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PERFORMANCE FEEDS

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MEAT & LIVESTOCK AUSTRALIA

For feedlot managers, feedmill operators and QA officers

Veterinary feed additive safety guidelines

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MLA acknowledges the Traditional Custodians of the lands on which we live, work and care for. We pay our respects to Elders past and present, as we recognise their history, culture, connection to land and water, and share in their commitment to caring for Country.

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1. Introduction

The guidelines are designed to provide a readily accessible reference source for the use of veterinary feed additives for participants within the feedlot supply chain. The information provided is relevant to feedlot managers, feedmill operators and feedlot Quality Assurance (QA) officer.

The information held within the guidelines can be used in a multiple of ways including as a reference, assist with reporting, contribute to the National Centre for Antimicrobial Stewardship (NFAS), provide framework for managing possible toxicity events, and assist with developing and implementing sample collection, storage and testing protocols.

2. Veterinary feed additive safety guidelines

2.1 Laboratory testing

Recommend contacting laboratory and confirm their confidence in testing veterinary feed additives, particularly when included in liquid supplements. Testing of ionophores in liquid supplements require additional sample preparation. Laboratories that do not routinely assess ionophores in liquid supplements will have low sensitivity (greater error) and poor recovery. A recovery of at least 90% is required in both finished feed and supplements (dry or liquid) to identify variation in concentration and/or cross contamination from veterinary feed additives.

2.2 Testing methods on-feedlot

2.2.1 Veterinary feed additive tests

The simplest on-feedlot testing is a dip test or coloured disc. These tests identify the presence of a feed additive but are not sensitive enough to determine concentration. They work by changing colour when the veterinary additive or group of additives is present. These tests are most useful for identifying cross-contamination when using in-feed medication.

Testing methods for identifying concentration of specific feed additives are more complicated and require greater sample preparation, involving multiple steps, calculations, and equipment (drying oven, reagents, filter paper). Commercially available methods and test kits include inhibitory analysis (Prem®Test) and binding reagent test (RIDASCREEN®).

2.2.2 Assessment of liquid supplement consistency

Benefit of on-feedlot sampling and assessment is greatest when results can be compared. The best comparison is using a sample taken directly from the delivery tanker known as a retention sample. Comparisons identify differences and these differences can be defined (acceptable or non-compliant). Retention samples should be taken from every delivery of liquid supplement, labelled (Date, Supplier, Liquid Supplement ID), and held in storage. Samples should be sufficient to undertake on-feedlot testing (500ml).

2.2.2.1 Bulk density

Bulk density uses the same method as assessing grain on receival. Bulk density defines the weight of a supplement sample within a known volume. Supplement manufacturers will know the expected bulk density of each supplement.

Require 500ml container or old half-litre grain measure and weigh scale. Place half a sheet of paper hand towel (protect scale from spillage, liquid supplements acidic) and container on scale. Tare scale to zero.

Fill container with liquid supplement. Record weight in grams. Bulk density = weight of liquid supplement, kg/ container volume, m^3. If liquid supplement weighed 615g, bulk density = 0.615/0.5 = 1.23kg/m^3 .Compare bulk density with retention sample or expected value from manufacturer and/or retention sample taken from delivery truck.

2.2.2.2 Brix level

Brix is the measure of molasses sugar content and dry matter. Liquid supplement manufactures measure Brix of raw molasses and may add water to reduce Brix to a desired level.

Brix is measured using a Brix sugar refractometer.

Brix values of supplement can be compared with retention sample collected from delivery tanker.

2.2.2.3 Viscosity

Viscosity is a measure of how resistant liquids are to flow. Viscosity can be measured manually, by timing the rate of descent of a weight such as a marble or a ball bearing through a column of supplement held in a glass measuring cylinder or measured using a Brookfield Viscometer. Important that viscosity is assessed at a constant temperature (warmer = less viscous, colder = more viscous).

2.2.2.4 pH

Supplement manufacturers use pH to determine manufacturing process and supplement stability. Supplement pH assessed using a pH probe. Diluting the supplement with 50% water can improve consistency of reading. Use pH probe calibration solutions (pH 4.0, 7.0, and 10.0) to ensure probe is reading accurately.

2.2.2.5 Visual

A visual assessment can be used for feedlots with supplement turnover rate of more than 30 days. Visual changes include colour and/or sediment accumulation. Samples of liquid supplement can be collected and stored in either a glass measuring cylinder or glass squib. This type of glassware provides calibrations for easy identification of changes throughout the sample.

2.2.3 Protocol for assessing mixing efficiency

A feed mixer test is completed on each mixer to assess the consistency of ingredient dispersion for a ration. The objective of the test is to determine the ability of a mixer to adequately mix a specific ration by assessing the presence of an ingredient or nutrient marker to calculate a coefficient of variation percentage (CV%). Target CV is less than 10%.

Requirements include:

- Mixer physical inspection:
  - Within mixing chamber.
    - build-up of material on mixer walls and mixer components
    - presence of foreign material (baling twine, netting, plastic)
    - broken, bent or missing mixer components
    - worn knives
    - worn mixer components.
  - External mixing chamber.
    - worn sections of wall
    - presence of rust
    - broken or worn load cell brackets and cables
    - loose hydraulic hoses or PTO shafts
    - loose conveyor chain or missing components
    - bent door, faulty operating arm
    - tyres and rims
    - leaf springs.
- Mixer operation:
  - Capacity (tonnes) for each ration.
  - Mechanical operation.
    - mixer component revolutions per minute – primary and secondary
    - define total mixer revolutions for each ration 10RPM per minute @ 1000RPM = 40 revolutions in 4minute mixing time
      RPM during loading/mixing, travel and delivery.
  - Mixing time (minutes) for each ration.

The sampling objectives are designed to reduce sampling bias and gain a representation of the complete quantity of feed mixed and delivered from first quantity to last. Collect ration samples in 10 x 20L buckets labelled 1 to 10.

The mixer load weight is calculated at the following percentages: 5%, 15%, 25%, 35%, 45%, 55%, 65%, 75%, 85% and 95% (e.g. 10t = 500kg, 1,500kg, 2,500kg, 3,500kg, 4,500kg, 5,500kg, 6,500kg, 7,500kg, 8,500kg & 9,500kg) to identify ration sample collection points.

To promote accuracy of collection, chalk can be used to mark points of collection from bunk to cover delivery of load.

Examples of suitable ingredient markers include whole corn, delinted whole cottonseed, sunflower seed or whole lupins. Ingredient used must not already be a diet component but can be consumed by cattle safely and without compromising food safety. Ingredient markers included at 0.2% inclusion or 20kg per 10,000kg load.

Once samples are obtained, the ingredient marker is separated and counted out from sample quantity. Placing sample on a clean flat surface (e.g. plywood sheet 1.2m x 1.2m) promotes ease of identification.

Once 10 samples are collected, the sample collector counts the individual ingredient markers in each sample bucket/bag, recording the number per bucket/bag to calculate the mixer coefficient of variation.

The feed ingredient marker is entered into an Excel spreadsheet for calculation of mean, standard deviation and co-efficient of variation (CV). The CV is a statistical term defining the level of variation from the mean or average of the samples counted. A coefficient of variation (CV) % of less than 10% is considered to be a homogenous diet mix.

Co-efficient of variation (CV) % = (standard deviation / mean) x 100.

An alternative method is to sample smaller quantities of ration (200-300g) as defined by bunk marks for external laboratory and nutrient marker validation. Examples of nutrient markers include zinc, calcium, or crude protein.

2.3 Feedlot veterinary feed additive safety guidelines

2.3.1 Interpreting labels and directions of use

Labels are provided on feed additive bags as described in **Figure 1**. Components of the label include:

- Signal heading (red circle).
  - » prescription animal remedy = schedule 4
  - » include other cautionary words or phrases
  - » direction to read directions before opening or using.
- Label displays the product name (Flaveco 40).
- Active constituent statement which includes name of constituent with concentration (purple circle).
- Statement of claims for use defining what it is for and what animal (green circle).
- Net contents statement (20kg NET).Directions for use (orange square).
  - » restraints
  - » includes the words DO NOT
  - » dosage and administration defined in table describing feeding rates for different livestock
  - » general directions
  - » these may include any other instructions or advice for the safe and effective use of the product (warnings, withholding periods, compatibility for mixing with other products).
- Withholding period statements and trade advice (black square).
  - » safety directions: provision of safety directions are a state and territory requirement under poison and pesticide legislation
  - » first aid instructions - specify initial action required to be taken to counteract the effects of exposure to the product
  - » disposal statements – provide advice on appropriate method of disposing unused product and used bags/containers
  - » storage statement – requirement to provide instructions for suitable storage.
- Contact details of the marketer (yellow rectangle) – requirement to provide name and address responsible for marketing of veterinary feed additive.
- APVMA label approval number (light blue rectangle), Expiry date and Batch number.

2.3.2 MRL, ESI and LD50

The current maximum residue limits (MRL), export slaughter intervals (ESI) and LD50 levels (oral lethal dose) at which mortality is 50% for veterinary feed additives is provided in **Table 1**.

Figure 1: Flaveco 40 bag label

DIRECTIONS FOR USE:
RESTRAINTS : DO NOT exceed recommended dose rates. DO NOT feed to any animal or bird not included in the label claims.
Beef Cattle: DO NOT USE for improvement of productivity, growth promotion o feed conversion efficiency on any cattle destined for the EU market.
Dairy Cattle: DO NOT USE in heifers or cows which are producing or may in the future produce milk or milk products which may be used or processed for human consumption.
DOSAGE AND ADMINISTRATION:
Mix thoroughly into the feed premix (vitamins, minerals and ingredients commonly used in commercial feeds), then add premix to bulk feed gradually and blend thoroughly.
Mix this preparation with all commercially available feeds at the following rates:

Animal Feed	Recommended inclusion of Flavophospholipol	Mixing rate of FLAVECO™40 per tonne of feed
Broilers: starter & finisher ration	1 - 2 ppm*	25 g - 50 g
Turkey: starter	2.5 ppm	62.5 g
finisher	1 ppm	25 g
Laying hens:	2 ppm	50 g
Piglets up to 25 kg	6 ppm	150 g
Pigs 25 kg or more	2 ppm	50 g
Beef Calves: milk replacer, starter	10 ppm	250 g
Beef Cattle	5 - 10 ppm	125 g - 250 g
IN SUPPLEMENTS:	Per head per day	Per head per day
Beef Calves: milk replacer, starter	10-20mg	0.25-0.5g FLAVECO™40
Beef Cattle	10-20mg	0.25-0.5g FLAVECO™40

1 ppm is equivalent to 1 g active/tonne.

GENERAL DIRECTIONS:
FLAVECO™40, an antibiotic developed exclusively for nutritional use as a feed supplement in animals. It has no therapeutic application in human and veterinary medicine.
FLAVECO™40 has the following benefits:
- no residue in animal tissues or organs as the product is not absorbed by animal digestive system.
- no metabolites are created as the product is excreted intact.
- no cross-resistance with other antibiotics has been found.
- the product can be mixed with all form of feeds.

COMPATABILITY:
FLAVECO™40 is compatible when mixed with feedstuffs, vitamins, minerals, amprolium, roxarsone, monensin, lasalocid, narasin, salinomycin and halofuginone.

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KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING
FOR ANIMAL TREATMENT ONLY

FLAVECO™ 40

FEED SUPPLEMENT

ACTIVE CONSTITUENT:
FLAVOPHOSPHOLIPOL 40 g/kg
FLAVECO™40 is a composition of dried strains of *Streptomyces* sp. mycelium and calcium carbonate.

For improvement of productivity by stimulating the growth rate and increasing feed conversion efficiency in poultry (broilers and turkeys), pigs, beef calves and beef cattle and increasing egg production in laying hens.

20 kg NET

®

WITHHOLDING PERIODS: MEAT: NIL, EGGS: NIL
Feed containing FLAVECO™40 may be provided to animals up to slaughter.
TRADE ADVICE:
Beef Cattle: Under EUCAS rules (in a segregated system), accredited cattle producers are not permitted to use this product for growth promotion or yield increase on animals destined for EU. Please consult the EUCAS rules for usage and documentation requirements.
EXPORT SLAUGHTER INTERVAL (ESI): This product does not have an ESI established. For advice on the ESI, contact the manufacturer on (02) 9672 7944 before using this product.

SAFETY DIRECTIONS:
The product will irritate the eyes and skin. Avoid contact with eyes and skin and do not inhale dust. When opening the container and mixing into feed wear cotton overalls buttoned to the neck and wrist, a washable hat, elbow-length PVC gloves, goggles and disposable dust mask. If product in eyes, wash it out immediately with water. After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves, goggles and contaminated clothing.
FIRST AID:
If poisoning occurs, contact a Doctor or Poisons Information Centre. *Phone Australia 131126; New Zealand (0800POISON) 0800764766.*
Additional information is included in the Material Safety Data Sheet (MSDS).
DISPOSAL:
Shake and empty contents into medicated feed. Do not dispose of undiluted chemicals on-site. Puncture or shred and bury empty bags in a local authority landfill. If no landfill available, bury the bags below 500 mm in a disposal pit specifically marked and set up for this purpose clear of waterways, vegetation and roots. Empty bags and product should not be burned.

STORE BELOW 30° C (Room Temperature) IN A WELL-CLOSED CONTAINER IN A DRY PLACE.
APVMA Approval Number : 54232
BATCH: ?????
EXPIRY: ??? 20??

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ECO ANIMAL HEALTH ®

Table 3: Veterinary feed additive toxic dose rates (LD50), export slaughter interval (ESI) and maximum residue limit (MRL)

Veterinary feed additive	Clinical signs of toxicity	LD 50* or reported toxicity	ESI Days	MRL mg/kg
Antibiotics				
Flavophospholipol (bambermycin)		>2,000mg/kg (mice, rat)	Nil	0.01 (fat, meat, kidney, liver)
Tetracyclines	The low oral absorption of both oxytetracycline and chlortetracycline makes oral toxicity highly unlikely (Riviere and Papich, 2009).	>5,000mg/kg (rat)		
Oxytetracycline (OTC)			5	
Chlortetracycline (CTC)		>2,314mg/kg (rat)	5 prophylactic 10 therapeutic	0.1(meat) 0.6 (kidney) 0.3 (liver)
Tylosin		600mg/kg (rat)	Nil	0.1 (meat, edible offal)
Virginiamycin		7,000mg/kg (mice, rat)	Nil	0.1 (meat) 0.2 (fat, edible offal)
Ionophores				
Monensin	- Scouring - Anorexia - Weakness, lethargy and possibly difficulty standing (ataxia) - Signs of respiratory distress (dyspnoea) - Recumbency and death (Potter et al, 1984)	26.4 mg/kg BW (Potter et al, 1984) For a 450kg steer = 11,880 mg At 25mg/kg dietary DM intake, and a DMI of 10kg/hd/d the intake would be 250mg/450kg = 0.6mg/kg. Therefore, the LD50 = 44 times the normal intake	Nil	0.05 (meat, edible offal)
Lasalocid	- Scouring - Anorexia (Galitzer, 1984) - Muscle tremors - Tachycardia - Rumen atony (Galitzer et al, 1986).	No deaths at 10mg/kg, deaths at 50mg/kg with severity and clinical signs (Galitzer 1984)	Nil	0.05 (meat) 0.7 (edible offal)
Salinomycin	- Dyspnoea - Exercise intolerance - Chronic cardiomyopathy (Bastianello et al, 1996)	90mg/kg diet DM resulted in the death of 8 out of 39 clinical cases out of a total of 380 cattle fed the TMR for 77 days. BW of the cattle was not reported (Bastianello et al, 1996)	Nil	0.05 (meat) 0.7 (edible offal)
Narasin	- Anorexia - Drooling and dysphagia - Lethargy, ataxia, and stiff gait - Possibly jugular pulse - Pulmonary oedema - Hydrothorax - Myopathy and cardiomyopathy	Approx. 15mg/kg (Wouters et al, 1997)	Nil	0.05 (meat, edible offal)

2.3.3 Calculations for addition of veterinary feed additives to finished feed

Accurately calculating concentrations of veterinary feed additives is critical to avoid toxicity events and ensure animals get the correct dose for a desired production/ health response as well as avoid risk of bacterial resistance.

Challenges for determining the required inclusion level includes:

- concentration level of veterinary feed additive
  - » descriptions may include a percentage (10%), a number (100) or a concentration (100g/kg or 100,000mg/kg)
- units
  - » concentrations are expressed as g/kg, mg/kg
  - » inclusion levels are expressed as kg/tonne
  - » ability to account for changes in units avoids errors and risk of over or under, including feed additives by 10, 100 or 1000 times
- dry matter of veterinary feed additive
  - » many additives are supplied in a dry form
  - » additives provided in a solution require adjustment to account for dry matter content
  - » determine whether stated concentration is provided on an as-fed or dry matter basis, ensure concentrations are compared on the same basis
- desired dose rate
  - » concentration in finished feed (mg/kg DM)
  - » daily requirement (mg/head/day)
  - » dose by body weight (mg/kg/body weight)
- average dry matter intake
- inclusion level of supplement in finished feed
- finished feed dry matter.

2.3.4 Veterinary feed additive records, storage, and safe handling

The National Feedlot Accreditation Scheme (NFAS) requires feedlots to ensure staff are competently trained in using veterinary products as well as establishing adequate storage, handling and disposal as described by product label. These requirements are referenced in elements QM5, FS2, FS3 and LM5 of the NFAS standards.

2.3.5 Prevention of cross contamination and mixer flush procedure

Cross contamination can occur within a mixer when finished feed containing a veterinary feed additive is inadvertently transferred to cattle that do not require medication. This can occur when carryover feed remains in the mixer or concentrated medication is loaded directly into the mixer rather than in a diluted premix form. Veterinary feed additives can also possess very small particle size, increasing surface area and resulting electrostatic charge. These particles can adhere to surfaces within the mixer.

Developing systems to eliminate or reduce the risk of cross contamination is critical for maintaining product integrity. Strategies which reduce risk of cross contamination include:

- Implementing dedicated batching and mixing equipment for feeding medicated feed
- cleaning protocols for equipment used for delivery of medicated feed
- implementing a flushing protocol

Addition of feed additive to finished feed:

Feed additive name	ZnMet50
Feed additive dry matter	98%
Feed additive concentration g/kg	50
Target level in finished feed DM	72.0mg/kg DM
Finished feed DM %	79.0%
Target level in finished feed as fed	= Target level x Finished feed DM
	= 72.0 x 79% = 56.88mg/kg
Inclusion level in finished feed	= 56.88 / additive DM x 1,000 / additive concentration
	= 56.88 / 98% x 1,000 / 50
	= 1,161mg/kg OR 1,161g/tonne OR 1.16kg/tonne

Flushing is the practice of cleaning and clearing equipment and systems after the addition of medicated additive. This may involve running a neutral product through the system to clear out any remnant ingredients and/or manufacture of a low-risk diet such as the starter ration.

- Controlled production scheduling:
  - » Group feeds with the same ingredients in succession.
  - » Schedule non-medicated feeds first, then move onto medicated feeds which reduces the number of flushes required.
- Quality control measures:
  - » Conduct routine testing of raw materials and finished products to detect contaminants or unwanted ingredients.
- Employee training:
  - » Train staff on the importance of cross-contamination and prevention methods including proper handling of veterinary feed additives.

2.3.6 Standard operating procedures

Standard operating procedures (SOP) are written instructions documenting a set of repeated activities, tasks or steps. Benefits of SOP include:

- minimise variation of outcome across different operators, increasing outcome quality
- generates consistent recording
- use as training tool.

An SOP for manufacture and mixing of a Macro is also included. A Macro is an on-site manufactured mineral supplement using ingredients readily available to the operation. Macro manufacture and use is a common strategy in Western Australia where many of the mineral components are mined on-site and/or are used in farming practices (limestone or lime sand, gypsum, potash, urea).

The macro uses a rolled grain, rolled lupins or canola meal as the carrier to support and promote mixing of the smaller inclusion ingredients. These components in combination with a purchased premix which contains ingredients less likely to be held on farm such as magnesium source and ingredients which are highly concentrated – such as trace minerals, vitamins, health and veterinary feed additives – are combined.

2.3.6.1 SOP Loading and mixing dry supplement

Purpose: Ensure accurate loading and even distribution of the dry supplement (meal or pellet) during ration manufacture and delivery.

Scope: Ration manufacture.

Procedures

1. Assess quality of stored dry supplement: ammonia smell, fines, separation of components, clumping, moisture exposure, colour change.
2. Identify appropriate ingredient loading order for the mixer in use.
3. Load dry meal or pellet supplement when loading of processed grain complete – provides similar particle size and density, facilitate distribution and reduce separation.
4. Load dry meal or pellet supplement on a weight basis.
5. Load dry meal or pellet supplement accurately, ±5.0kg. It is the most concentrated ingredient on hand.
6. Dry meal or pellet supplement formulated for inclusion is sufficient to promote distribution and creation of homogenous finished feed.
7. Ration uniformity minimises separation and promotes nutrient consistency.
8. To meet mixing objectives, each ration is mixed for a set period (minutes/revolutions) after the last ingredient has been loaded.
9. Assess mixing time by running mixing efficiency assessment for each ration.
10. Use Mixing Efficiency Protocol to compare mixing times.
11. Mixing efficiency assessment ensures each ration for each mixer type is not over or under mixed.

2.3.6.2 SOP Loading and mixing liquid supplement

Purpose: Ensure accurate loading and even distribution of the liquid supplement during ration manufacture and delivery.

Scope: Ration manufacture.

Procedures

1. Identify appropriate ingredient loading order for mixer in use. Liquids are generally loaded last except for horizontal reel where loading can be first.
2. Load liquid supplements on a weight basis. Do not use a volume measure such as a flow meter. Density (tonnes/m^3) of molasses-based liquid supplements can vary.
3. Load liquid supplement accurately, ±5.0kg. It is the most concentrated ingredient on hand.
   Small errors in loading can result in large changes in nutrient content of final ration.
4. Objective of mixing is to promote even distribution of ingredients which vary in physical characteristics to create a homogenous (uniform appearance and composition) ration.
5. To meet mixing objectives, each ration is mixed for a set period (minutes/revolutions) after the last ingredient has been loaded.
6. Assess mixing time by running mixing efficiency assessment for each ration.
7. Use Mixing Efficiency Protocol to compare mixing times.
8. Mixing efficiency assessment ensures each ration for each mixer type is not over or under mixed

2.3.6.3 SOP Manufacture and mixing of a macro

Purpose: Ensure accurate loading and even distribution of ingredients during mixing of MACRO and avoid separation during MACRO storage.

Scope: MACRO manufacture.

Procedures

1. Ensure mixer is completely empty.
2. Ensure sufficient ingredients and quantities for MACRO are on hand to complete manufacture of the desired tonnage quantity.
3. Load ingredients of MACRO in the following order:
   i) carrier – cracked grain, lupins or protein meal
   ii) vegetable oil
   iii) limestone
   iv) MICRO – contains trace mineral and vitamins, veterinary feed additives, other additives, mineral not accessible on farm, e.g. magnesium oxide
   v) urea
   vi) Gypsum
   vii) salt
   viii) potash.
4. Loading order is designed to promote homogenous MACRO by combining ingredients with similar particle size and density, load in order of inclusion, from highest to lowest.
5. Load accurately, +5.0kg.
6. Request MICRO manufacturer provide in bag a batch quantity. For example, if standard manufactured MACRO quantity is 5t and MICRO is included at 10%, then manufacturer provides MICRO in 500kg bulker bag.
7. Use MICRO bag per batch to assess mixer scale accuracy.
8. The mineral ingredients of MACRO provide fine particle size and high density.
9. Objective of mixing is to create uniformity and minimise separation.
10. Mix MACRO for 12 minutes after the last ingredient has been loaded.
11. Ration uniformity minimises separation and promotes nutrient consistency.
12. Remove 10 small 100g samples from manufactured MACRO to count prilled urea particles to assess mixing efficiency, targeting a CV of no greater than 10% (see mixing efficiency protocol).
13. Halve the inclusion of MACRO when loading ingredients for first ration following MACRO manufacture.

2.3.6.4 SOP Sampling and testing dry supplement

Purpose: Collect a representative sample of dry supplement (meal or pellet) for nutrient analysis.

Scope: Ingredient quality.

Procedures

1. Dry supplement (meal or pellet) sampling can occur at two locations:
   a. At receival – collect samples directly from delivery transport.
   b. From feedlot storage – bulk bay or bin.
2. At receival, request the driver fills two 200ml volume plastic jars with screw top lids of dry supplement.
3. Label jars with Date, Feedlot Name, Ingredient and Sample identification (1 or 2).
4. Store samples on shelves or in a carboard in a storage room protected from direct sunlight.
5. When collecting samples from feedlot storage it is critical that samples are representative of the quantity stored.
6. Collect ten grab samples from multiple locations of bulk quantity and drop in 10L bucket.
7. Mix samples collected in bucket with hand.
8. Take two 200ml samples from the bucket and store in plastic jars with screw top lids.
9. Label jars as described in Procedure #3.
10. Store sample jars as described in Procedure #4.
11. Send both (duplicate) dry supplement samples collected to a reputable laboratory for analysis.
12. Request samples NOT be analysed using NIRS.
13. Request the following nutrient analysis: Dry matter%, Crude protein%, Ionophore mg/kg, Calcium g/kg, Magnesium g/kg, Zinc mg/kg and Copper mg/kg.
14. Compare the analysis results on a dry matter basis with theoretical nutrient values provided by the feedmill or nutritionist.
15. The difference between the two duplicate samples identifies laboratory error, indicating accuracy and sensitivity of laboratory methodology.

2.3.6.5 SOP Sampling and testing liquid supplement

Purpose: Collect a representative sample of liquid supplement for nutrient analysis.

Scope: Ingredient quality.

Procedures

1. Liquid supplement sampling can occur at two locations:
   a. At receival – collect sample directly from delivery transport.
      Assess nutrient levels across multiple loads to compare with theoretical formulated values.
   b. From feedlot storage – bulk storage tank.

Identify specific issue with load, storage period or supplement management.

2. At receival, request the driver fill two 200ml volume plastic jars with screw top lids with liquid supplement.
3. Label jars with Date, Feedlot Name, Ingredient and Sample identification (1 or 2).
4. Store samples on shelves or in a carboard in a storage room protected from direct sunlight.
5. When analysis is required, multiple samples across period relevant to specific supplement formulation can be combined in a 4L bucket, by emptying approximately half the sample from each jar.
6. Combined samples are stirred in the bucket.
7. Remove sufficient quantity to fill two 200ml plastic jars with screw top lids.
8. Label jars as described in Procedure #3.
9. Place jars in zip lock plastic bag for postage.
10. Collect supplement samples from the tank in use. If the feedlot has multiple tanks, the tank in use should be the only tank with open taps.
11. Fill two 200ml plastic jars with screw top lids from bleed valve or from receival line.
12. Label jars as described in Procedure #3.
13. Store sample jars as described in Procedure #4.
14. Place jars in zip lock plastic bag for postage.
15. Send both duplicate liquid supplement samples collected to reputable laboratory for analysis.
16. Request the following nutrient analysis: Dry matter%, Crude protein%, Ionophore mg/kg, Calcium g/kg (suspension supplement), Magnesium g/kg, Zinc mg/kg and Copper mg/kg.
17. Compare analysis results on a dry matter basis with theoretical nutrient values provided by the feedmill or nutritionist.
18. The difference between the two duplicate samples identifies laboratory error, indicating accuracy and sensitivity of laboratory methodology.

2.3.6.6 SOP Sampling and testing finished feed

Purpose: Collect a representative sample of finished feed for nutrient analysis.

Scope: Ration quality, integrity and safety.

Procedures

1. Collect defined ration from at least five different locations (random pens) around the feedlot.
2. Sample ration from the bunk using full hand grabs and drop into a 10L bucket.
3. With all samples collected in bucket, lightly mix ration by hand.
4. From bucket remove approximately a 300g sample and place in plastic sealable bag.
5. Where possible, use the sample bags provided by laboratory.
6. Before placing 300g sample in the bag, fill out required information on the bag.
7. Minimum requirements include date; feedlot name; ration type.
8. Store collected samples in the fridge until ready for postage.
9. At postage, pack samples within a postage box, ensuring appropriate and completed paperwork is included.
10. Ensure type of analysis required is identified and contact information of feedlot.

2.3.7 Managing suspected toxicity event

Beef cattle have considerable tolerance to overdosing of S4 antibiotics, but this is not the case with ionophores, as described in Table 1. The clinical signs and pathology of a toxicity event are similar across all registered ionophores.

Initial signs of a possible ionophore toxic consumption in cattle is depressed feed intake. Prolonged exposure may result in the following clinical signs:

- scouring
- anorexia
- weakness, lethargy and possible difficulty standing (ataxia)
- elevated rate and laboured breathing
- recumbency and death.

List of actions if feedlot suspects an ionophore toxicity event:

- Remove feed from affected pens.
- Avoid moving affected cattle.
- Cease feeding the supplement to all cattle.
- Consider flushing feed mixers with a combination of milled grain and straw/hay to remove any remaining contaminated feed.
- Consider feeding finisher cattle a heat load ration or alternative intermediate diet (higher roughage).
- Contact feedlot veterinarian.
- Contact feedlot nutritionist.
- Review requirements and actions defined by LM8 – Livestock Incident Reporting within the Feedlot NFAS QA manual and follow instructions provided by Trigger Level 2, even if morbidity / mortality levels have not exceeded trigger levels.
  - » Veterinarian to undertake postmortem observation and diagnostic sampling of tissue.
  - » Notify ALFA of incident within 12 hours.
  - » Maintain a diary of observations, actions and deaths.
  - » Provide required information to ALFA as described by LM8.
- Contact processor and notify cancelation of exits.
- If possible, delay arrival of purchased cattle.
- Contact supplement manufacturer to request availability for testing of retention samples of suspected load.

The effects of an ionophore toxic event can extend for several days after the initial episode. Morbidity and mortalities can continue even after the suspected feed has been removed or suspect supplement removed.

Sampling and testing are a key component to assist with identifying the cause and understanding the event.

Recommended sampling includes:

- Collect samples of the supplement currently in use (see SOP for supplement sampling)
  If the supplement is liquid, remove samples from top and bottom of tank to identify if ionophore has separated.
  - » Consider testing for all ionophores used by supplement manufacturer to determine cross contamination.
  - » Send all supplement samples in duplicate.
    - › Collect samples of rations from affected pens (see SOP for sampling finished feed).Collect samples from multiple sites as the ionophore concentration may be unevenly distributed due to compromised equipment or mixing.
  - » Send all ration samples in duplicate – for each point at which samples taken, take two (2) samples.
- Post-mortem diagnostic samples (with veterinary presence)
  - » Undertake feedlot SOP for post-mortem examination and tissue sampling.
  - » Formalin-fixed tissues for histopathology: heart, lung, skeletal muscle, liver, rumen
  - » Gastrointestinal content and liver for ionophore residue analysis to determine what quantity of ionophore the animal ingested, whether the type of ionophore detected matches the type of ionophore fed and to determine the presence or absence of other ionophores that should not have been present in the feed.
  - » Liver ionophore concentrations are the most reliable diagnostic measure.
  - » Require a 2cm x 2cm liver sample. Place in yellow-top container. Label container with appropriate information (Date, Feedlot, Sample, Animal ID, Pen, DOF, Name of person sampling).
- Live animal diagnostic samples:
  - » Serum/plasma for clinical chemistry: creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP)

2.3.8 Antimicrobial use and surveillance

Antimicrobial reporting of use and surveillance of resistance in agricultural livestock is critical to:

- maintain effectiveness of "last line" antibiotics
- minimise transfer of resistance to other bacteria
- avoid creating multi-resistant bacterial strains such as Staphylococcus aureus (MRSA)
- maintain functionality and availability of antibiotics for disease control in both humans and livestock.

In Australia, antimicrobial resistance surveillance occurs in two areas:

1. Bacteria that effect health of cattle:
   Primary focus on bacteria involved in the bovine respiratory disease (BRD) complex. Specific bacterial include Mannheimia haemolytica, Pasteurella multocida and Histophilus somni.
2. Bacteria which infect food and effect human health:
   Primary focus on E.coli, Enterococcus, Salmonella and Campylobacter species.

In Australia, antimicrobials for use in feedlots can only be provided through prescription from a veterinarian. Veterinarians implement Prudent Use strategies when determining appropriate treatment regime. Prudent use strategies include identifying appropriate antibiotic for diagnosis, treatment program, dose rate and method of administration. They alsodefine WHP and ESI, and include review and monitor outcomes of treatment strategies.

Within the feedlots, antimicrobial use is also supported by the industries QA program NFAS. The NFAS standards include two elements concerning antimicrobial use:

- Food Safety Management Element 2
  - » Safe and Responsible Animal Treatment Systems have been implemented to ensure that animal treatments are stored and administered in a safe and responsible manner to minimise the risk of chemical residues and physical hazards intended for human consumption.
- Livestock Management Element 5
  - » Animal Health Systems have been implemented to ensure the health of livestock is routinely monitored and maintained whilst ensuring the judicious use of antimicrobials.

Australia does not have a national surveillance program for monitoring antimicrobial resistance, but relies on periodic surveillance with the objective of identifying presence and trends of antimicrobial resistance.

Antimicrobial surveillance focuses on bacteria which are present in livestock (faecal, caecum or carcass) pose a threat to human health by transfer along the food chain and provide indicator to the presence of resistance or resistant gene as described in **Table 2**.

Table 2: Bacteria selected for surveillance of antimicrobial resistance in cattle (Webber & Valois, 2003)

Pathogens	Zoonotic	Commensal or indicator
Pasteurella spp (gram -ve)	Salmonella spp	Escherichia coli
Haemophilus somnus (gram -ve)		Enterococcus faecium
Staphylococcus aureus (gram -ve)		Enterococcus faecalis
Streptococcus agalactiae (gram -ve)		Enterococcus hirae
Streptococcus uberis (gram +ve)		

Antimicrobial resistance is assessed across a specific range of antimicrobials (Webber and Valois, 2003), including:

- Aminoglycosides
- amphenicols
- beta-lactams
- cephalosporins
- glycopeptides
- lincosamides
- macrolides
- quinolones
- streptogramins
- sulfonamides
- tetracyclines.

The antimicrobials of interest to beef cattle are those that have been rated as high importance and include the cephalosporins (ceftiofur antibiotics – Excenel and Excede) and streptogramins (virginiamycin). The other high importance antibiotic is Polymyxin B, used to treat eye infections. Polymyxin is a last line treatment option for control of multi-resistance gram positive bacteria.

Although the ceftiofur and virginiamycin are not currently used in human health, both risk potential to select resistance across groups of antimicrobials. All antimicrobials defined as high importance are classified as Schedule 4 and require a veterinary prescription for use. Off-label use is strictly prohibited.

The outcomes of the periodic surveillance conducted to determine antimicrobial resistance level, and trends are described in **Table 3**. Results show that bacterial isolates resistant to highly important antimicrobials are low and remaining low.

The survey results show there is limited evidence of production practices such as grain feeding which increases risk of antimicrobial resistance. The ceftiofur and virginiamycin are the only antimicrobials likely to cause resistance and be a public health risk. However, any use of antimicrobials poses a risk to the development of antimicrobial resistance with the ability of bacteria to acquire and/or transfer antimicrobial mechanisms.

Table 3: Survey summary of antimicrobial resistance to bacterial isolates in beef cattle

Year	Bacterial isolates	Head#	Resistance %	Conclusion
DAFF 2007	E.coli Enterococcus	194	Both isolates: 1% Florfenicol and Tetracycline 9.5% Erythromycin and Virginiamycin	Low resistance to high importance antimicrobials
Barlow et al 2015	E.coli Salmonella	910	Nil resistance identified	As above
Barlow et al 2017	Enterococcus	910	80.2% Flavomycin 94.2% Lincomycin	High resistance to antimicrobials not critically important Low resistance to high importance antimicrobials
Barlow et al 2020	E.coli Salmonella Enterococcus	591	3.6% Ceftiofur 0.2% Ceftiofur 10% Virginiamycin	Low resistance to high importance antimicrobials

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4. Appendix

4.1 Feedlot liquid supplement storage management and use

A survey of Australian feedlots was conducted to identify how liquid supplements are managed at the feedlot. Lot feeders were asked to take five duplicate samples from their storage tank during use at 95%, 75%, 50%, 25% and 5% of tank capacity. An additional duplicate sample was taken directly from the delivery tanker to provide a reference level.

The key storage and management practices which influence supplement integrity and consistency are outlined below. Monensin concentration (mg/kg DM) was used to quantify the effects.

Key takeaways

- Greatest variability occurs when tanks are refilled before being completely emptied or when multiple tanks are used without full clean-out.
- Shared transfer lines can alter concentration through product carryover.
- Short-term storage (≤3 weeks) has minimal impact on concentration when supplements are managed correctly.
- To maintain consistent monensin delivery, tanks should be fully emptied where practical, regularly cleaned, and dedicated transfer systems used where possible.

Scenario	Key observation	Impact on monensin concentration
Flat bottom tank	Tank refilling occurs before complete emptying. Monensin concentration decreases immediately after refill and increases as the tank empties.	Variable concentration (+100 mg/kg DM range observed).
Multiple tanks in use	Residual supplement remains in tanks or tanks are not fully emptied/cleaned before refilling.	Progressive increase in concentration over time due to accumulation.
Shared delivery	Different liquid products (e.g. water, molasses, glycerol, vegetable oil or liquid supplements) are delivered through the same line. Variations in density and viscosity create carryover effects.	Moderate variation in concentration within the tank.
Usage within three weeks	Suspension supplements remain relatively stable when used within three weeks of delivery.	Minimal change in concentration, remaining within specification.

Liquid supplement storage

Cone bottom tank

Fitted with a shut-off valve, an isolation valve and a sampling outlet

Flat bottom tank

Not suitable – Outlet on the side of the tank; therefore, the supplement does not empty evenly, resulting in accumulated residues

Multiple tanks

Fully empty one tank before re-filling

Single tank

Flush cone annually with water

Multiple liquid ingredients

Water, vegetable oil, molasses, liquid supplement, glycerol

Shared delivery line

Increase shrink

Separate delivery lines for each ingredient

Turnover period

More than three weeks

Mix by recirculation, avoid prolonged mixing or aeration, regularly check supplement consistency, and reformulate for higher inclusion if required

Within three weeks

No additional management needed

Reliable and stable supplement

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