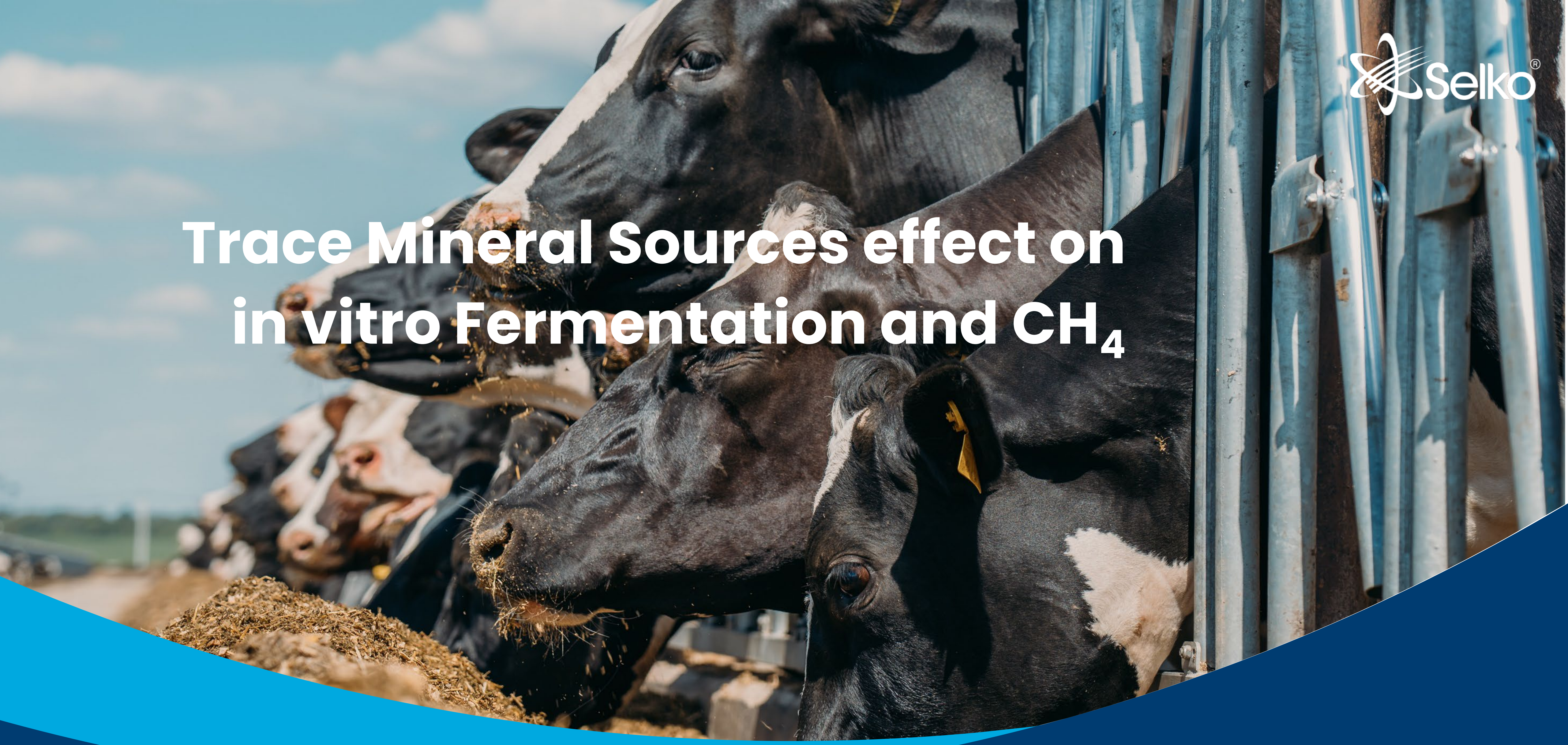
Trace Mineral Sources effect on in vitro Fermentation and CH_4



Carlyn Peterson

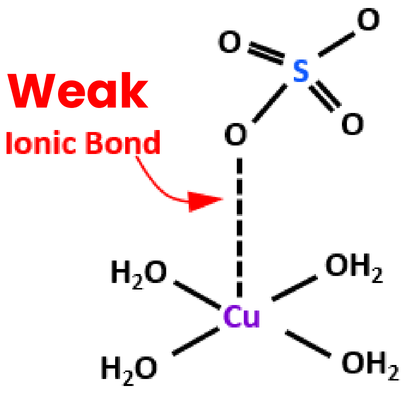
DairyNutriVision, September 10, 2024

Background: Trace Mineral Sources

Improved Trace Mineral Sources

INORGANIC

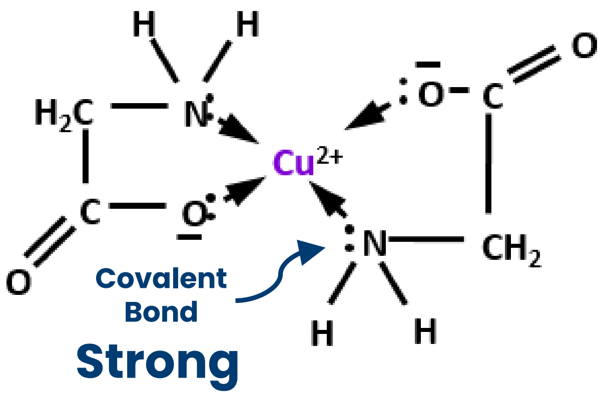
Sulfate



A specific metal bound to a non-carbon containing sulfate ligand.
Developed in the 1930's

$CuSO_4$; $ZnSO_4$; $MnSO_4$

Organic

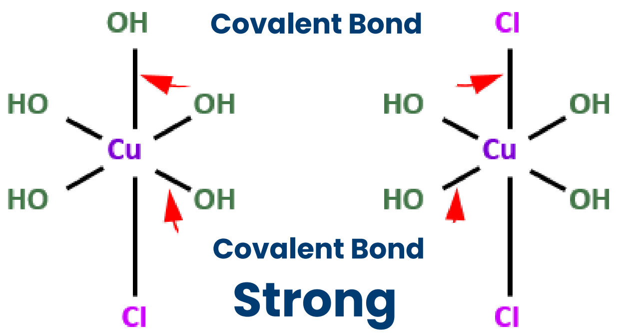


A specific metal bound to a carbon/nitrogen-containing ligand.
Developed in the 1970's

ZnAA; CuProteinate; ZnPolysaccharide

INORGANIC

Hydroxy



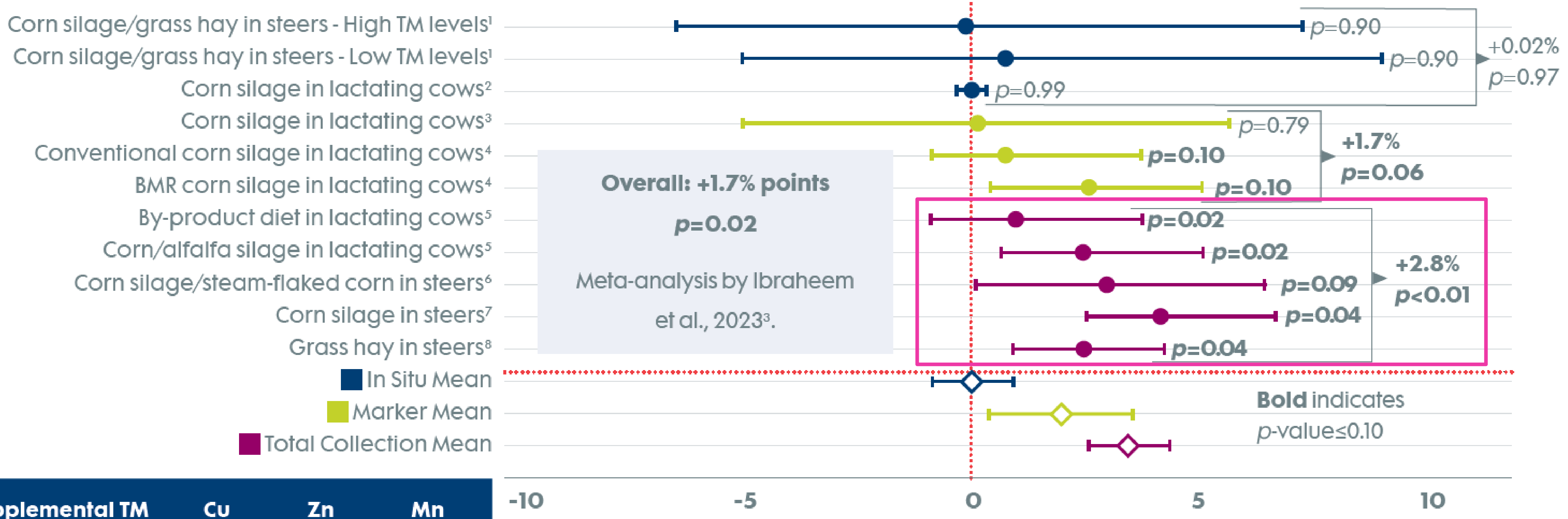
A specific metal bound via a coordinated covalent bond with a hydroxyl ligand.
Developed in late 1990's

IntelliBond C^{II} - $Cu_2(OH)_3Cl$
IntelliBond M - $Mn_2(OH)_3Cl$
IntelliBond Z - $Zn_5(OH)_8Cl_2 \bullet (H_2O)$

IntelliBond Meta-analysis: NDF Digestibility

Results

The meta-analysis found that feeding IntelliBond trace minerals increased NDF digestibility by 1.7 percentage points compared to animals fed sulfate trace minerals (*p* =0.02).



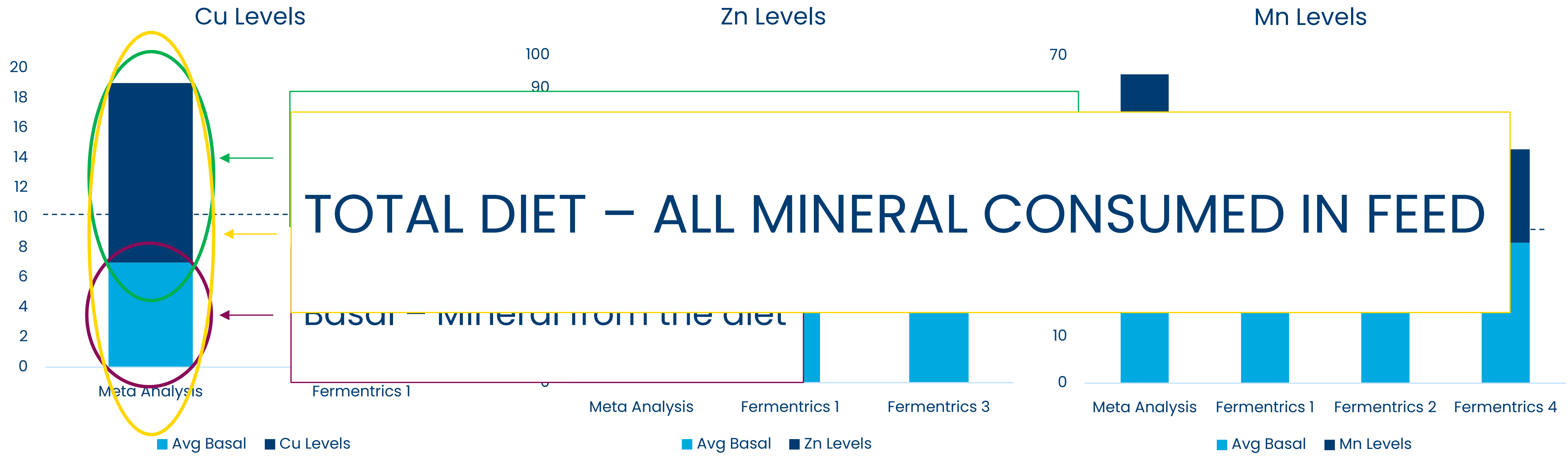
Supplemental TM	Cu	Zn	Mn
Average ppm	12	56	36

[1] Spears et al., 2004. Anim. Feed Sci. Technol. 116:1-13
[2] Shaeffer et al., 2017. Anim. Feed Sci. Technol. 232:1-5
[3] Ibraheem et al., 2023. J. Dairy Sci. 106, no. 4:2386-2394
[4] Genther and Hansen. 2015. J. Dairy Sci. 98:566–573
[5] Micronutrients Trial #2017D103CACZM
[6] Micronutrients Trial #2017D123USCZM
[7] Miller et al., 2020. J. Dairy Sci. 103:3147-3160
[8] Faulkner and Weiss. 2017. J. Dairy Sci. 100:5358-5367
[9] Caldera et al., 2019. J. Anim. Sci. 97:1852-1864
[10] Guimaraes et al., 2020. J. Anim. Sci. MWASAS Abstract #226
[11] Guimaraes et al., 2021. J. Anim. Sci. 99:1-7
[12] Souza et al., 2016. J. Dairy Sci. Vol. 99 E-Suppl 1.
[13] Yasui et al., 2014. J. Dairy Sci. 97: 3728-3738

Total Diet Mineral Levels

--- NASEM Guidelines

Cu	10
Zn	66
Mn	31



Aim of the studies:

Evaluate the effects of different trace mineral sources on 48h in-vitro fermentation

- All minerals (copper (Cu), zinc (Zn), and manganese (Mn))
- Zinc and Manganese separately
- Effects on Methane

Multi-trial assessments:

- **Trial 1: All IntelliBond vs Sulfates**
  - inclusion of copper, zinc, and manganese comparing sulfate to IntelliBond.
- **Trial 2: Mn Sources**
  - inclusion of Mn from Mn oxide, Mn sulfate, Optimin Mn, and IntelliBond M.
- **Trial 3: Zn Sources**
  - 7 different sources of Zn were tested: Control (no TM), Zn oxide, Zn sulfate, IntelliBond Zn, Vistore Zn, Availa Zn, and Mintrex Zn.
- **Trial 4: Expanded Mn Sources**
  - 7 different sources of Mn were tested: Control (no TM), Mn oxide, Mn sulfate, IntelliBond Mn, Vistore Mn, Availa Mn, and Mintrex Mn.

Study Objective Trial 5:

Objective: Understand the effects of Hydroxy Trace Mineral (HTM) source on rumen fermentation characteristics including CH_4.

Multi-trial assessment:

- **Experiment 1: CH_4 Experiment – IntelliBond® vs Sulfates**
  - Control (no TM) plus inclusion of Cu, Zn, and Mn, individually and in combination, comparing sulfate to IntelliBond.
- **Experiment 2: OHTM Experiment**
  - Control (no TM) plus inclusion of Cu, Zn, and Mn in combination from: sulfate, IntelliBond, Nutrilock Chemlock, Phibro Vistore, SAM Nutrition, Orffa Excential Smart, EcoTrace Glycinate.
- **Experiment 3: Zn Sources**
  - Control (no TM), Zn oxide, Zn sulfate, IntelliBond Zn, Oxide Zn (@ level of Oxide in NL Low Hydroxy), Oxide Zn (@ level of oxide in NL High Hydroxy), Chemlock Zn (with low Hydroxy Zn), Chemlock Zn (with High Hydroxy Zn).

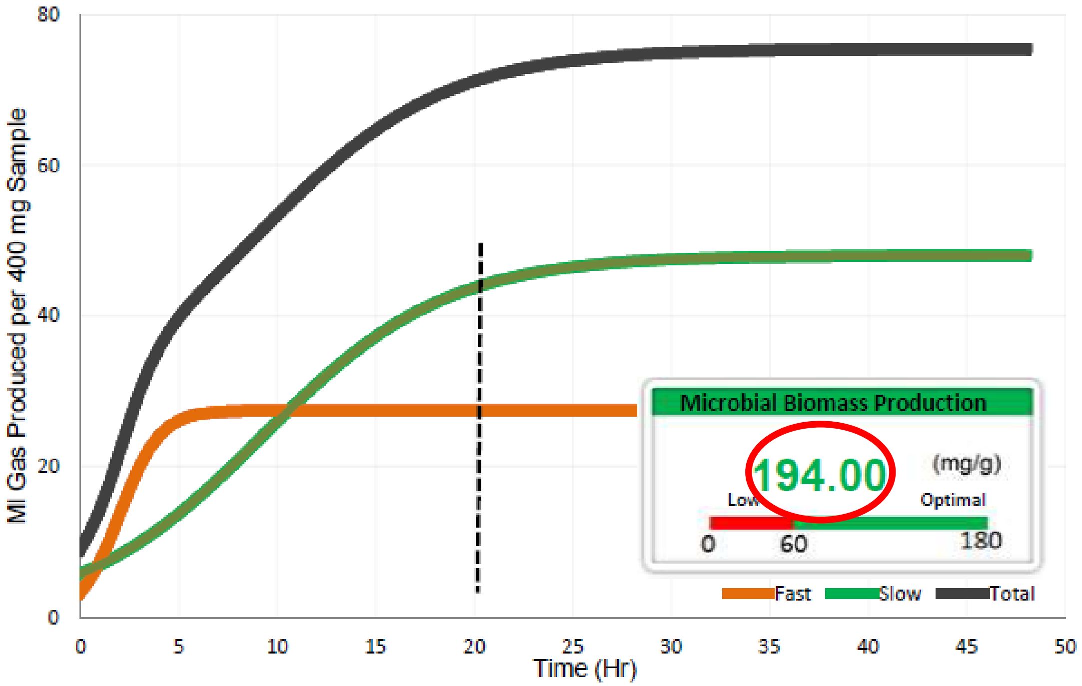
Example Fermentrics Output

Fermentrics™
Gas Fermentation System

Manganese Trial

Description: Intel Mn
Sample #: Selko

Origin: Selko
Run Date: Feb 4 2023



Digestion Rates

Fast Pool (Kd/hr)	24.35
Slow Pool (Kd/hr)	5.40
C:B1 (Kd/hr)	22.71
C:B3 (Kd/hr)	5.40

Relative Pool Contributions

	ml gas	% of total
	27.42	36.35%
Slow Pool	48.01	63.65%
2-Pool Total	75.43	

Relative Times to Max Rate

	Max (hr)
Fast Pool	2.50
Slow Pool	9.50

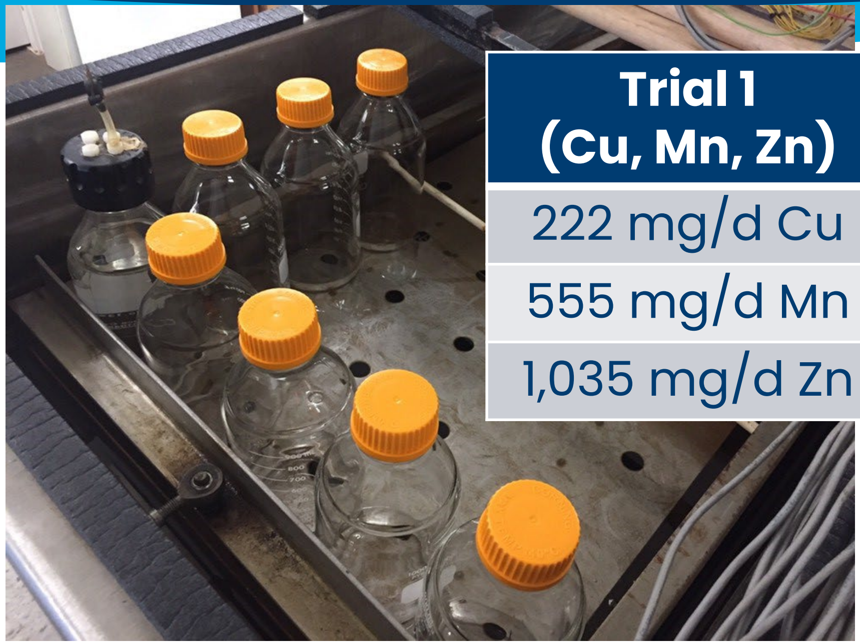
18.69		DM
Moisture, %		100.0
Dry Matter, %		
Crude Protein, %		18.3
AD-ICP, %CP		0.6
ND-ICP, %CP		1.98
SP (BB), % CP		29.87
SP (Microbial), %CP		34.57
Lignin %		3.06
ADF %		18.21
aNDF %		30.2
peNDF		23.10
EE %		3.91
Sugar %	WSS	3.9
Starch%		26.27
NFC%		
aPartitioning Factor		3.32
aOMD (%DM)		57.21
TDN (Est.)		
NE/Lact Mcal/kg		
NE/Main Mcal/kg		
NE/Gain Mcal/kg		
Ash %		8.05
Calcium (Ca), %		
Phosphorus (P), %		
Potassium (K), %		
Magnesium (Mg), %		
Sodium (Na), %		
uNDF%NDF		22.77
uADF48om		

Fermentrics™
www.fermentrics.com

Materials & Methods:

- **Samples**
  - 400 mg TMR Substrate ground to 6mm
  - Placed in 5x10 cm bags (50 ± 10 micron; Ankom #R5x10)
- **Rumen Fluid collected at 8am**
  - Preheated thermos and bathed in CO_2
  - Rumen fluid filtered through 3 layers cheese cloth
- **Glass Fermentation vessels**
  - 80% KSU buffer and 20% rumen fluid
  - Mineral treatments are added to the vessels simultaneously with the rumen fluid
- **Fermentation bottles are placed in insulated reciprocating water bath heated to 39.5°C for a 48-h incubation**

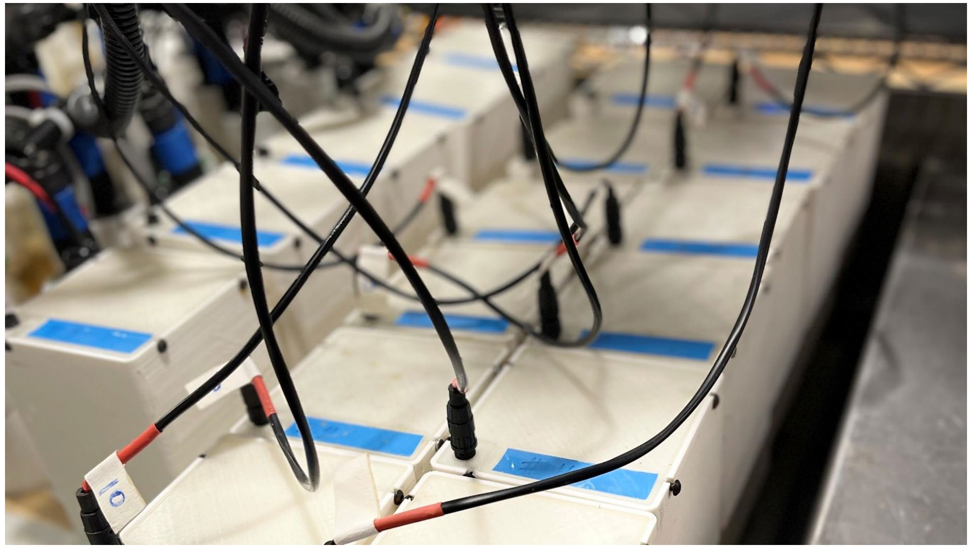
Materials & Methods:



Trial 1 (Cu, Mn, Zn)	Trial 2 (Mn)	Trial 3 (Zn)	Trial 4 (Mn)	Trial 5 (Cu, Mn, Zn)
222 mg/d Cu	-	-	-	125 mg/d Cu*
555 mg/d Mn	555 mg/d Mn	-	500 mg/d Mn*	500 mg/d Mn*
1,035 mg/d Zn	-	750 mg/d Zn*	-	750 mg/d Zn*

Statistics:

- Data analyzed with PROC MIXED (SAS)
- Completely Randomized Design
- Bottle is experimental unit
- Significance equaled P ≤ 0.05



*Levels changed to account for updated requirements in NASEM 2021/Industry Recommendations

Measurements:

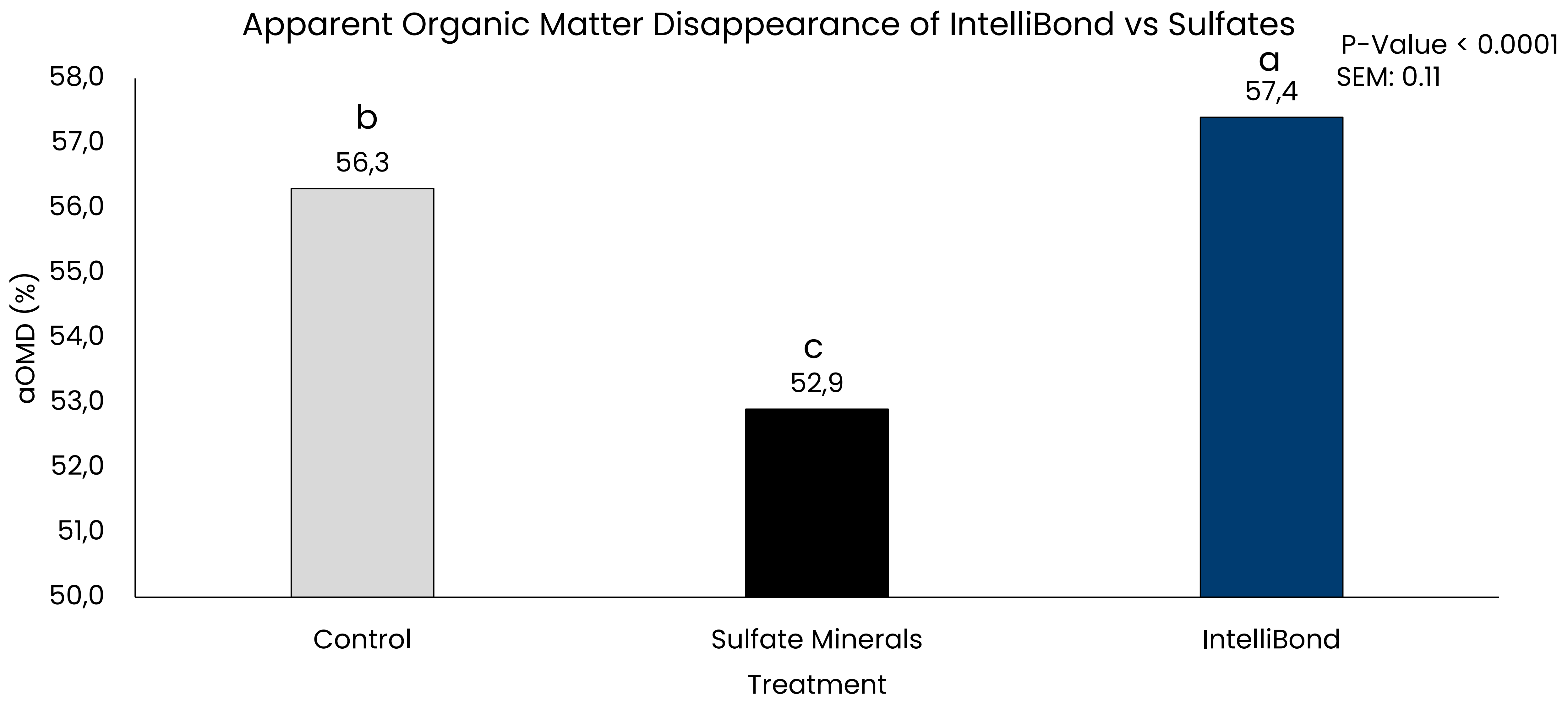
- **Fermentrics™:** a full-service agricultural testing and research laboratory specializing in fully automated in-vitro gas fermentation analysis of feeds and forages that has proven effective in reviewing ingredients, TMR's and feed additives.
  - Allow understanding of digestion kinetics of feeds and forages and can be used to estimate milk production.
  - Provide the end user with options on how to adjust rations.

- **Apparent Organic Matter Disappearance (aOMD):** Apparent organic matter digestibility is the percent of organic matter digested
- **Apparent Microbial Biomass Production (aMBP):** Microbial biomass production is measured directly by analyzing the substrate that remains after 48-hour incubation with a NDF analysis (w/o amylase or sodium sulfite). The difference between the weight of the substrate before and after NDF analysis at the end of fermentation is the microbial biomass after accounting for microbial protein.
- **CH_4/aOMD:** Methane/apparent organic matter digestibility as a proxy for methane yield.

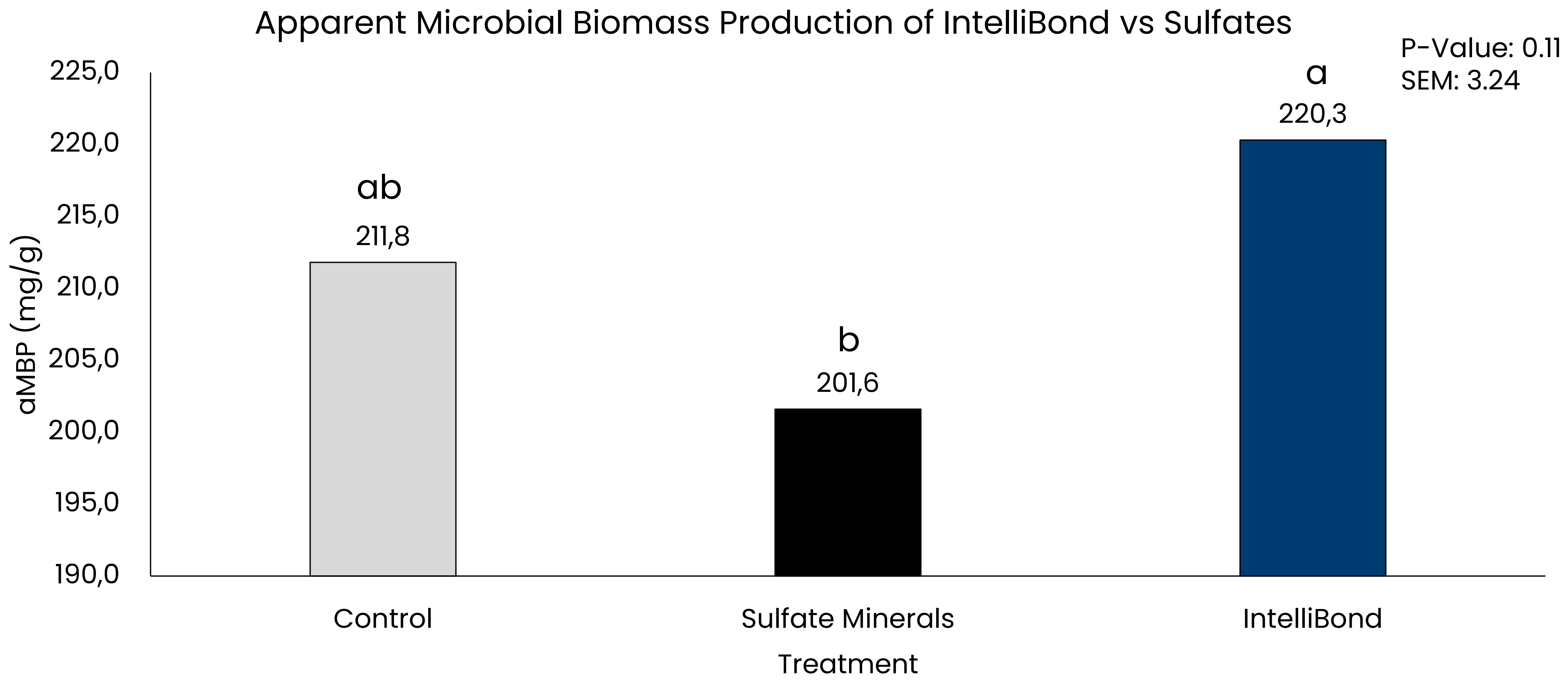
Trial 1: All IntelliBond vs Sulfates

- inclusion of copper, zinc, and manganese comparing sulfate to IntelliBond.

Trial 1: All IntelliBond vs Sulfates



Trial 1: All IntelliBond vs Sulfates



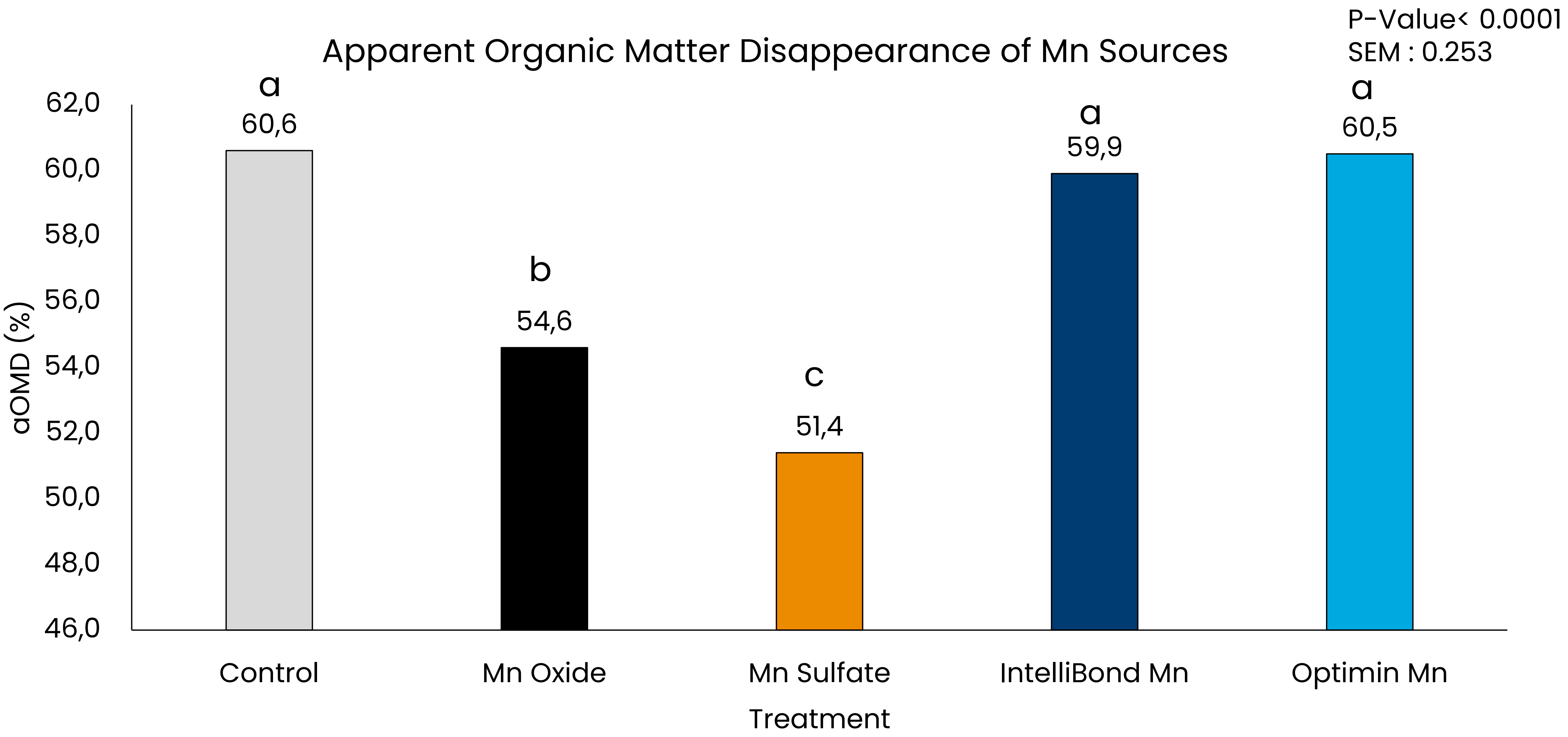
Trial 1: IB vs Sulfates Conclusions

- IntelliBond, an improved mineral source of Cu, Zn, and Mn, did not negatively affect organic matter disappearance (aOMD) whereas the sulfate treatment reduced aOMD ($P < 0.01$).
- The IntelliBond treatment also tended to have higher microbial biomass production (aMBP) compared to the sulfate mineral treatment ($P = 0.11$).

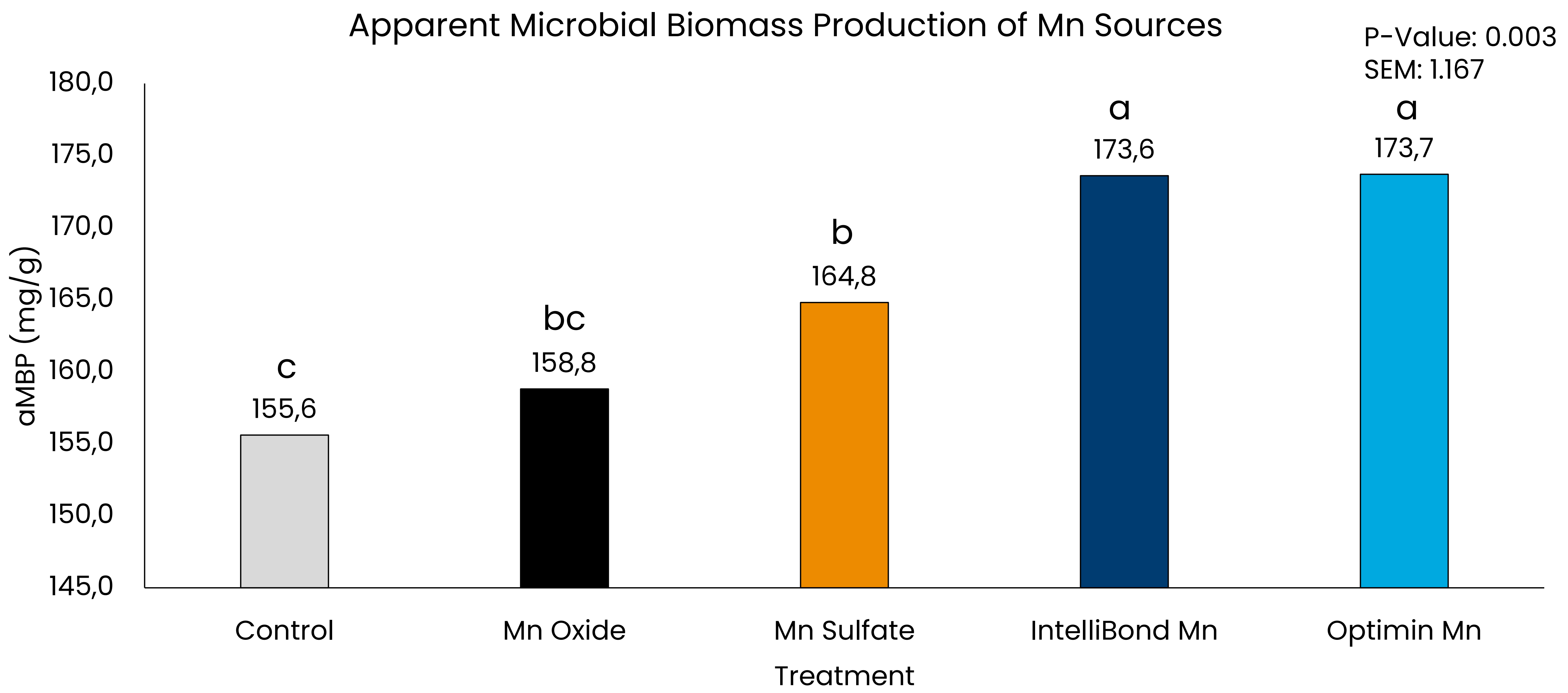
Trial 2: Mn Sources

- inclusion of Mn from Mn oxide, Mn sulfate, Optimin Mn, and IntelliBond M.

Trial 2: Mn Sources



Trial 2: Mn Sources



Trial 2: Mn Sources Conclusions

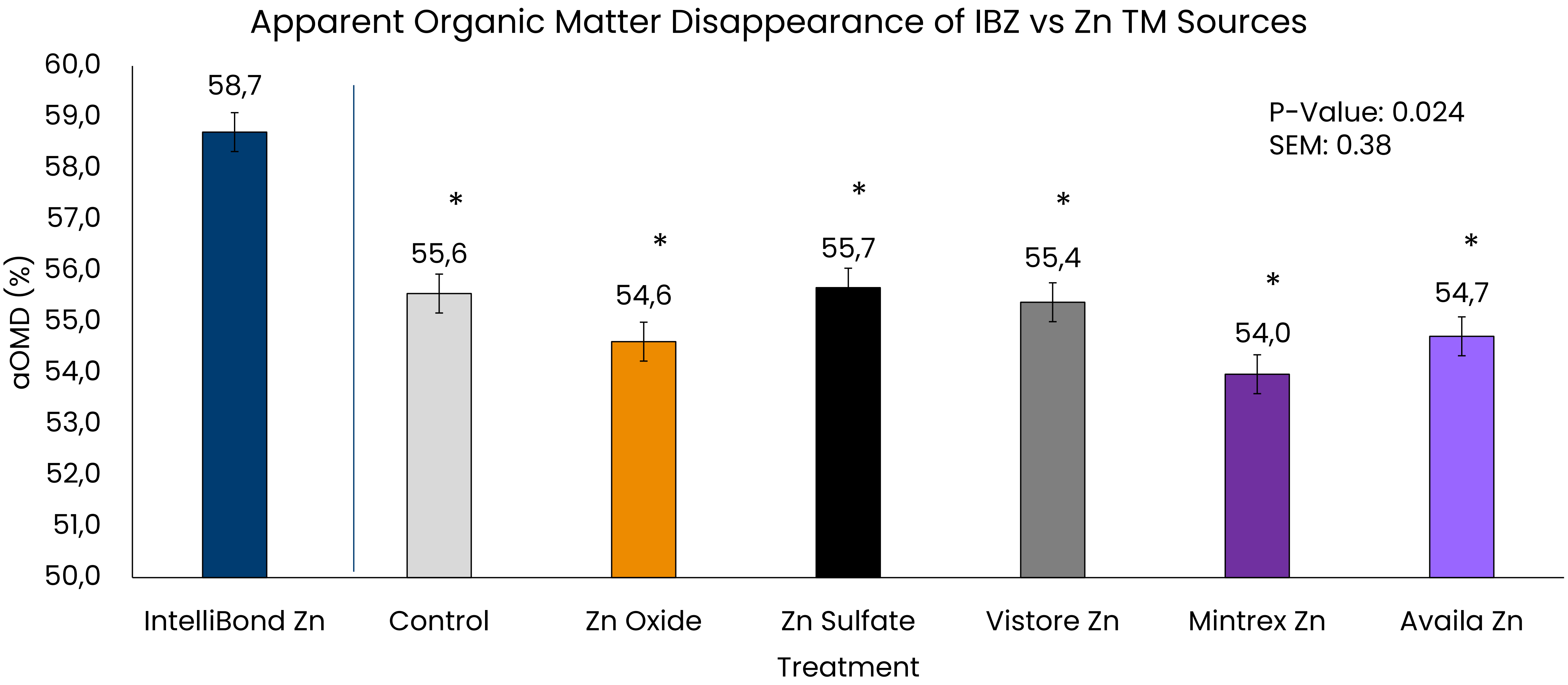
- Improved Mn sources (IntelliBond and Optimin) did not negatively affect organic matter disappearance (aOMD) whereas Mn sulfate and oxide reduced aOMD ($P < 0.010$).

- Improved Mn sources (IntelliBond and Optimin) had higher microbial biomass production compared to control, sulfate, and oxide ($P < 0.003$).

Trial 3: Zn Sources

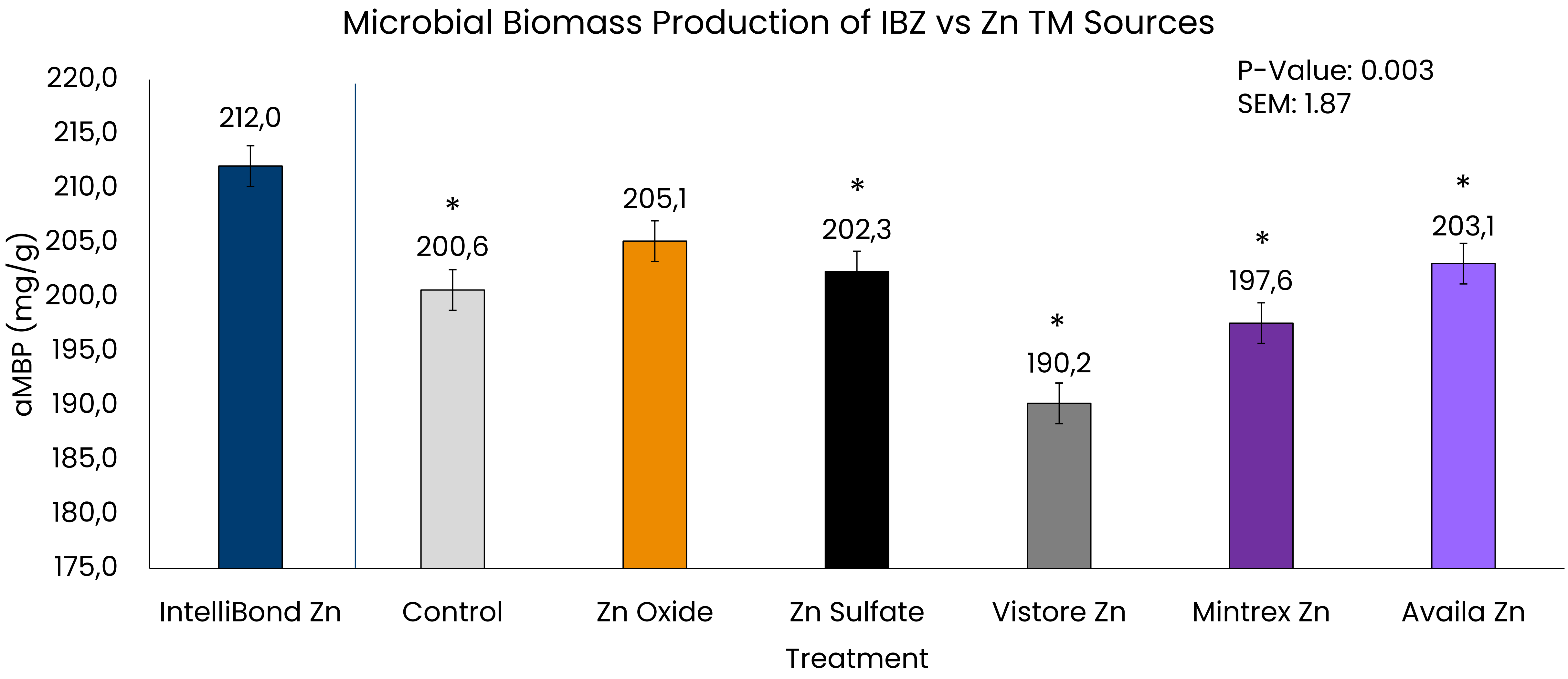
- 7 different sources of Zn were tested: Control (no TM), Zn oxide, Zn sulfate, IntelliBond Zn, Vistore Zn, Availa Zn, and Mintrex Zn.

Trial 3: Zn Sources



Means notated with a * are significantly different from the IntelliBond treatment.

Trial 3: Zn Sources



Means notated with a * are significantly different from the IntelliBond treatment.

Trial 3: Zn Sources Conclusions

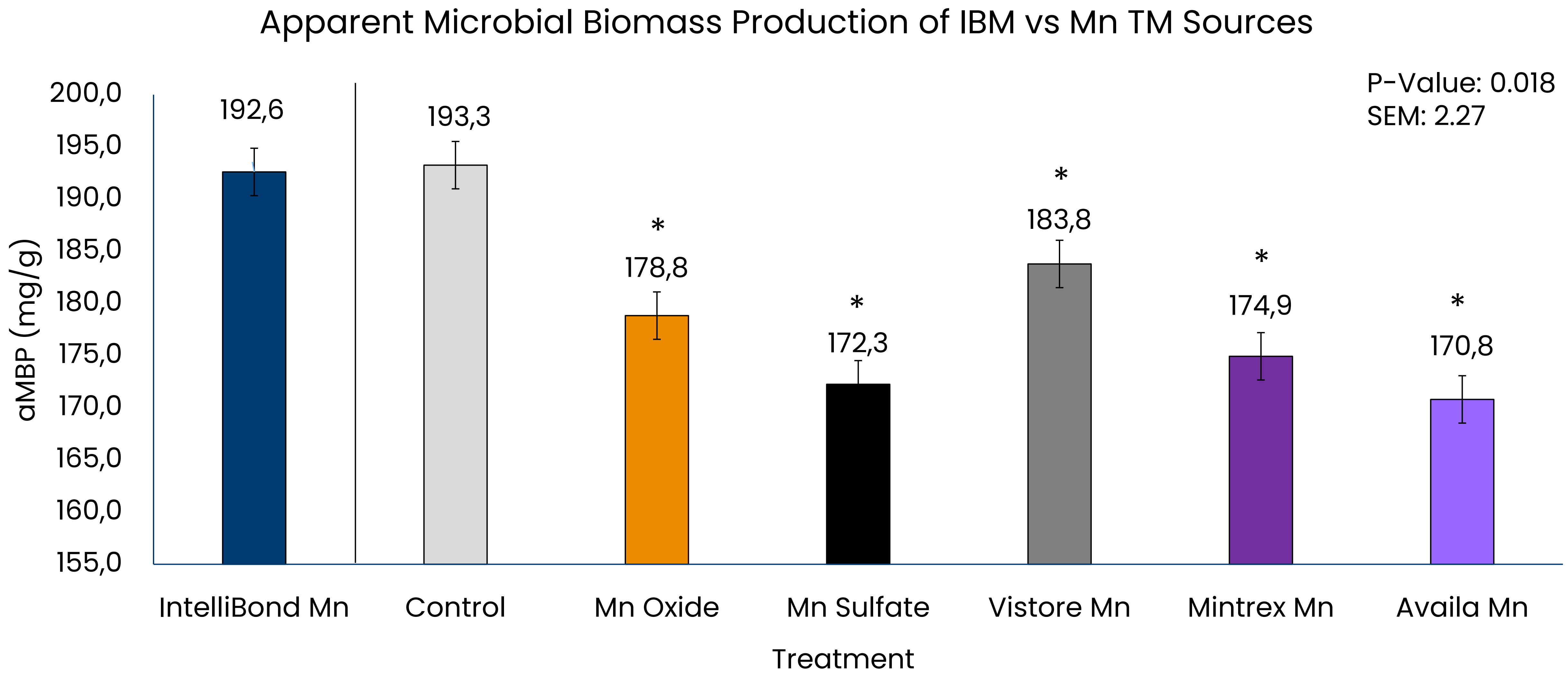
- IntelliBond Zn did not negatively affect organic matter disappearance (aOMD) whereas all other treatments significantly reduced aOMD ($P<0.024$).

- IntelliBond Zn improved microbial biomass production compared to sulfate Zn, Vistore Zn, Mintrex Zn and Availa Zn ($P<0.003$).

Trial 4: Expanded Mn Sources

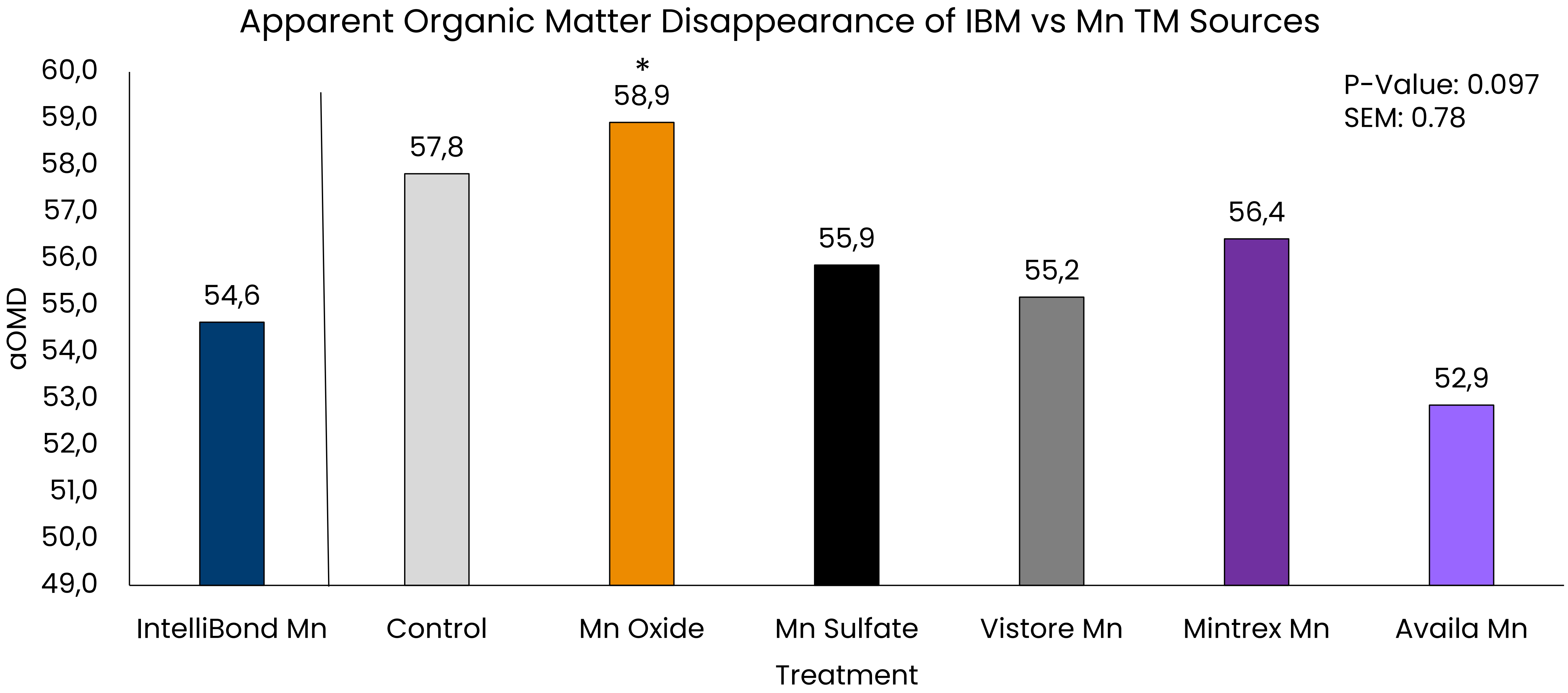
- 7 different sources of Mn were tested: Control (no TM), Mn oxide, Mn sulfate, IntelliBond Mn, Vistore Mn, Availa Mn, and Mintrex Mn.

Trial 4: Expanded Mn Sources



Means notated with a * are significantly different from the IntelliBond treatment.

Trial 4: Expanded Mn Sources



Means notated with a * are significantly different from the IntelliBond treatment.

Trial 4: Expanded Mn Sources

- IntelliBond Mn (similar to the control) improved microbial biomass production compared to all other treatments ($P<0.018$).
- IntelliBond Mn did not negatively affect organic matter disappearance (aOMD) compared to the control ($P<0.097$).

Trial 5: Methane Experiments

Experiment 1: CH_4 Experiment - IntelliBond® vs Sulfates

- Control (no TM) plus inclusion of Cu, Zn, and Mn, individually and in combination, comparing sulfate to IntelliBond.

Experiment 2: OHTM Experiment

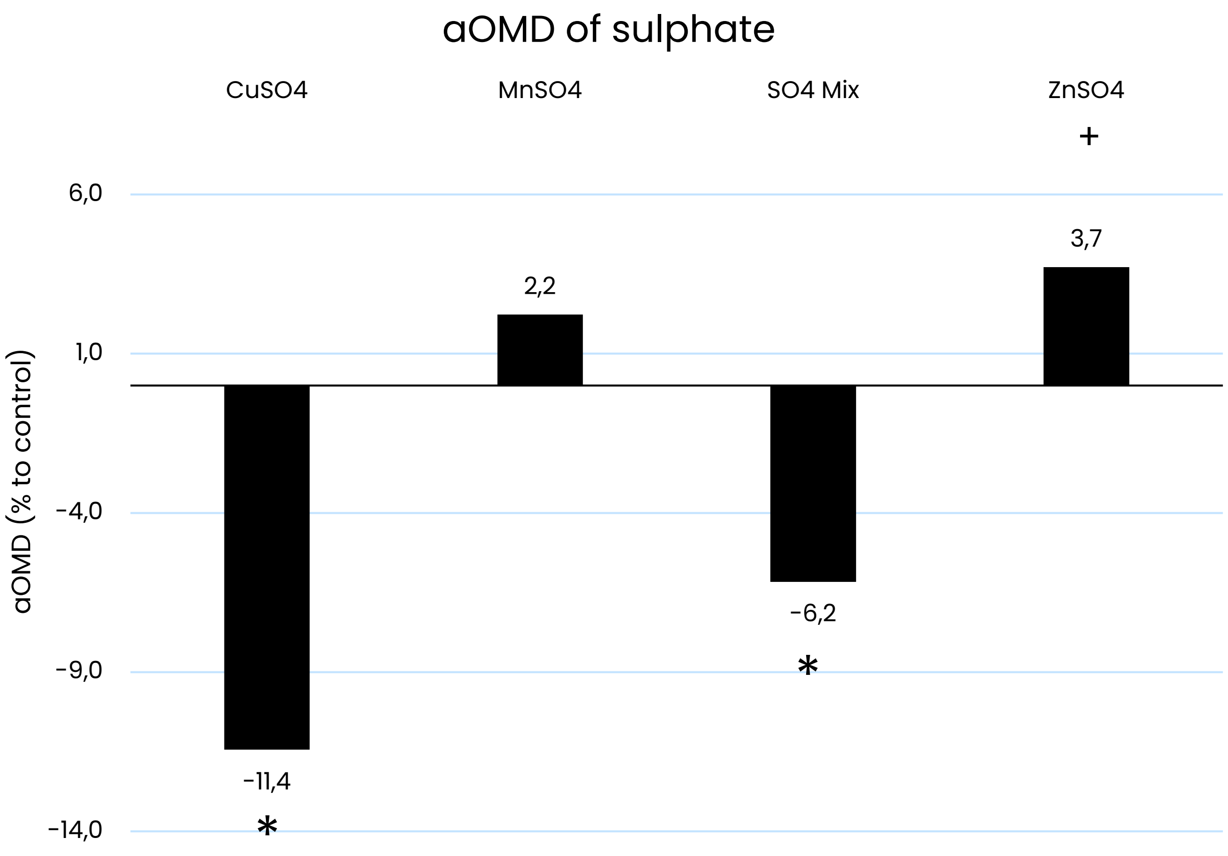
- Control (no TM) plus inclusion of Cu, Zn, and Mn in combination from: sulfate, IntelliBond, Nutrilock Chemlock, Phibro Vistore, SAM Nutrition, Orffa Excential Smart, EcoTrace Glycinate.

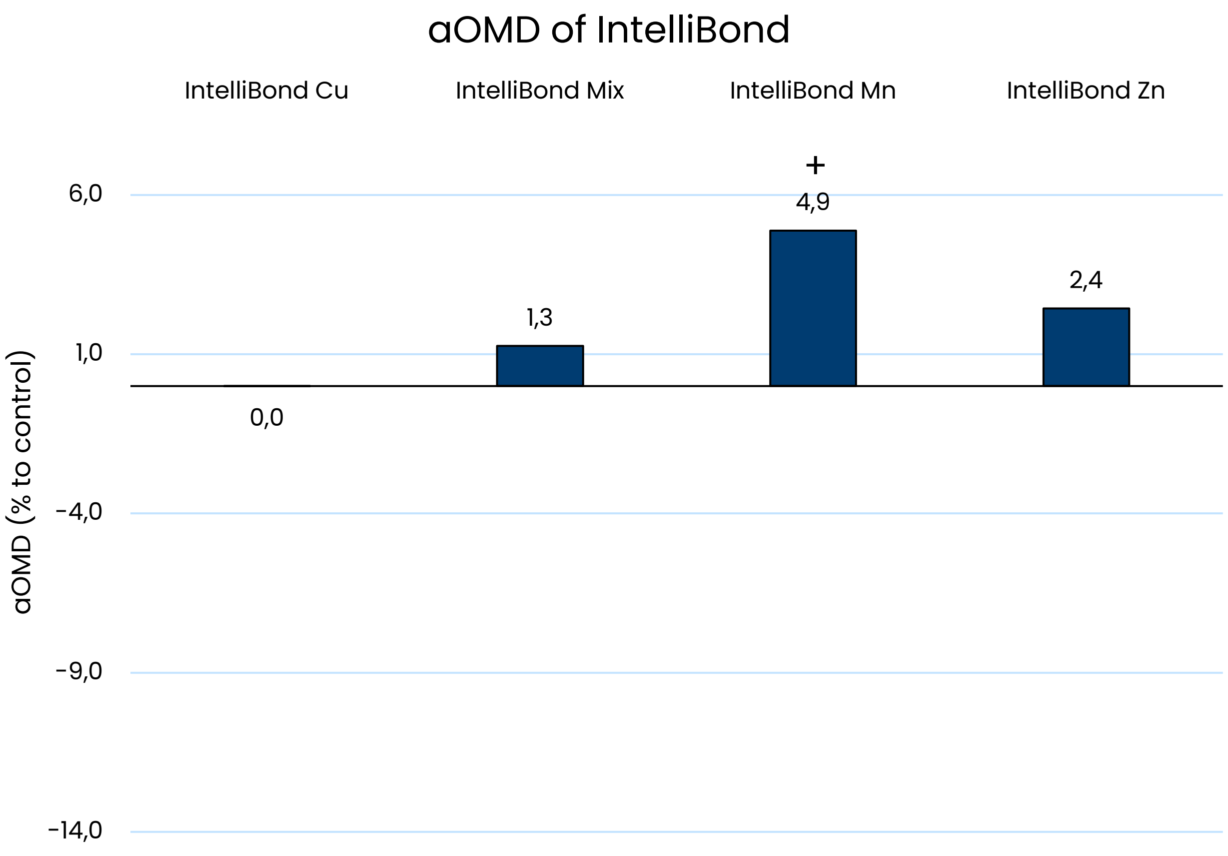
Experiment 3: Zn Sources

- Control (no TM), Zn oxide, Zn sulfate, IntelliBond Zn, Oxide Zn (@ level of Oxide in NL Low Hydroxy), Oxide Zn (@ level of oxide in NL High Hydroxy), Chemlock Zn (with low Hydroxy Zn), Chemlock Zn (with High Hydroxy Zn).

Trial5Exp1: CH_4 Experiment – aOMD

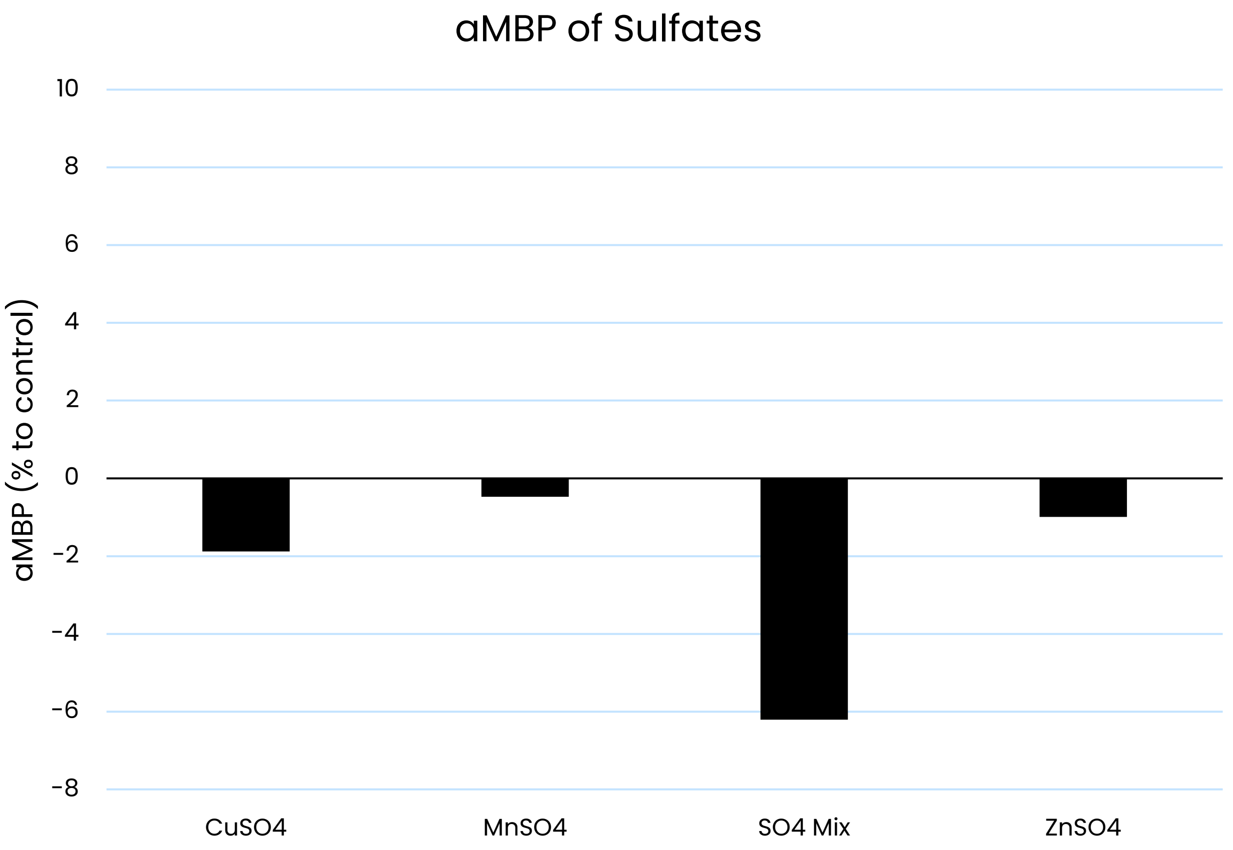
*P ≤ 0.05
+0.05>P≤0.2

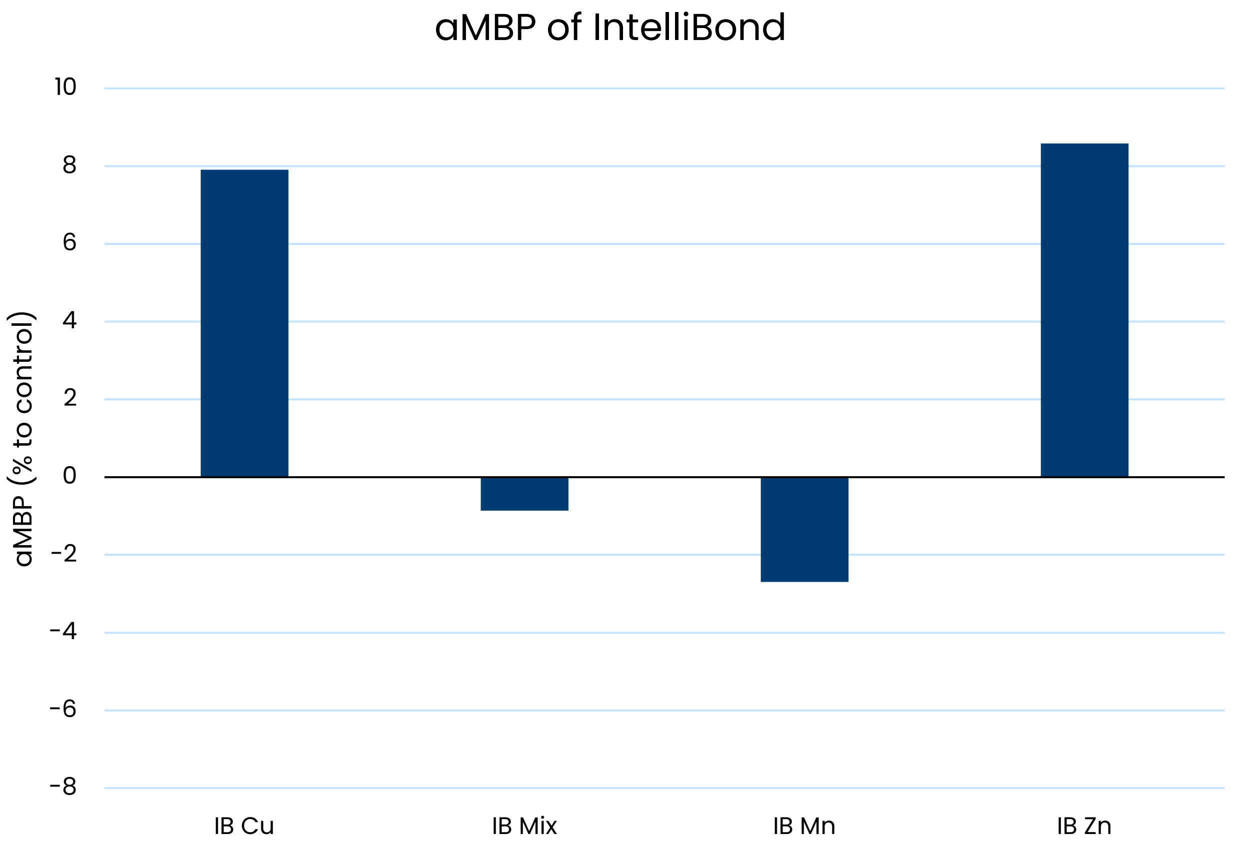




Trial5Exp1: CH_4 Experiment – aMBP

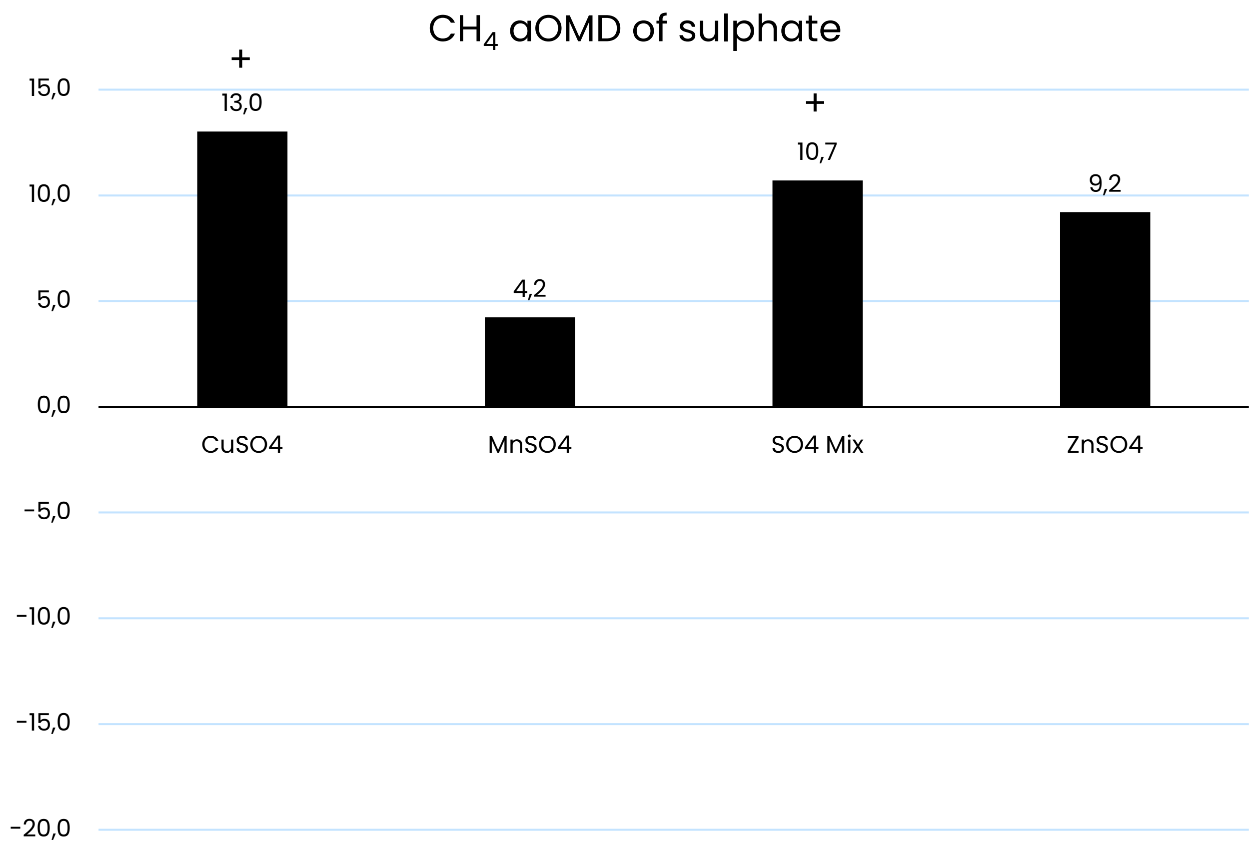
*P ≤ 0.05
+0.05>P≤0.2

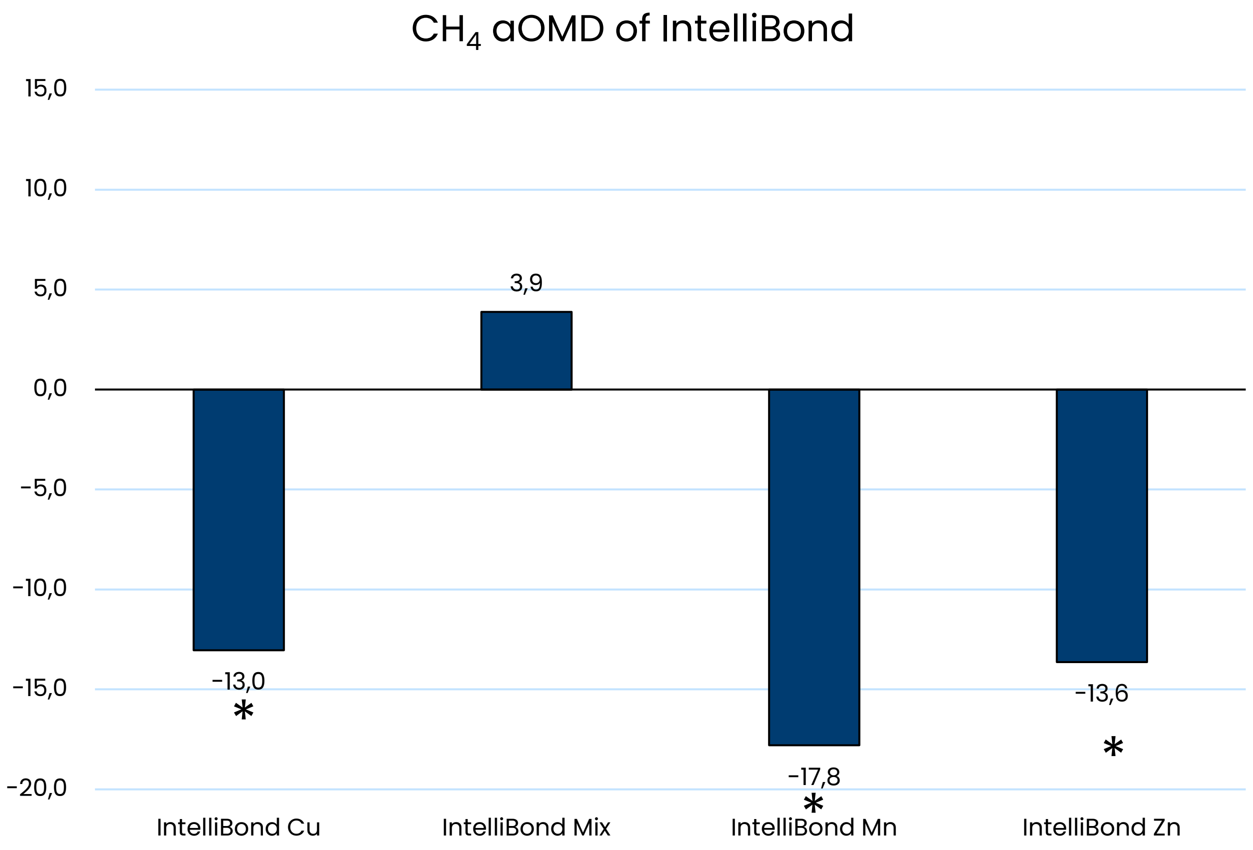




Trial5Exp1: CH_4 Experiment – CH_4/aOMD

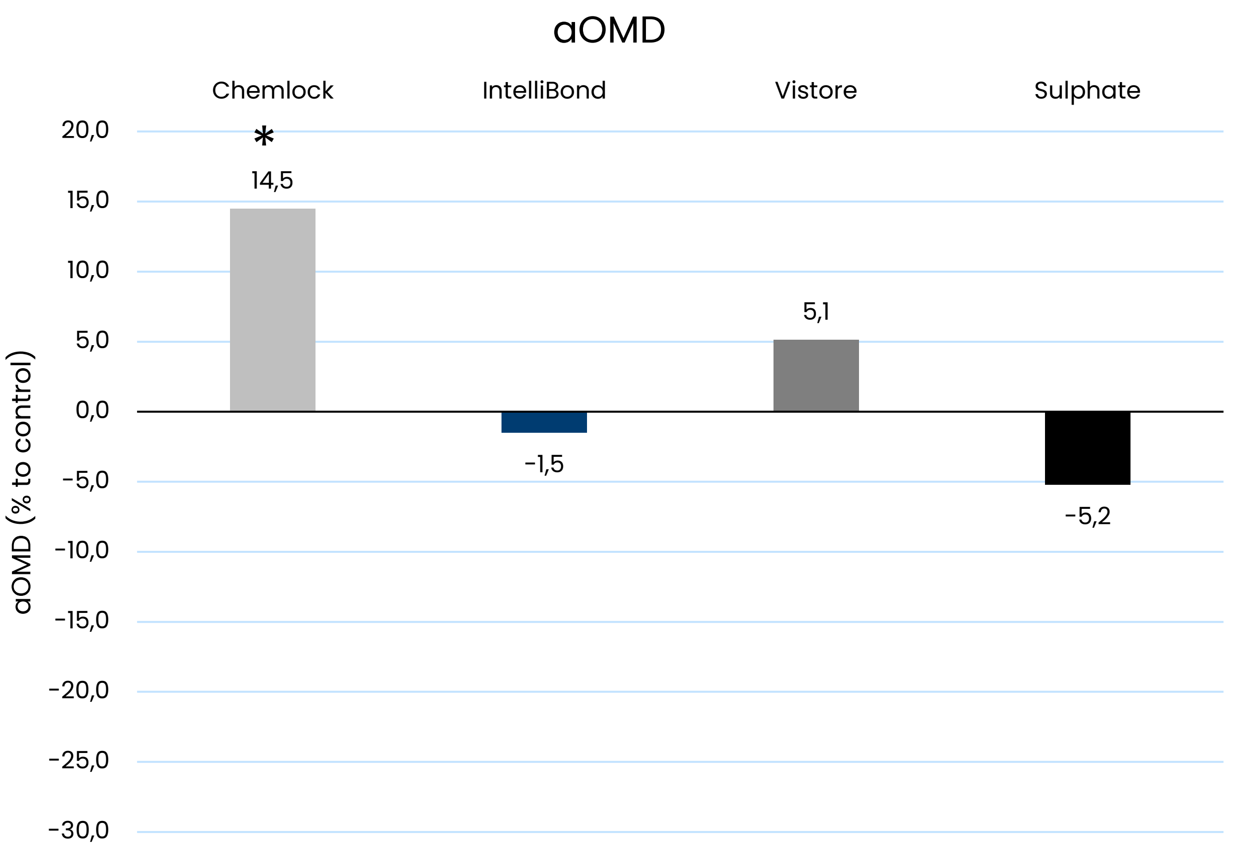
*P ≤ 0.05
+0.05>P≤0.2

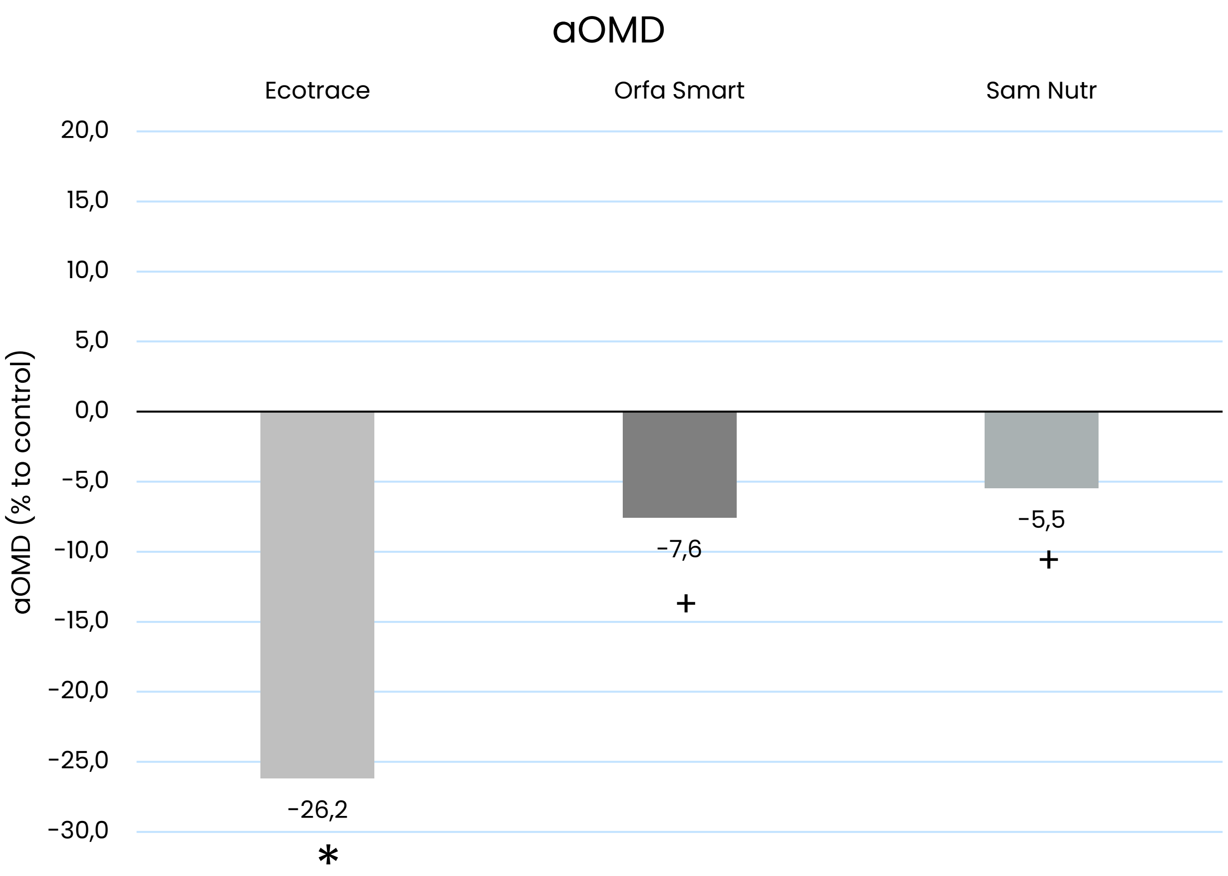




Trial5Exp2: OHTM Experiment – aOMD

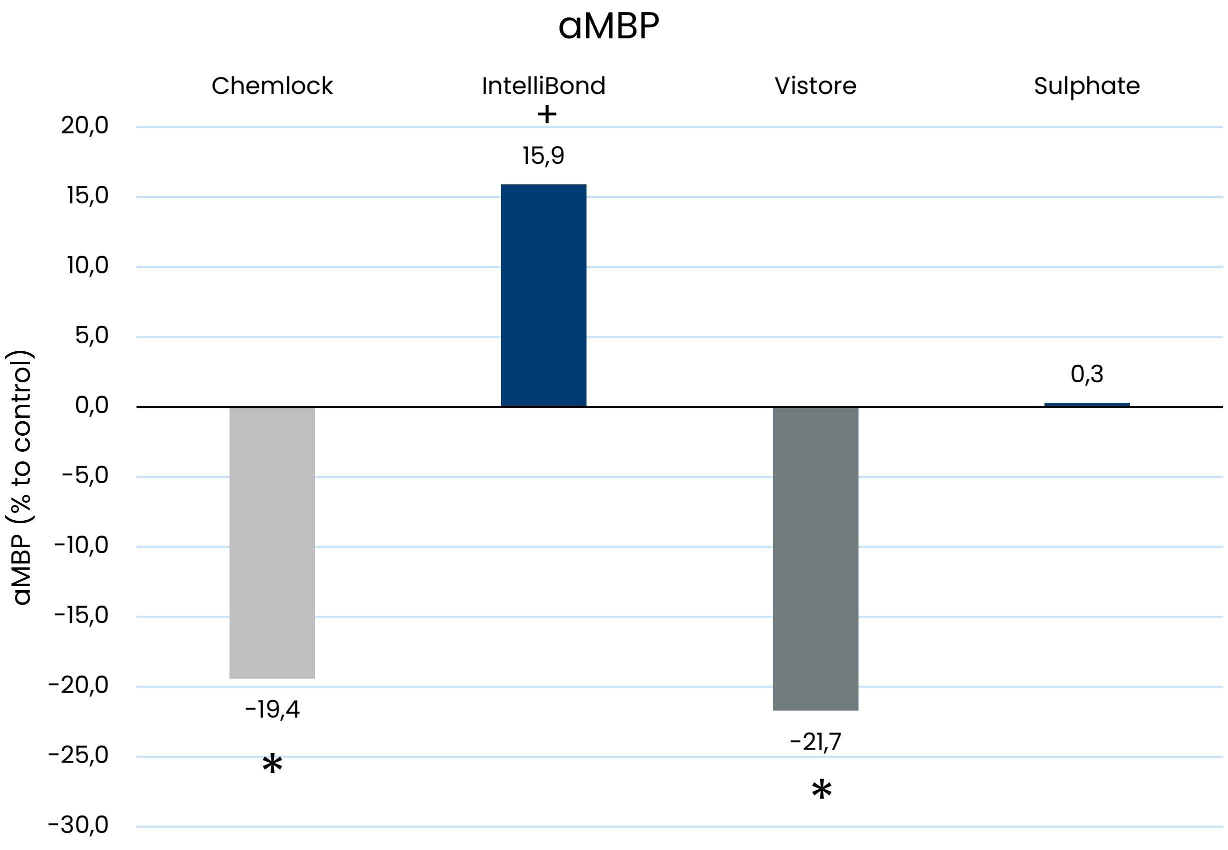
*P ≤ 0.05
+0.05>P≤0.2

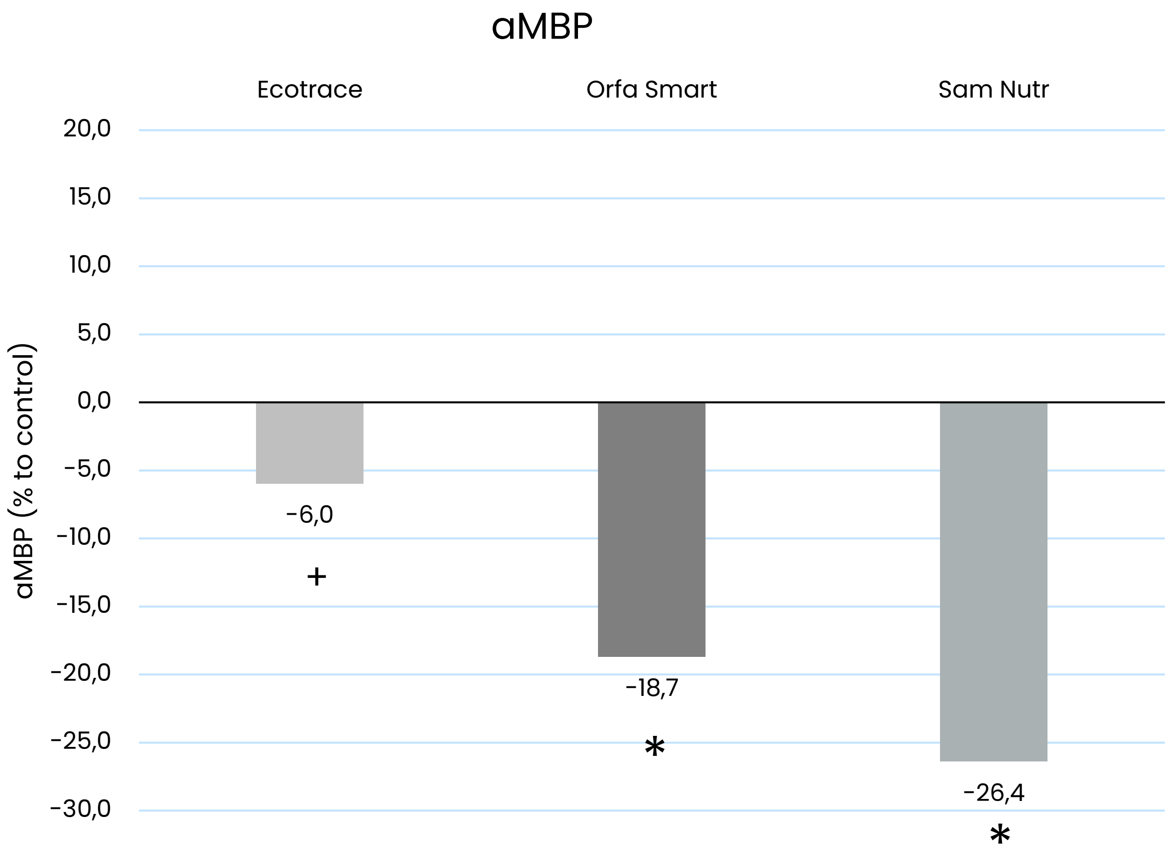




Trial5Exp2: OHTM Experiment – aMBP

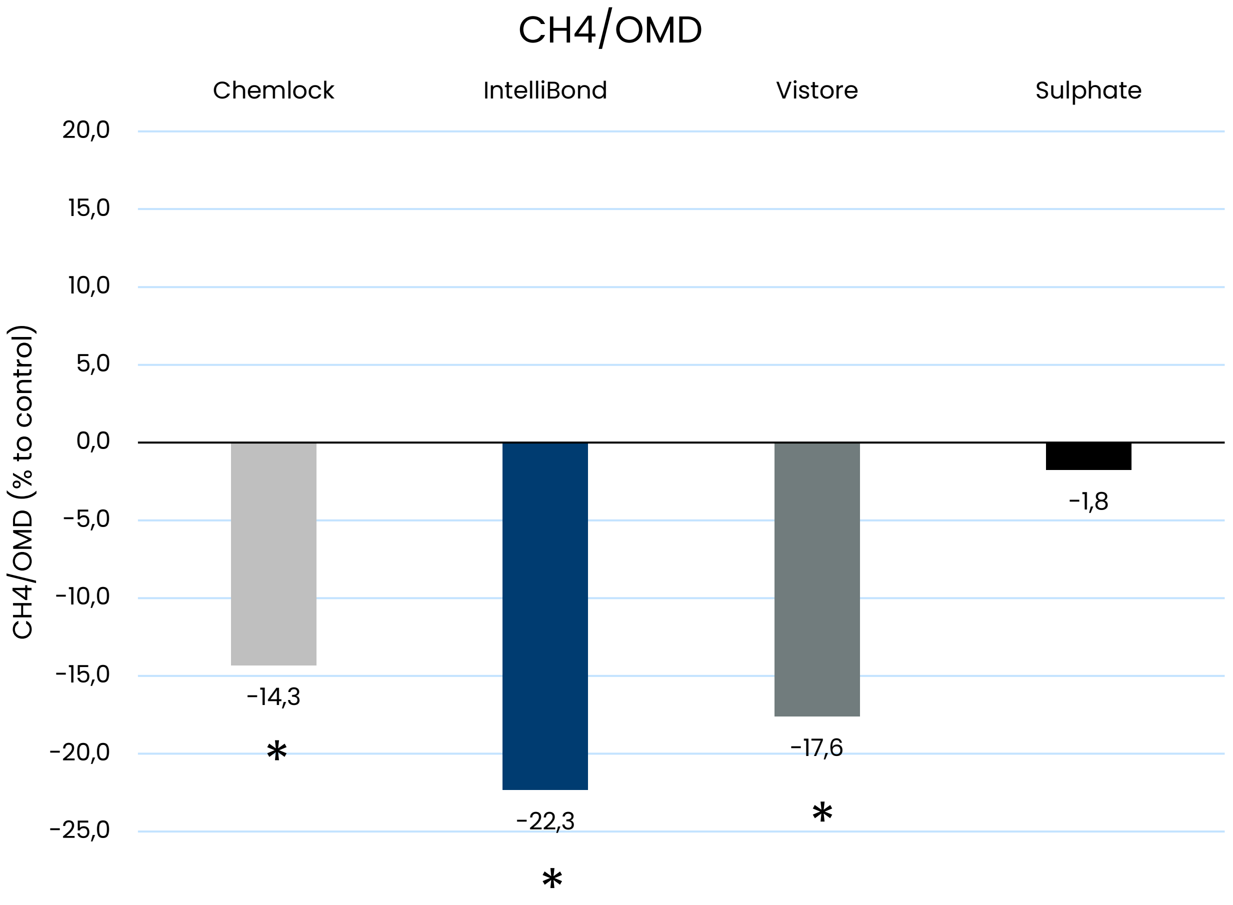
*P ≤ 0.05
+0.05>P≤0.2

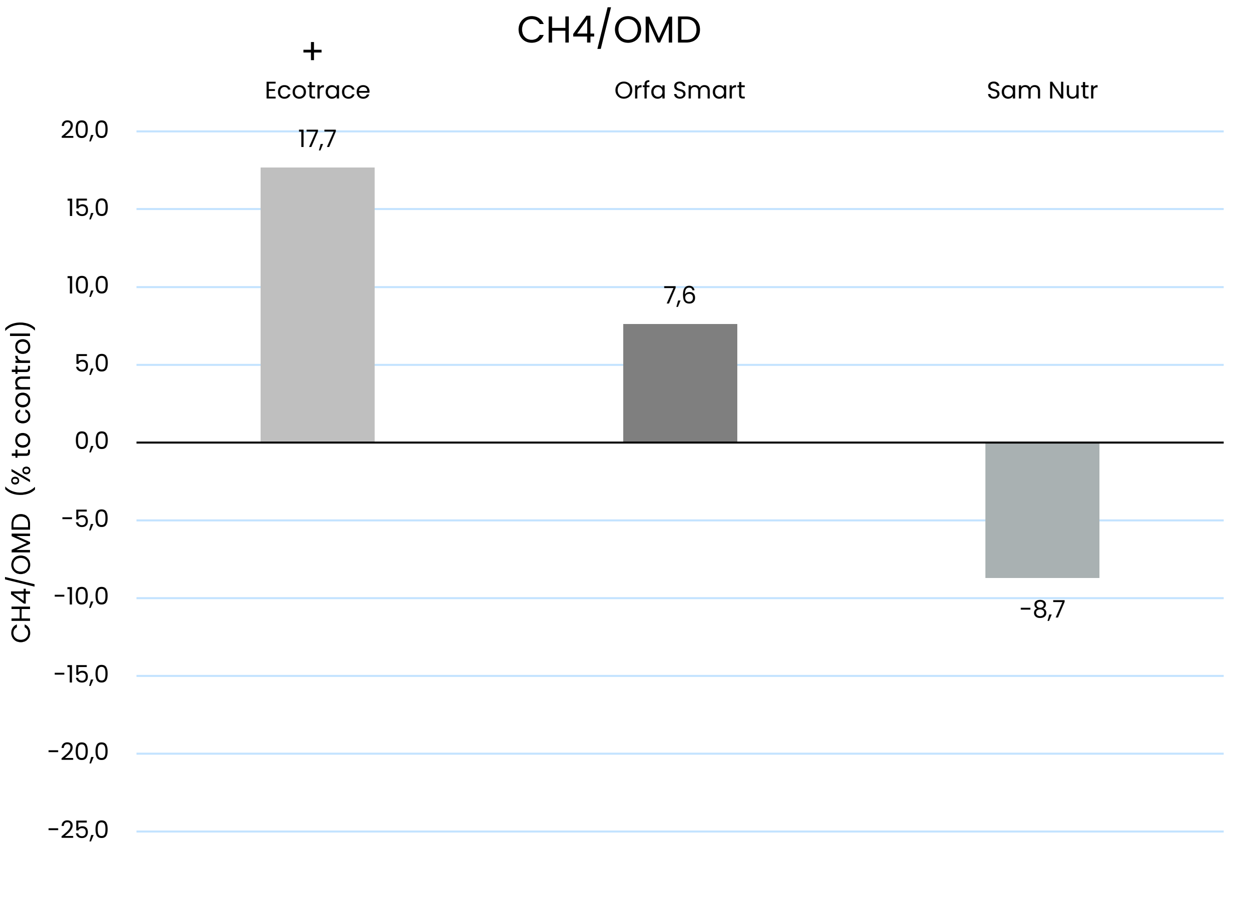




Trial5Exp2: OHTM Experiment - CH_4/aOMD

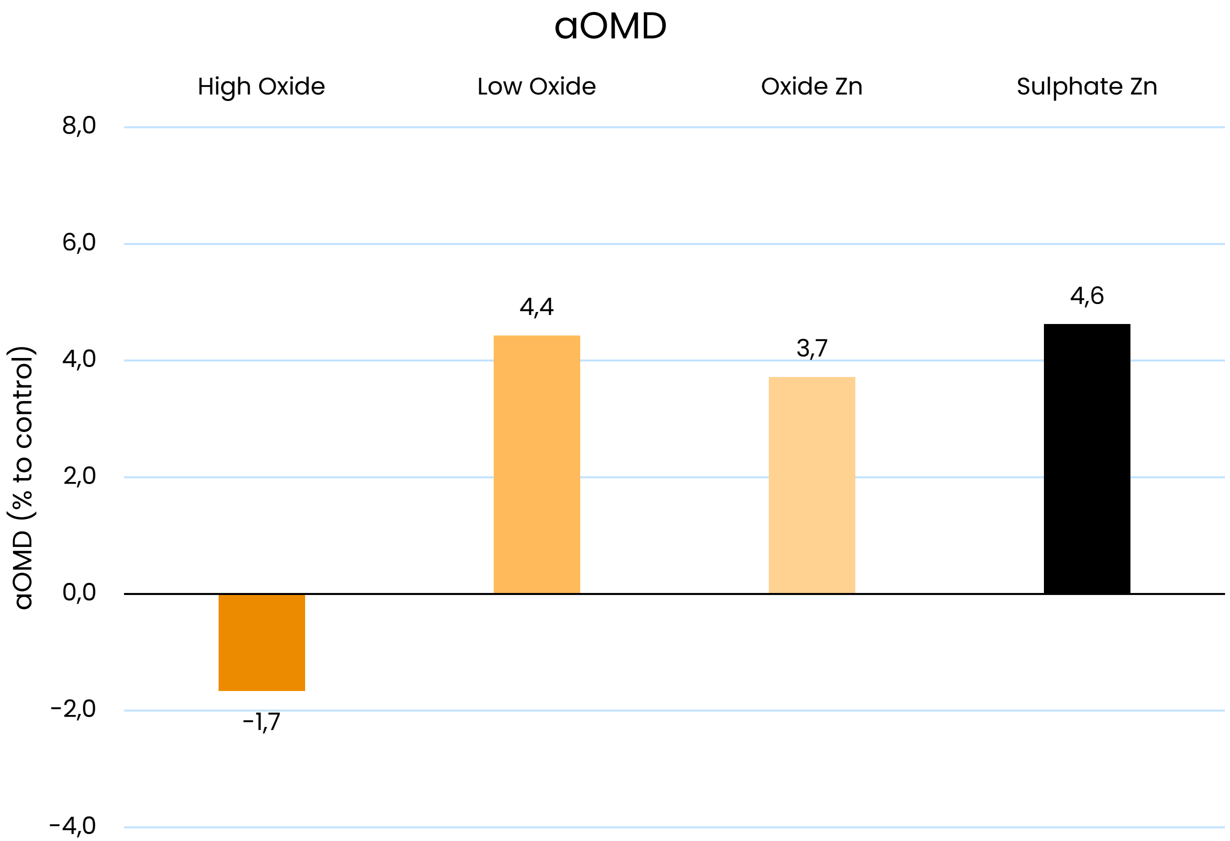
*P ≤ 0.05
+0.05>P≤0.2

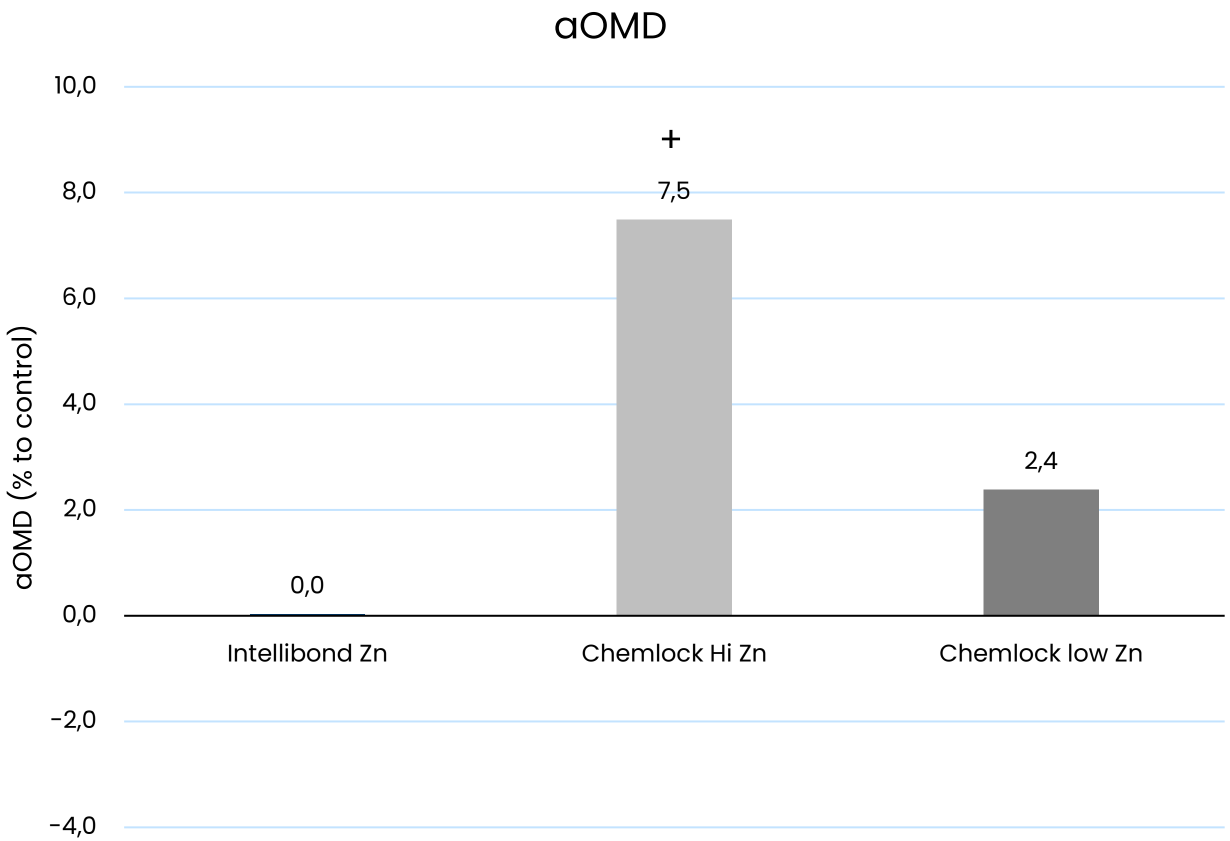




Trial5Exp3: Zn Sources – aOMD

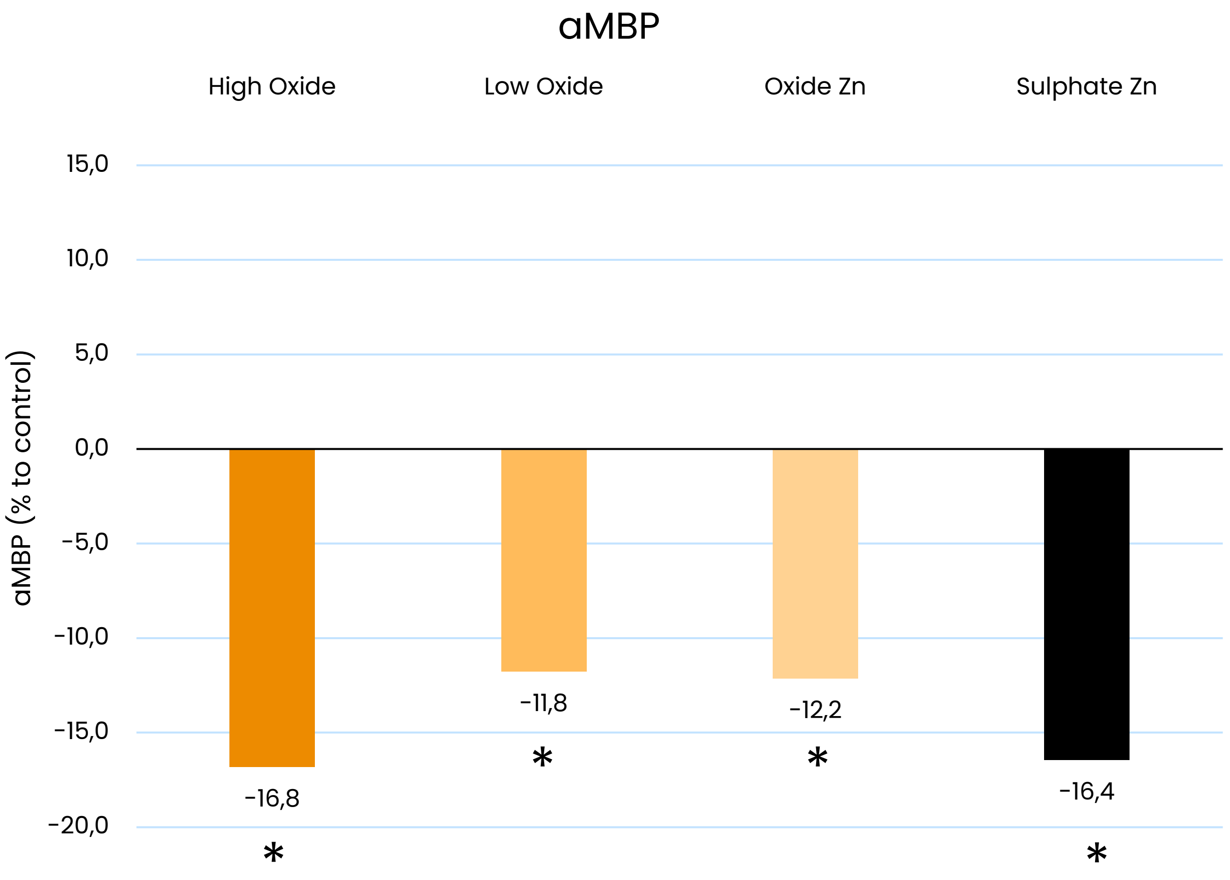
*P ≤ 0.05
+0.05>P≤0.2

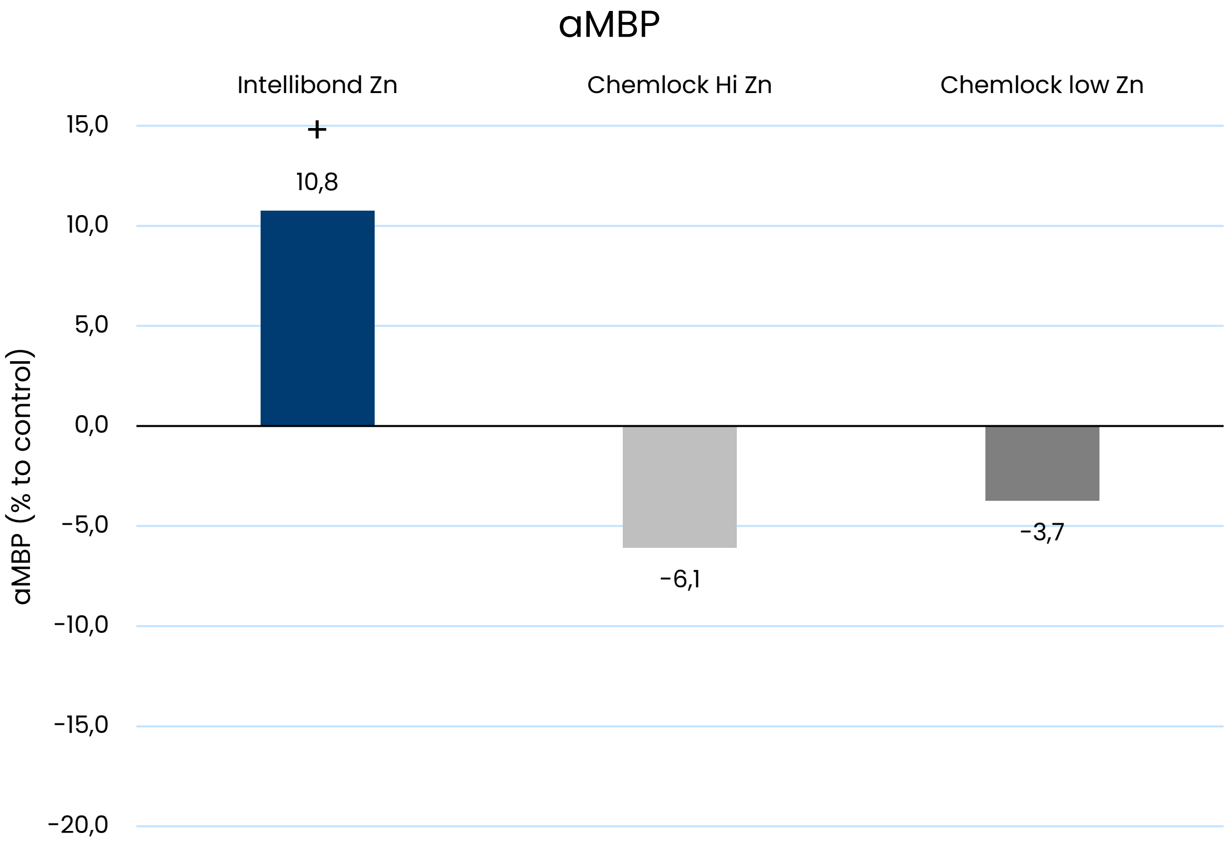




Trial5Exp3: Zn Sources – aMBP

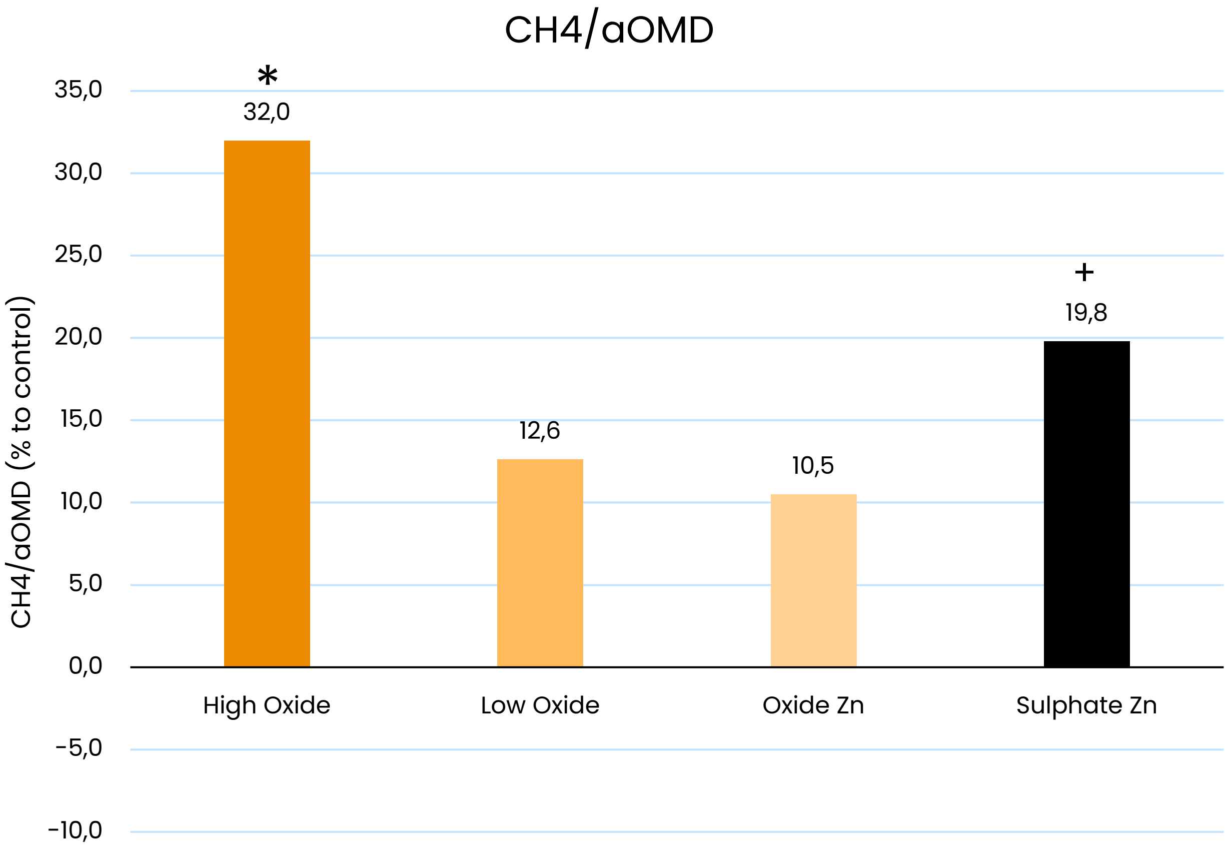
*P ≤ 0.05
+0.05>P≤0.2

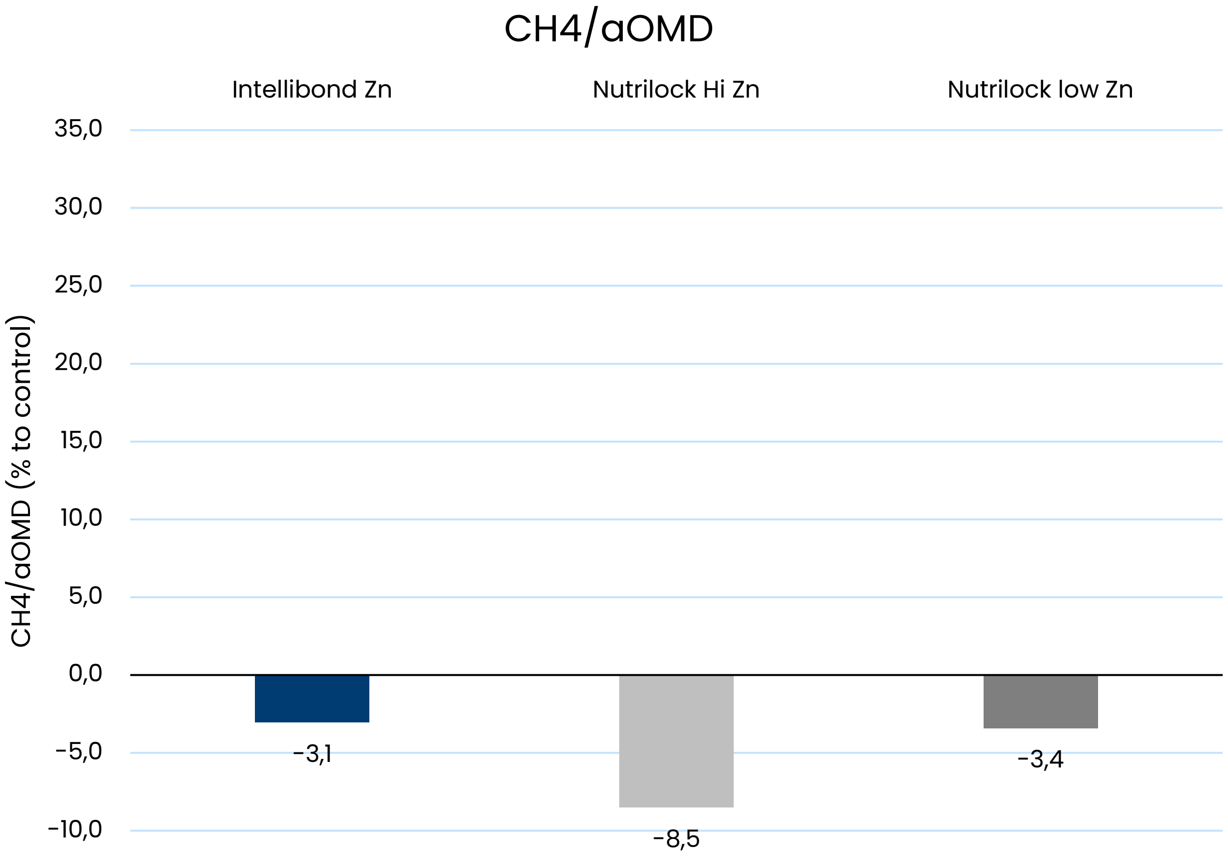




Trial5Exp3: Zn Sources - CH_4/aOMD

*P ≤ 0.05
+0.05>P≤0.2





Fermentrics Trials 1-4 Conclusions:

- The combination of IntelliBond trace minerals did not inhibit microbial growth compared to inorganic mineral solutions.

IntelliBond trace minerals are an improved source of trace mineral that increased in-vitro fermentation relative to other Cu, Zn, and Mn sources.

- IntelliBond Z did not negatively affect organic matter disappearance (aOMD) whereas all other treatments significantly reduced aOMD

Fermentrics Methane Trials Conclusions:

- Experiment 1 showed that a mix of sulfate trace minerals and $CuSO_4$ reduced apparent organic matter disappearance (aOMD), whereas the IntelliBond Mn improved aOMD.

IntelliBond improved in vitro fermentation parameters of aOMD and aMBP (proxy for milk production) compared to other trace mineral sources.

- aMBP is the main driver for milk production used by the Fermentrics system.

Thank you!

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carlyn.peterson@selko.com