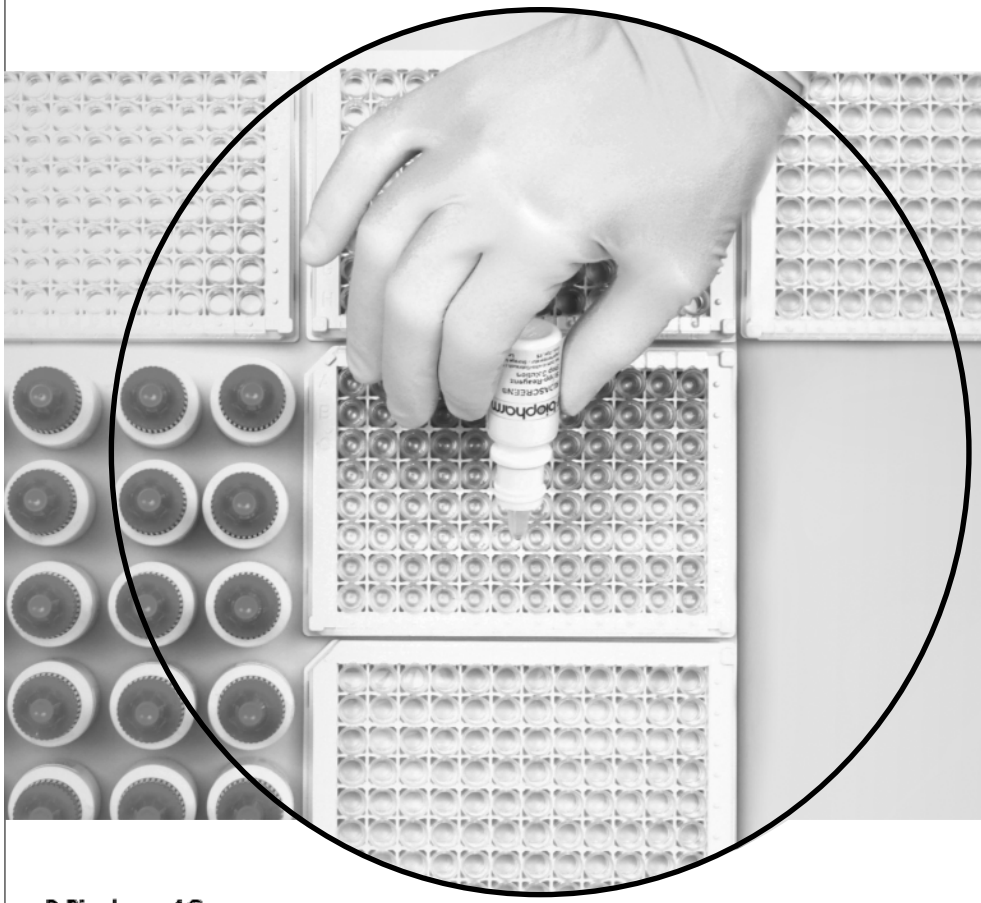

MLGA (MGA) in Feed

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MLGA

RIDASCREEN MELENGESTROL ACETATE (MLGA)

- Competitive Enzyme Immunoassay
- Sample Extraction: Homogenize, Dilute, Centrifuge, Solid-phase Cleanup- (Approx 2.5 hours per sample.)
- Incubation time 2.5 hours
- Cross-Reactivity: Melengestrol Acetate 100%, Megestrol Acetate 10%, Medroxyprogesterone Acetate 6.6%,

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Overview

- **Synthetic gestagens:** Used for estrus inhibition or synchronization.
- **Melengestrol acetate** belongs to the most active synthetic gestagens.
- **Oral bioactivity:** 10 to 100 times higher than gestagens chlormadinone acetate (CMA) or medroxyprogesterone acetate.
- **MLGA** is a licensed growth promoting feed additive for heifers in the USA and Canada.
- **Admitted dose** is 0.5 mg per day and head, given as a feed premix.
- **Mode of anabolic action** is unclear, but it stimulates the ovarian synthesis of the endogenous anabolic steroid estradiol which may have androgenic side effects.
- **Parenterally,** hormonal activity of MLGA is still 125 times higher than that of progesterone.
- **Strongly lipophilic**, MLGA is accumulated in fat 200x higher than in blood plasma.
- **European Union:** Use of sexual hormones for growth promoting purposes is generally forbidden since 1988, as well as the import of meat from hormone treated cattle.

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Extraction for Feed and Premixes

- Homogenize approximately 25 g of feed sample.
- Transfer 1.0 g of the homogenized sample into a glass tube.
- Add 7.0 mL of 80 % methanol in distilled water.
- Mix for 30 sec using a vortex.
- Mix for another 10 min using a shaker.
- Add 4.0 mL of distilled water and mix again for 30 sec using a vortex.
- Centrifuge sample for 6 minutes at 4000 rpm.
- Transfer 5.5 mL of the clean extract onto an activated C18 cartridge.
- Conduct the solid phase clean-up procedure

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Solid Phase Clean-Up

- Activate solid phase cartridges (C18, R-Biopharm Cat. No. R2002)
  -wash the cartridges subsequently with 5 mL MeOH 2x and with 5 ml 50 % MeOH in distilled water two time.
- Transfer 5.5 mL of feed sample extract onto activated solid phase cartridge.
- Wash the cartridges using 7.0 mL 50 % methanol in distilled water.
- Dry the cartridges under vacuum.
- Elute using 1 ml 80 % methanol in distilled water with a speed of 1 mL per min.
- Dilute extract 1+1 with distilled water.
- Use 20 µL per well in the assay.

Depending on the MGA concentration in the sample, further dilutions may be necessary. In this case use methanol/distilled water (40/60 v/v) for all further dilutions (i.e. always maintain 40% methanol

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Test Procedure

• Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate.
• Add 20 µL of each standard solution or prepared sample to separate duplicate wells.
• Add 50 µL of diluted enzyme conjugate to each well.
• Add 50 µL of the diluted anti-MLGA antibody solution to each well. Mix and incubate 2 h at room temperature.
• Pour the liquid out of the wells and rinse 3x with wash solution.
• Add 50 µL of substrate and 50 µL of chromogen to each well. Mix and incubate for 30 min at room temperature in the dark.
• Add 100 µL of the stop solution to each well.
• Mix and measure the absorbance at 450 nm.

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Results:

Initial studies were performed using feed and premix samples provided by SDSU.

Subsequent data was created with a commercially available feed, with and without addition of a premix. LOD was calculated using this feed only.

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MGA	Claim (ppb)	Spike added (ppb)	Result	Dilutions	RPD & spike recovery
09F-04167A	2200		3743	(1:1250)	4.1
09F-04167B	2200		3594	(1:1250)	
09F-04167C (spike)	2200	2000	5261	(1:1250)	94%
09F-04167D (spike)	2200	2000	5358	(1:1250)	
09F-03896A	882				
09F-03896B (spike)	882	1000			
08S-20786A	881		824	(1:625)	14.6
08S-20786B	881		712	(1:625)	
08S-20786C (spike)	881	1000	1540	(1:625)	91%
08S-20786D (spike)	881	1000	1661	(1:625)	
Steak-Maker (40-28) A	880		364	(1:625)	8.3
Steak-Maker (40-28) B	880		335	(1:625)	
Steak-Maker (40-28) C (spike)	880	1000	965	(1:625)	73%
Steak-Maker (40-28) D (spike)	880	1000	1010	(1:625)	
11 ppm Spiking Solution	11000		11190	(1:6250)	10.7
12 ppm Spiking Solution	11001		10050	(1:25000)	
Premix A	220000		296800	(1:125000)	2.5
Premix B	222000		289600	(1:125000)	
Premix C Spike	220000	110000	376000	(1:178600)	97%
Premix D Spike	222000	110000	403857	(1:178600)	

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SDSU Summary:

- **RPD (Range):**
  2.5-14.6
- **RPD (Mean):**
  8.0
- **Percent Recovery (Range):**
  73%-97%
- **Percent Recovery (Mean):**
  89%

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Sample	Claim	Dilution	Replicates	Results	
With MLGA	**880 ppb**	1:625	20	Mean	**845.35**
				SD	47.27
				CV	5.59
No MLGA	**0 ppb**	1:4	20	Mean	**0.62**
				SD	0.03
				CV	5.52
				LOD	**0.72**
Premix	**220000 ppb**	1:125000	10	Mean	**228730.00**
				SD	3369.82
				CV	1.47

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Heifers
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Melanie Hageleit, Andreas Daxenberger and Heinrich H.D. Meyer
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Thank you for your attention!