, was destroyed by the sulphur dioxide. Previous testing of a range of pet meats and unrefrigerated pet rolls from four states of Australia found 64 out of 74 contained quantities of SO_2 likely to inactivate a considerable portion of the endogenous thiamine present in these products, with an average concentration of 380 mg SO_2/kg. (Studdert & Labuc 1991; Steele 1997; Singh et al. 2005).

Unlike the human food supply chain, the Australian pet food industry has traditionally been self-regulated, operating under a voluntary code of practice developed by the Pet Food Industry Association of Australia (PFIAA). To ensure the quality, safety and labelling of pet foods sold in Australia, a new Australian Standard (AS 5812) - Manufacturing and Marketing of Pet Food, has now been published by Standards Australia. This has followed industry consultation involving representation from the Pet Food Industry Association (PFIAA),The Australian Veterinary Association (AVA), the Royal Society for the Prevention of Cruelty to Animals (RSPCA) and Federal and State government authorities. This standard specifies requirements for the production and supply of multi-ingredient, manufactured food for domesticated dogs and cats, and applies to both domestic and imported manufactured pet food products.

Section 3.1.10 of the standard states:

> "Where sulphur dioxide or sodium or potassium sulphites are used the common, prescribed, proprietary name or the FSANZ Food Standards Code number shall be included on the label. In this instance, to avoid acute thiamine deficiency in pets, sufficient thiamine shall be present throughout the shelf life of a pet food product. If necessary this may be achieved by thiamine supplementation. Such supplementation may not of itself render the product nutritionally complete, but is to ensure the product is not deficient in thiamine according to AAFCO Official Publication guidelines."

Unprocessed pet meat is subject to a separate industry standard (PISC 2009 Standard for the Hygienic Production of Pet Meat). The standard (Section 1) requires that:

> "Where used, additives (including preservatives) shall be applied at such levels that they do not cause adverse impact or undue risk to the well-being of pets."

During 2011 and 2012, a series of trials were conducted by Biologic Pty Ltd, with assistance from the technical staff at the Department of Agriculture, Fisheries and Forestry (DAFF), Coopers Plains, QLD. These trials explored the relationship between exogenous sulphites included in kangaroo meat

and resultant thiamine assays over a period of time broadly corresponding to the usual recommended shelf life ("best before" date) of uncooked kangaroo meat products for pet consumption. These studies were funded by the Rural Industries Research and Development Corporation and the Kangaroo Industry Association of Australia (KIAA).

The role of thiamine in companion animal nutrition

Thiamine (Vitamin B1) is not synthesized in the tissues of dogs and cats and therefore, must be ingested in the food these animals consume. While thiamine is present in many foods of both animal and plant origin, it is only abundant in a few of these foods. Recognised rich sources of thiamine are: yeast, wheat germ, liver and legume seeds.

Thiamine plays an important role in metabolism. Thiamine pyrophosphate is a coenzyme in two types of active aldehyde transfer reactions: oxidative decarboxylation of α-ketoacids and transformation of α-ketols, and these reactions are important in energy and carbohydrate metabolism. Thiamine is absorbed from the small intestine by both an active and passive process, with active absorption requiring sodium (Na+). In humans, 20-30% of thiamine in plasma is protein-bound and is taken into erythrocytes by a facilitated diffusion process and into other cells by active transport. In these cells, around 80% of thiamine is present as thiamine pyrophosphate. (NRC, 2003).

Signs of thiamine deficiency in companion animals

Dogs:

Thiamine is water soluble and the body has a limited ability to store thiamine. Clinical signs of thiamine deficiency appear in a shorter time after exposure to a thiamine–deficient diet than for most other vitamins. (NRC, 2003)

Acute thiamine deficiencies tend to involve the brain and produce severe neurological signs as pathology can include necrosis of grey matter. Chronic deficiencies produce pathological changes of the myocardium and peripheral nerves with diffuse myelin degeneration and axonal disintegration. (NRC, 2003).

Cats:

Cats are more susceptible to thiamine deficiency than dogs, as they require about four times as much thiamine in their diet than dogs (NRC, 2003).

Thiamine deficiency induces pathological changes in the central nervous system of the cat. Three stages of thiamine deficiency have been reported by Everett (Everett,1944). The first stage, which frequently occurs within one to two weeks of consumption of a deficient diet (NRC 2003) is characterised by anorexia. The second stage is characterised by the appearance of neurological signs including those involving posture (typically with ventroflexion of the head) and short convulsive seizures. The third, or terminal stage, is characterised by progressive weakness, prostration and death.

Nutritional requirements of dogs and cats for thiamine

The Association of American Feed Control Officials (AAFCO):

The AAFCO Dog and Cat Food Nutrient Profiles and the AAFCO Feeding Protocols are the only AAFCO recognised methods for substantiating the nutritional adequacy of "complete and balanced" pet foods (on a dry matter basis).

Table 1. Thiamine requirements of dogs and cats (AAFCO 2011)

Species	Units DM basis	Growth & reproduction (minimum)	Adult maintenance (minimum)	Maximum
Dog	mg/kg	1.0	1.0	Not defined
Cat	mg/kg	5.0	5.0	Not defined

The AAFCO Handbook (2011) includes the statement: "Because processing may destroy up to 90% of the thiamine in the diet, allowances in formulation should be made to ensure the minimum nutrient level is met after processing".[1]

The nutrient levels are expressed on a dry matter basis at a defined caloric density. For dogs, this is 3500 kcal Metabolisable Energy perkg Dry Matter (ME/kg DM); for cats, this is 4000 kcal ME/kg DM. AAFCO advise that the above food nutrient profiles based on dry matter may require adjustment as follows:

For dogs: presumes an energy density of 3500 kcal ME/kg. Rations greater than 4000 kcal ME/kg should be corrected for energy density, rations less than 3500 kcal ME/kg should not be corrected for energy.

For cats: presumes an energy density of 4000 kcal ME/kg. Rations greater than 4500 kcal ME/kg should be corrected for energy density, rations less than 4000 kcal ME/kg should not be corrected for energy (AAFCO, 2011).

[1] *It is assumed by the author of this report that this statement refers to the potential loss of thiamine during thermal (heat) processing, which is used in making a variety of formats of pet foods (including canned and dry pet foods). However, it is noted that the nature of 'processing' is not specified in the AAFCO (2011) official publication.*

Objectives

The objectives of these studies is to improve the quality, safety and reputation of kangaroo meat as a healthy pet food, expanding knowledge within the kangaroo pet meat sector and amongst key industry stakeholders, including veterinarians, animal welfare bodies and pet owners with regards to sulphur preservatives and the supplementation of pet meat with exogenous thiamine. This is to ensure that operators in the pet food industry continue to comply with relevant industry standards.

The objectives of this series of research projects:

1. Determine the moisture content (dry matter) of lean ground kangaroo meat.

2. Determine the endogenous thiamine content in fresh kangaroo meat.

3. Measure the liberation of sulphur dioxide (SO_2) from sodium metabisulphite when added at varying levels to ground lean kangaroo muscle meat while refrigerated at 4^oC.

4. Investigate the extent and efficiency of thiamine degradation in the presence of varying levels of sulphites over a time frame that represents the expected shelf life of chilled, uncooked kangaroo pet meat products.

5. Investigate the amount of exogenous thiamine required to be included to offset the inactivation of thiamine by varying levels of SO2 in fresh kangaroo meat, by undertaking controlled trials over a period of time reflecting the approximate shelf life of uncooked kangaroo pet meat products.

Methodology

This series of RIRDC studies were undertaken at the Department of Agriculture, Fisheries and Forestry (DAFF), Coopers Plains, Queensland and included PRJ-007258 (completed August 2011), PRJ-008154 (completed January 2012), PRJ008402 (completed April 2012), and PRJ-008460 (completed August 2012).

Each of these trials involved broadly similar design. All trials extended for a minimum of 28 days, a duration chosen to approximate the likely shelf life or best before date for chilled pet foods made from fresh kangaroo meat. The initial project (PRJ-007258) extended for a period of 56 days.

The research projects involved sourcing 20-50kg of fresh kangaroo muscle meat for each trial, assayed to ensure that it was free from exogenous sulphites. The trial commenced on Day 0. The meat was ground and divided into 300g (PRJ-008154, PRJ-008402, PRJ-008460) or 600g samples (PRJ-007258). The meat samples in each trial (except negative controls) were treated with differing levels of sodium metabisulphite to achieve liberation of target SO_2 levels in the meat. Exogenous thiamine hydrochloride was added to meat samples (except negative controls) at varying amounts at the commencement of each trial, with each project including negative controls for sulphites and thiamine. Samples were vacuum packed in polythene bags to replicate the retail packaging utilized for some kangaroo meat products and once prepared, samples were refrigerated at 4^0C (equivalent to refrigerators in commercial retail outlets where pet food kangaroo meat is sold). Samples were submitted for analysis of sulphur dioxide and thiamine assays at weekly intervals throughout each trial, commencing 24 hours after sample preparation (Day 1). Detailed information about each project's design is presented in the completed RIRDC reports for these projects.

Figure 2. Sample preparation

Results

1. Assessment of dry matter content of fresh, ground, lean kangaroo meat

The series of studies (PRJ-007258, PRJ-008154, PRJ-008402 and PRJ-008460) undertaken as part of this study included assessment of dry matter of fresh, ground kangaroo meat in three separate studies.

The mean value for dry matter content (%) of all ground kangaroo meat samples assessed throughout the projects is 22.7% (w/w), equivalent to 77.3% moisture (w/w) (Tables 2,3,4. Appendix).

2. Assessment of endogenous thiamine content of kangaroo meat

The endogenous thiamine content of kangaroo meat was assessed in the above trials. The results of thiamine content of negative controls (no added thiamine and no added sulphites) for day 0 of trials PRJ-007258, PRJ-008154, PRJ-008402 and PRJ-008460 are presented in table 4 of the Appendix.

Some variability in endogenous thiamine results was noted, with day 0, negative control thiamine assays on an “as is” basis ranging from a minimum of 34 mcg/100 g to a maximum of 83.6 mcg/100g. These results return a mean endogenous thiamine level of 65.7 mcg/100g for day 0 controls.

Using this mean value (65.7 mcg/100g ‘as is’) for endogenous thiamine and the mean value measured for dry matter content of kangaroo meat (22.7%), results in an estimated (mean) endogenous thiamine content of 2.9 mg/kg (DM basis) for fresh kangaroo meat. This exceeds the AAFCO recommended thiamine level for dog nutrition for adult growth and reproduction (AAFCO 2011). This calculated mean observed endogenous thiamine level represents approximately 60% of the AAFCO nutrient guideline for cats for thiamine on a dry matter basis (5 mg/kg D.M), although the variability of individual thiamine assays as indicated above should be recognised.

This level of endogenous thiamine in kangaroo meat is compared with a range of meats referenced in the 1985 Nutrient Requirements of Dogs and Cats (NRC, 1985) (Table 6, Appendix).

3. Liberation of sulphur dioxide (SO_2) from sodium metabisulphite when added to fresh ground kangaroo meat

A series of “calibration trials” were undertaken as part RIRDC projects PRJ-007258 and PRJ-008154 (see appendix), to provide data regarding the extent of liberation of sodium dioxide (SO2) from sodium metabisulphite when added to ground, fresh kangaroo meat. Prior to these RIRDC studies, there was only limited published data (Wedzicha B.L, Mountfort K.A, 1990) regarding the liberation of SO_2 from sodium metabisulphite added to meat. This previous research suggested a relationship of 1.33 SMBS to SO_2 liberated in ground meat, although it should be noted that this study was undertaken in the United Kingdom using sausage meat. While the authors did not define the nature of the meat used it is considered highly unlikely that kangaroo meat was used in this prior published study. The results from the calibration trial conducted as part of RIRDC project PRJ-008154 indicates that the relationship of SO_2 liberated from SMBS in ground, lean kangaroo meat is more accurately reflected in a liberation ratio of 2.5 units of SMBS added to one unit of SO_2 liberated. This relationship is demonstrated in the graph below (figure 3) showing a meta-analysis of the extent of

SO_2 liberation from SMBS added to meat samples at Day 1 of three RIRDC projects (PRJ-008154, PRJ-008402 and PRJ-008460). Data is presented in Table 7 in the Appendix to this report.

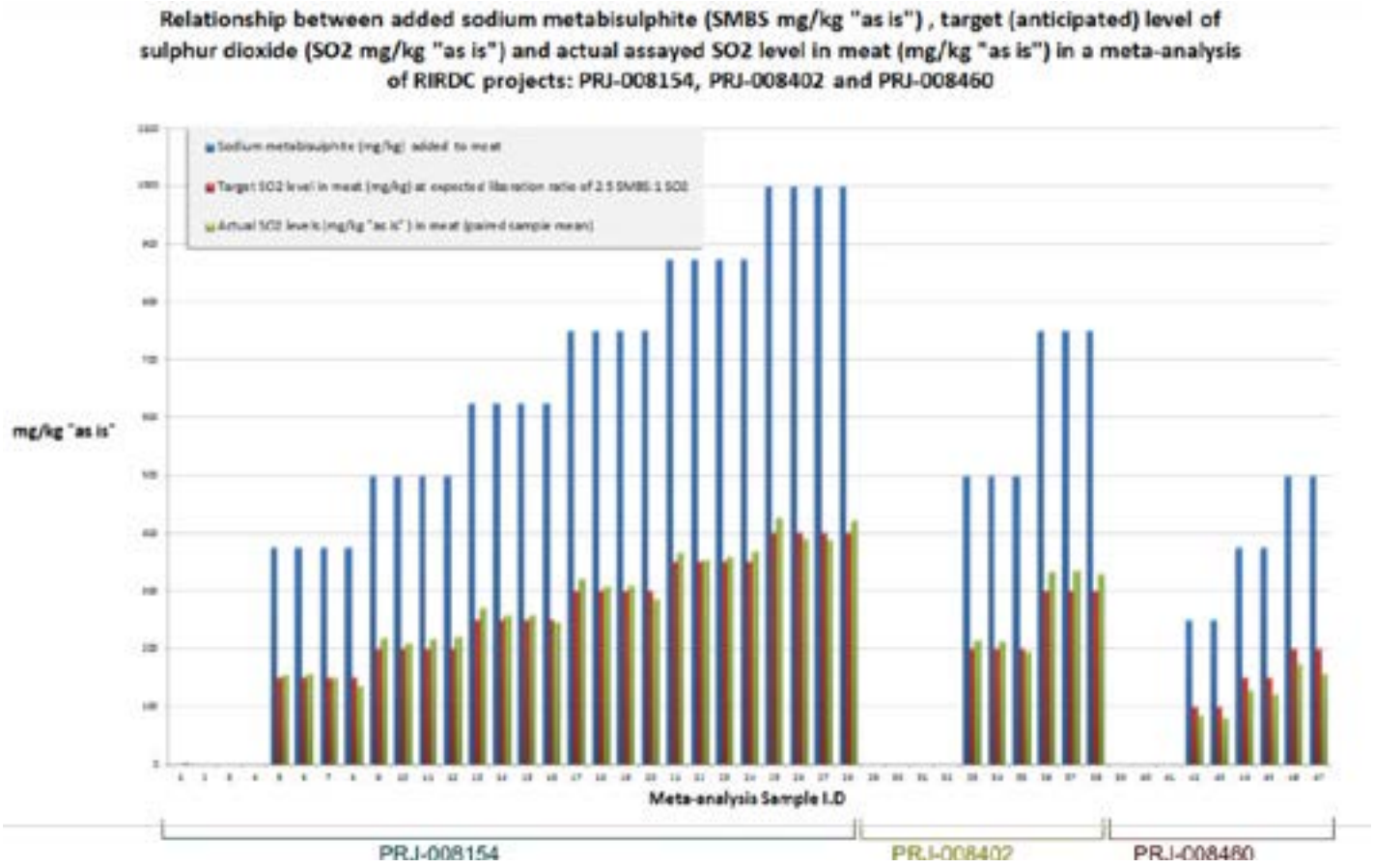

Figure 3. Relationship between added SMBS, target SO2 & actual SO2 in samples, Day 1

4. Investigate the extent and efficiency of thiamine degradation in the presence of varying levels of sulphites

The results presented in figure 4 are a meta-analysis of three trials: PRJ-008154, PRJ-008402, PRJ-008460 to provide an analysis of the relationship between thiamine and sulphur dioxide measured in samples on Day 1 and Day 28 of these trials. This time frame represents the expected shelf life of chilled, uncooked kangaroo pet meat products. The data for these graphs is presented in tables 7 & 8 in the Appendix.

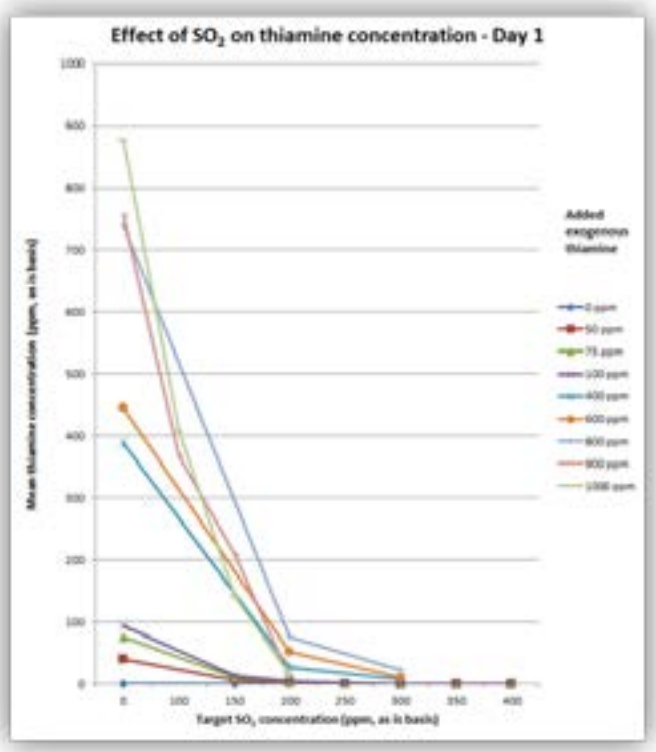

Figure 4. Effect of SO2 on thiamine concentration, Day 1

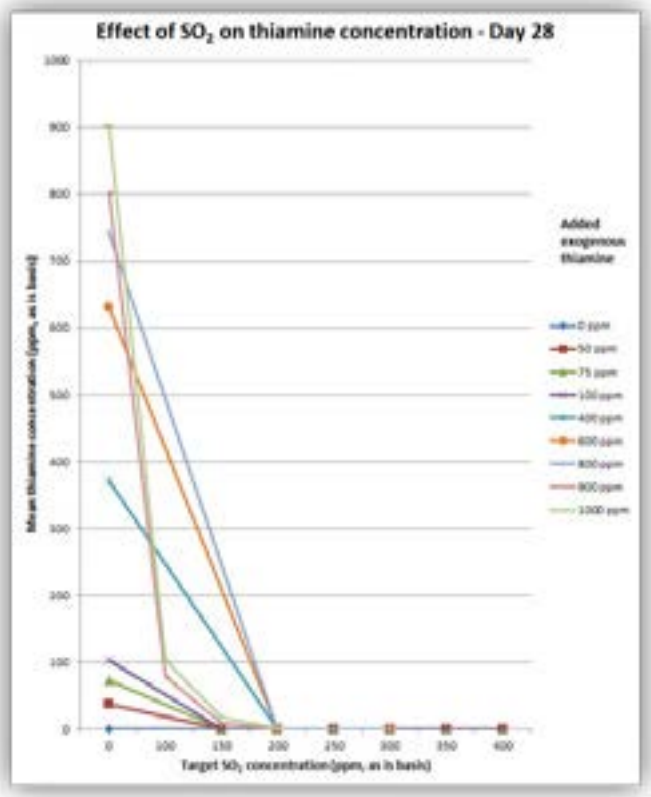

Figure 5. Effect of SO2 on thiamine concentration, Day 28

Figures 4 and 5 demonstrate the extent of thiamine degradation in the presence of sulphur dioxide. This meta data includes the results of project PRJ-008460 “Kangaroo sulphur dioxide and thiamine relationship study – supplemental study #2”. This project specifically involved inclusion of SMBS at levels to achieve target levels of sulphur dioxide in meat of 0, 100, 150 and 200 ppm while including thiamine at levels of 0, 900 and 1000 ppm. The results for Day 1 and Day 28 of this trial are included in tables 9 and 10 of the Appendix to this report.

At Day 28 of the trial, levels of thiamine in kangaroo meat comfortably exceeded the published AAFCO nutrient profile of both cats and dogs, when meat was supplemented with exogenous thiamine at levels of either 900ppm or 1000ppm sodium metabisulphite where concentration of SO_2 in meat was targeted at 150ppm or below. For instance, in samples targeting 150ppm sulphur dioxide with 900ppm of exogenous thiamine, the measured thiamine at Day 28 of the trial was 9.0ppm on an ‘as is’ basis and 38ppm on a DM basis. Where 1000ppm thiamine was added to samples targeting 150ppm sulphur dioxide, the result at Day 28 of this trial returned mean sample thiamine content of 17.9ppm on an ‘as is’ basis, equivalent to 76.8ppm on a DM basis. This is well in excess of the AAFCO published nutrient profile for both dogs and cats.

Where targeted sulphur dioxide concentration was 200ppm (mg/kg), supplementation with 1000ppm thiamine resulted in a thiamine concentration of 1.6ppm on an ‘as is’ basis, equivalent to 6.7ppm (mg/kg) thiamine on a dry matter basis, which exceeds the AAFCO published nutrient profile for both dogs and cats.

At a level of SMBS targeting a sulphur dioxide concentration of 200ppm, addition of 900ppm exogenous thiamine resulted in a measured concentration of 0.7ppm (on an ‘as is’ basis), equivalent to 3.0ppm on a dry matter basis. This exceeds the AAFCO nutrient profile for dogs by a factor of three, but is below the published requirement for cats of 5.0mg/kg DM basis.

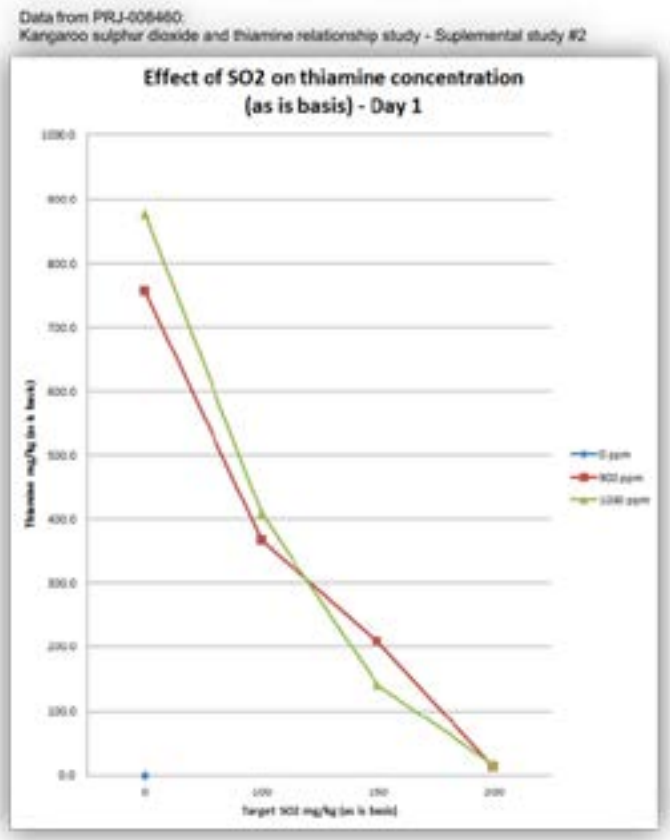

Figure 6. Effect of SO_2 on thiamine concentration, Day 1 (from project PRJ-008460)

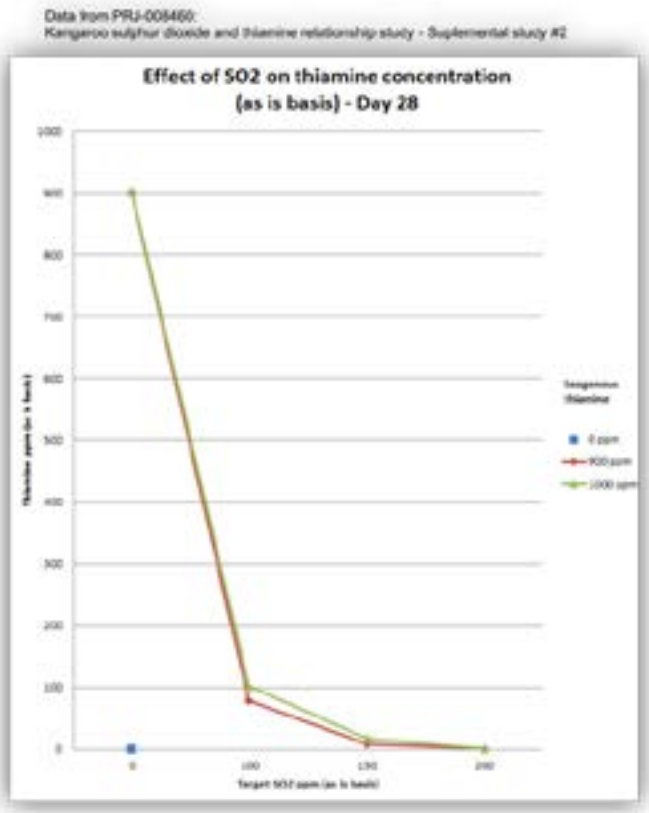

Figure 7. Effect of SO_2 on thiamine concentration, Day 28 (from project PRJ-008460)

Recommendations

This project collates the data of a series of controlled studies (PRJ-007258, PRJ-008154, PRJ-008402 and PRJ-008460), which explore the relationship between the addition of sulphites and thiamine in kangaroo meat products. The addition of sulphites assists the preservation of kangaroo meat used for chilled kangaroo petmeat. The addition of exogenous thiamine is important to ensure that these products meet the nutritional requirements of dogs and cats, as defined by the American Association of Feed Control Officials (AAFCO) nutrient profiles.

Each of the studies included in this series of research project had limited numbers of samples, due to the high cost of biochemistry analysis (in particular, the high cost of thiamine testing). Therefore, these projects, while providing considerable new data regarding the relationship between sulphites and thiamine in kangaroo meat, should be regarded as pilot studies. Due care is advised when interpreting and applying results within an industrial manufacturing environment. Given the very limited number of samples included in data sets, no statistical analysis has been applied to trial results.

The results of this study were obtained under controlled laboratory conditions. Therefore, in the author's opinion, it would be prudent and appropriate to implement studies within commercial kangaroo meat processing facilities. This is necessary to assess the relevance, repeatability and practicality of these findings within a complex manufacturing environment. Extending this applied research through the chilled supply chain nationally would validate the results and findings of this series of trials.

Disclaimer

The material presented in this report is based on research conducted and published materials and sources that are believed to be reliable. Whilst every care has been taken in the preparation of the report, the author provides no warranty that the cited references, sources or interpretations included in this report are correct and accepts no responsibility for any resultant errors contained herein or any damages or loss, whatsoever caused or suffered by any individual or corporation.

Appendices

Dry Matter (DM) assessment

The results of individual dry matter assessment assays undertaken are shown in tables 2 to 4 below:

Table 2. DM assessment: Project PRJ-008154

Project PRJ-008154	Sample I.D	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	**Mean**
Moisture	% w/w	77.9	77.6	78	78	78.9	77.6	78.1	77.1	79.3	78.2	**78.1**
Dry matter	% w/w	22.1	22.4	22	22	21.1	22.4	21.9	22.9	20.7	21.8	**21.9**

Table 3. DM assessment: Project PRJ-008402

Project PRJ-008402	Sample I.D	A	B	C	D	E	F	**Mean**
Moisture	% w/w	76.9	76.8	75.2	77.1	77.6	76.6	**76.7**
Dry matter	% w/w	23.1	23.2	24.8	22.9	22.4	23.4	**23.3**

Table 4. DM assessment: Project PRJ-008460

Project PRJ-008460	Sample I.D	A	B	C	D	E	F	**Mean**
Moisture	% w/w	76.9	76.8	76.5	76.9	76.6	76.8	**76.7**
Dry matter	% w/w	23.1	23.2	23.5	23.1	23.4	23.2	**23.3**

Table 5. Endogenous thiamine content of fresh, ground, lean kangaroo meat, Day 0

Trial number	Sample I.D	SO2 assay in sample Mg/kg	Thiamine assay in sample mcg/100g
PRJ-007258	Sample 1 (Day 0)	<5	83.6
PRJ-008154	Sample 1(Day 0)	5.2	68.5
	Sample 2 (Day 0)	<5	79.5
PRJ-8460	Sample 1(Day 0)	<5	59
	Sample 2 (Day 0)	<5	68
PRJ-008460	Sample 1 (Day 0)	<5	39
	Sample 2 (Day 0)	<5	34
Mean			**61.65**

Table 6. Thiamine content of selected meat raw materials

Raw material name / type	Thiamine content (mg/kg) on a dry matter (D.M) basis
Kangaroo (muscle meat)*	2.9
Cattle lungs (fresh)^	2.8
Broiler flesh (fresh)^	2.9
Whole hen (fresh)	2.4

*Average result from RIRDC trials 2011-12 as above

^Nutrient Requirements of Dogs (NRC) 1985

Liberation of sulphur dioxide (SO2) from sodium metabisulphite added to fresh, ground, kangaroo meat.

The results of the main trial conducted as part of RIRDC project PRJ-008154 reflect the relationship between included sodium metabisulphite (SMBS) and resultant liberated sulphur dioxide (SO2) in fresh kangaroo meat. The relationship that was evident in the main trial is demonstrated in figure 8, which shows the effect of added SMBS on SO_2 concentration achieved in this trial. The trend line overlaid on this graph shows the almost linear relationship between SMBS and liberated SO2 assays and the fact that this relationship is close to the ration of 2.5:1 (SMBS added to resultant liberated SO_2).

Figure 8. Effect of added SMBS on SO_2 concentration in meat

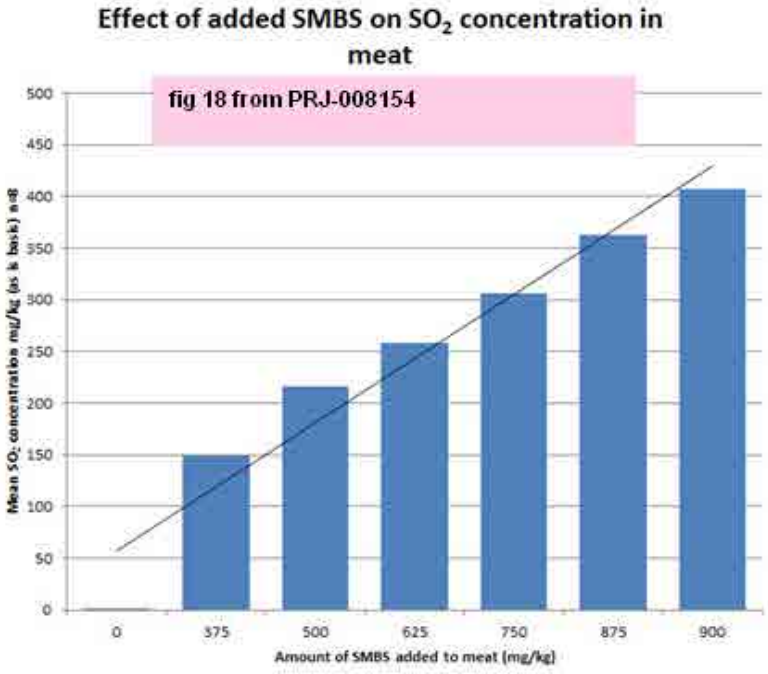

Table 7. Meta data of projects PRJ-008154, PRJ-008402 and PRJ-008460 (Day 1) investigating the extent and efficiency of thiamine degradation in the presence of varying levels of sulphites, over a time frame that represents the expected shelf life of chilled, uncooked kangaroo.

Meta-analysis of combined project data (compiled results of trials PRJ-008154, PRJ-008402 and PRJ-008460)

Day 1 results of mean values of paired samples (combined results of 3 trials)

Trial number	Paired sample I.D	Sodium metabisulphite (mg/kg) added to meat	Target SO2 level in meat (mg/kg) at expected liberation ratio of 2.5 SMBS:1 SO2	Actual SO2 levels (mg/kg "as is") in meat (paired sample	Thiamine (exogenous) added (ppm)	Paired sample mean Thiamine on an "as is" basis	Paired sample mean Thiamine (ppm) on a dry matter basis"	% Dry matter (mean) for trial
PRJ 008154	1	0	0	2.6	0	0.7	3.2	21.9%
	2	0	0	0	50	40.7	185.8	
	3	0	0	0	75	75.8	346.1	
	4	0	0	0	100	94.1	429.7	
	5	375	150	155	0	0.2	0.9	
	6	375	150	157	50	6.5	29.7	
	7	375	150	149	75	10.1	46.1	
	8	375	150	136	100	13.7	62.6	
	9	500	200	218.5	0	0.1	0.5	
	10	500	200	209.5	50	2.3	10.5	
	11	500	200	218	75	3.3	15.1	
	12	500	200	221	100	4.8	21.9	
	13	625	250	271	0	0	0.0	
	14	625	250	258.5	50	0.9	4.1	
	15	625	250	258	75	1.2	5.5	
	16	625	250	247	100	1.9	8.7	
	17	750	300	322	0	0	0.0	
	18	750	300	308.5	50	0.3	1.4	
	19	750	300	310	75	0.5	2.3	
	20	750	300	286.5	100	0.9	4.1	
	21	875	350	366.5	0	0	0.0	
	22	875	350	354	50	0.1	0.5	
	23	875	350	359.5	75	0.1	0.5	
	24	875	350	368.5	100	0.3	1.4	
	25	1000	400	427.5	0	0	0.0	
	26	1000	400	391	50	0	0.0	
	27	1000	400	389.5	75	0	0.0	
	28	1000	400	422	100	0.1	0.5	
PRJ 008402	29	0	0	0	0	0.64	2.7	23.3%
	30	0	0	0	400	389	1669.5	
	31	0	0	0	600	445	1909.9	
	32	0	0	0	800	739.5	3173.8	
	33	500	200	215	400	27	115.9	
	34	500	200	212.5	600	53.3	228.8	
	35	500	200	195.5	800	76.6	328.8	
	36	750	300	333	400	7.1	30.5	
	37	750	300	335.5	600	11.1	47.6	
	38	750	300	328.5	800	23.4	100.4	
PRJ 008460	39	0	0	0	0	0.4	1.7	23.3%
	40	0	0	0	900	755.5	3242.5	
	41	0	0	0	1000	876.5	3761.8	
	42	250	100	85.9	900	367	1575.1	
	43	250	100	73.6	1000	408	1751.1	
	44	375	150	127.5	900	209	897.0	
	45	375	150	122	1000	141.8	608.6	
	46	500	200	174	900	12.8	54.9	
	47	500	200	156.5	1000	15.8	67.8	

Table 8. Meta data of projects PRJ-008154, PRJ-008402 and PRJ-008460 (Day 28) investigating the extent and efficiency of thiamine degradation in the presence of varying levels of sulphites, over a time frame that represents the expected shelf life of chilled, uncooked kangaroo.

Meta-analysis of combined project data (compiled results of trials PRJ-008154, PRJ-008402 and PRJ-008460)

Day 28 results of mean values of paired samples (combined results of 3 trials)

Compiled results of Trials PRJ 008154, PRJ 008402 and PRJ 008460

Trial number	Paired sample I.D	Sodium metabisulphite added (mg/kg) to meat	Target SO2 in meat (ppm) at expected liberation ration of 2.5 SMBS:1 SO2	Paired sample mean SO2 (mg/kg) "as is"	Thiamine (exogenous) added (ppm)	Paired sample mean Thiamine on an "as is"	Paired sample mean Thiamine (ppm) on a dry matter basis	% Dry matter (mean) for trial
PRJ 008154	48	0	0	0	0	0.0	3.7	21.9%
	49	0	0	0	50	37.9	173.1	
	50	0	0	0	75	72.8	332.4	
	51	0	0	0	100	103.1	470.8	
	52	375	150	129	0	0.3	1.4	
	53	375	150	139.5	50	0.2	0.9	
	54	375	150	149	75	0.4	1.8	
	55	375	150	146.5	100	0.6	2.7	
	56	500	200	198.5	0	0	0.0	
	57	500	200	191	50	0.1	0.5	
	58	500	200	130.5	75	0.1	0.5	
	59	500	200	216	100	0.1	0.5	
	60	625	250	268	0	0	0.0	
	61	625	250	252	50	0	0.0	
	62	625	250	266.5	75	0	0.0	
	63	625	250	249.5	100	0.1	0.5	
	64	750	300	305	0	0.1	0.5	
	65	750	300	300.5	50	0	0.0	
	66	750	300	314.5	75	0	0.0	
	67	750	300	308.5	100	0	0.0	
	68	875	350	358	0	0.1	0.5	
	69	875	350	355	50	0	0.0	
	70	875	350	370	75	0	0.0	
	71	875	350	379.5	100	0	0.0	
	72	1000	400	454	0	0	0.0	
	73	1000	400	456.5	50	0	0.0	
	74	1000	400	404	75	0	0.0	
	75	1000	400	413	100	0	0.0	
PRJ 008402	76	0	0	0	0	0.53	2.3	23.3%
	77	0	0	0	400	372	1596.6	
	78	0	0	0	600	631	2708.2	
	79	0	0	0	800	743	3188.8	
	80	500	200	173	400	0.32	1.4	
	81	500	200	183.5	600	0.45	1.9	
	82	500	200	168.5	800	0.93	4.0	
	83	750	300	232	400	0	0.0	
	84	750	300	254.5	600	0	0.0	
	85	750	300	252.5	800	0.14	0.6	
PRJ 008460	86	0	0	0	0	0.248	1.1	23.3%
	87	0	0	0	800	802	3442.1	
	88	0	0	0	1000	903	3875.5	
	89	250	100	70.6	900	79.2	339.9	
	90	250	100	65.2	1000	102.5	439.9	
	91	375	150	90.4	900	9	38.6	
	92	375	150	89.9	1000	17.9	76.8	
	93	500	200	152	900	0.7	3.0	
	94	500	200	128.5	1000	1.6	6.9	

Table 9. Results of trial PRJ-008460 supplemental study #2 (August 2012) with target SO2 levels of 0, 100, 150 and 200ppm with thiamine inclusion levels of 900ppm and 1000ppm at Day 1

DAY 1 RESULTS

Data point	Represents paired mean of samples	Target SO2 in meat (ppm)	Mean SO2 assay of sample pairs (ppm)	Thiamine added (ppm)	Mean Thiamine assay of sample pairs (ppm) on "as is" basis	Paired sample mean Thiamine (ppm) on "D.M" basis (23.3% DM basis)	Comment
1	1+2	0	0.0	0	0.4	1.6	negative control
2	3+4	0	0.0	900	755.5	3242.5	
3	5+6	0	0.0	1000	876.5	3761.8	
4	7+8	100	85.9	900	367.0	1575.1	
5	9+10	100	79.6	1000	408.0	1751.1	
6	11+12	150	127.5	900	209.0	897.0	
7	13+14	150	122.0	1000	141.8	608.4	
8	15+16	200	174.0	900	12.8	54.9	
9	17+18	200	156.5	1000	15.8	67.6	

Table 10. Results of trial PRJ-008460 supplemental study #2 (August 2012) with target SO_2 levels of 0, 100, 150 and 200ppm with thiamine inclusion levels of 900ppm and 1000ppm at Day 28

DAY 28 RESULTS

Data point	Represents paired mean of samples	Target SO2 in meat (ppm)	Mean SO2 assay of sample pairs (ppm)	Thiamine added (ppm)	Mean Thiamine assay of sample pairs (ppm) on "as is" basis	Paired sample mean thiamine (ppm) on "D.M" basis (23.3% DM basis)	Comment
28	55+56	0	0.0	0	0.248 (*)	1.1	* ONLY SINGLE SAMPLE INCLUDED
29	57+58	0	0.0	900	802.0	3442.1	
30	59+60	0	0.0	1000	903.0	3875.5	
31	61+62	100	70.6	900	79.2	339.7	
32	63+64	100	65.2	1000	102.5	439.9	
33	65+66	150	90.4	900	9.0	38.8	
34	67+68	150	89.9	1000	17.9	76.8	
35	69+70	200	152.0	900	0.7	3.2	
36	71+72	200	128.5	1000	1.6	6.7	

* Due to equipment failure at analytical lab, only one sample in this data set (sample 55) is included, so this figure does not represent a mean of paired sample.

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Building Confidence in Kangaroo Meat for Pet Nutrition

By Duncan Hall

Pub. No. 13/006

This project collates the data of this series of controlled studies, which explore the relationship between the addition of sulphites and thiamine in kangaroo meat. The addition of sulphites assists the preservation of kangaroo meat used for chilled kangaroo pet meat.

This information should be of interest to kangaroo pet meat products supply chain operators, veterinarians, and key industry stakeholders. This information should also be of interest to pet owners, with regard to the safety of feeding fresh kangaroo meat products to companion animals, and reducing the risk of adverse impacts to the health of pets due to thiamine deficiency.

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