Final report

Bovine appeasing substance: A meta-analysis of the effects on production, health, and stress indicators

Project code: B.FLT.4027

Prepared by: Ian J Lean & Helen M Golder
Scibus

Date published: 23 March 2025

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Objective

To use meta-analytical methods to evaluate the effectiveness of treatment of cattle with bovine appeasing substance (**BAS**) to improve production, health, and stress indicators.

Materials and Methods

A search of three search engines identified experiments that examined the effectiveness of treatment of cattle with BAS to improve production, health, and stress indicators. Results of experiments were evaluated to ensure that these were: from peer-reviewed journals or theses published in English; in vivo and evaluated use of BAS; randomized; had appropriate analysis of data; and contained data to determine the effect size for outcomes. The standardized mean difference (**SMD**) was estimated for continuous data and for the dichotomized disease data the risk difference between groups was estimated. Publication bias was assessed through funnel plots. Multilevel models were used for SMD and risk difference estimates when experiments were nested within studies. The potential influence of pseudo-replication and duration of experiment were assessed through meta-regression.

Results and Discussion

Thirteen studies with up to 18 experiments were included. There was no significant difference in initial BW (P = 0.434) or evidence of publication bias for any outcome. For the stress outcomes, only blood cortisol was reduced by treatment by 3.85 ng/ml (95% CI -7.60 to -0.09; P = 0.045), supporting the proposed mode of action of BAS, but did not result in overall production or health benefits. However, there were positive results in some individual experiments, particularly for final BW, mortality, and virus antibodies. Examination at the univariate level for final BW, which is less robust than at the multilevel as it does not account for the confounding effects of studies nested within experiments showed treatment produced cattle 1.72 kg heavier (95% CI 0.06, 3.39; P = 0.043), suggesting a potential benefit if study power is increased with well replicated studies. The limited number of experiments and large variation in study design prevented extensive exploration of potential sources of heterogeneity. A decrease in heterogeneity when the effect of experiment within study was accounted for indicates that the variation at the study level was influential and needs further exploration.

Implications and Applications

The reduction in blood cortisol concentrations by treatment indicates the potential for BAS to mitigate the effects of routine animal husbandry stress on cattle. Positive point directions for final BW, mortality, and virus antibodies suggest the need for more experiments to determine whether the intervention can be justified on a cost-efficacy or ethical basis.

Executive summary

Background

Bovine appeasing substance is a pheromone that has the potential to moderate adverse effects of routine husbandry procedures in cattle. The intent of this study was to evaluate the efficacy of the intervention on production performance, health, and blood measures related to stress and immune response in cattle. The target audience is cattle producers, researchers, and those interested in the well-being of cattle. The results of the research will inform adoption of the intervention and the need for further investigations.

Objectives

To use meta-analytical methods to evaluate the effectiveness of treatment of cattle with bovine appeasing substance to improve production, health, and stress indicators.

Methodology

- A search of 3 search engines identified studies that examined the effectiveness of treatment of cattle with bovine appeasing substance (**BAS**) to improve production, health, and stress indicators.
- Results of studies and experiments were evaluated to ensure that these were; from peer-reviewed journals or theses published in English; experiments were *in vivo* and evaluated use of BAS; randomized; had appropriate analysis of data; contained the data to determine the effect size (**ES**) for outcomes.
- The standardized mean difference (**SMD**) was estimated for continuous data and for the dichotomized disease data the risk difference between groups was estimated. When experiments were nested within studies multilevel modelling was used to provide a more correct measure of the SMD or risk difference.

Results/key findings

- Thirteen reports or studies with up to 18 experiment comparisons were included.
- There was no evidence of publication bias in the funnel plots for any variable.
- There was no significant difference in initial body weight (**BW**) ($P = 0.434$) nor was overall final BW increased for treated cattle when examined using a multi-level model (ES = 1.31 kg; 95% CI -1.303 to 3.930 kg).
- Examination at the univariate level for final BW, which is less robust than at the multilevel as it does not account for the confounding effects of studies nested within experiments showed treatment produced cattle 1.72 kg heavier (95% CI 0.06, 3.39; P = 0.043), suggesting a potential benefit if study power is increased with well replicated studies.
- ADG, DMI, G:F, diarrhea, respiratory disease, overall mortality, and virus antibodies were not improved with treatment. However, there were positive results in some individual experiments, particularly for final BW, mortality, and virus antibodies.
- For metabolic variables studied only blood cortisol was reduced by treatment with a 3.85 ng/ml reduction (95% CI -7.60 to -0.09; P = 0.045), supporting the proposed mode of action of BAS.

- The limited number of experiments and large variation in study design prevented extensive exploration of potential sources of heterogeneity.

Benefits to industry

- Blood cortisol concentrations were significantly reduced by treatment indicating the potential for BAS to mitigate the effects of routine animal husbandry stress on cattle.
- However, despite positive experimental results in some studies, overall, no other results of the meta-analysis were significant.
- At present, there are no outstanding benefits for industry, but some promise of potential benefit based on positive results on individual studies, positive point directions, and significance at the univariate level for final BW.

Future research and recommendations

- Positive point directions for final BW and mortality suggest the need for more studies to determine whether the intervention can be justified on a cost-efficacy or ethical basis.
- These studies should utilise existing information on timing of sampling to detect treatment effects.
- Suggested areas for funding would be castration, weaning, feedlot entry, and pre-slaughter transport interventions which could all be addressed in a single well replicated study under Australian conditions and standard management practices.
- To detect a reduction in mortality or other health outcomes, and/or an improvement in production with an effect size of 40% with a study power of 0.6, an α of 0.05 pen studies on 8 lots, each with 6 randomised pens containing 80 head would be required. The study power is driven by number of lots or pens per lot rather than the number of cattle in the pen.

Optimal time of application

- We were not able to identify an optimal time of application, but suggest for prolonged stressors, treatment be applied 4-6 hr before stress event every 14 days as per label directions for Secure® (IRSEA Group, France).
- There is the potential for cattle to self-apply with an oiler, but this requires further research.

Comparison of regulatory environments

- It is our understanding that BAS is currently marketed in the USA and Europe with minimal to no regulation required.
- Product registration would be required in Australia which would be a barrier to adoption.

Suitability for use in Australian cattle production

- More experiments are required before BAS could be recommended for use in Australia.

Table of contents

1. Background

Pheromones are chemical substances excreted or secreted that provide communication within a species. The species that respond to pheromones are extensive and vary from complex mammalian organisms through to eukaryotic organisms and plants. Studies have evaluated the effects of bovine maternal appeasing substance (**BAS**) on production, health, and blood chemistry of cattle exposed to "stressful" conditions (Cappellozza et al., 2020; Colombo et al., 2020; Vieira et al., 2023). Other studies evaluated the immune responses of cattle to common respiratory viruses by monitoring blood antibodies to infectious bovine rhinotracheitis (**IBR-1**), bovine viral diarrhea or pestivirus (**BVD**), bovine respiratory syncytial virus (**BRSV**), and parainfluenza virus-3 (**PI-3**). The "stressful" conditions studied include routine husbandry procedures such as castration, weaning, and vaccination (Angeli et al., 2020; Vieira et al., 2023), entry to feedlots (Colombo et al., 2020), and transport (Cappellozza et al., 2020). Studies were conducted on calves, steers, and heifers at feedlot entry. The BAS is excreted by cows and the studies conducted used a commercially developed form of this pheromone that the patent states is a composition or a solution comprising 24.9% to 28.6% (w %/w %) oleic acid, 19.2% to 23.1% (w %/w %) palmitic acid, 20.5% to 24.3% (w %/w %) linoleic acid, 1.9% to 4.2% (w %/w %) lauric acid, 3.2% to 5.6% (w %/w %), myristic acid, and 18.4% to 22.8% (w %/w %) 1-docosanol and derivatives thereof (Pageat, 2000). The results of the BAS interventions vary with some studies showing significant effects in improving some outcomes (Angeli et al., 2020; Cappellozza et al., 2020; Colombo et al., 2020), but other outcomes were not significantly improved.

When the results of studies vary and where there is a body of literature that utilizes a consistent intervention, there is the potential to use meta-analytical methods to evaluate the information and produce pooled estimates of the effect (Glass, 1976). Meta-analysis, the quantitative analysis of previous studies, provides opportunities to investigate previously proposed hypotheses and to develop new hypotheses from large databases (Lean et al., 2009). The method increases statistical power to evaluate responses, and this is especially valuable when the expected effect sizes of an intervention may be modest. An evaluation of the literature on BAS suggested that this would be a suitable intervention for quantitative review and meta-analysis.

The intervention, BAS, has the potential to benefit cattle, producers and enhance animal well-being.

We hypothesized that BAS would increase production responses, improve health, and modify the blood chemistry of cattle exposed to the intervention.

2. Objectives

To use meta-analytical methods to evaluate the effectiveness of treatment of cattle with bovine appeasing substance to improve production, health, and blood chemistry indicators.

These objectives were met.

3. Methodology

We utilized meta-analytical methods described by Lean et al. (2009), Sargeant and O'Connor (2020), and Page et al. (2021) to undertake the review and analyses. The latter are consistent with those of the review by Tempelman (2025).

3.1 Reports and experiments included

A comprehensive search of the English language literature used the US National Library of Medicine National Institutes of Health through PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Google Scholar (http://scholar.google.com/), and the ISI Web of Science (http://apps.webofknowledge.com).

The following key words with no limits included: maternal bovine appeasing substance; appeasing substance and maternal or cattle or calves or steers or cows or bulls or heifers; pheromones or bio-stimulation and maternal or cattle or calves or steers or cows or bulls or heifers; and appeasing substance and preconditioning program.

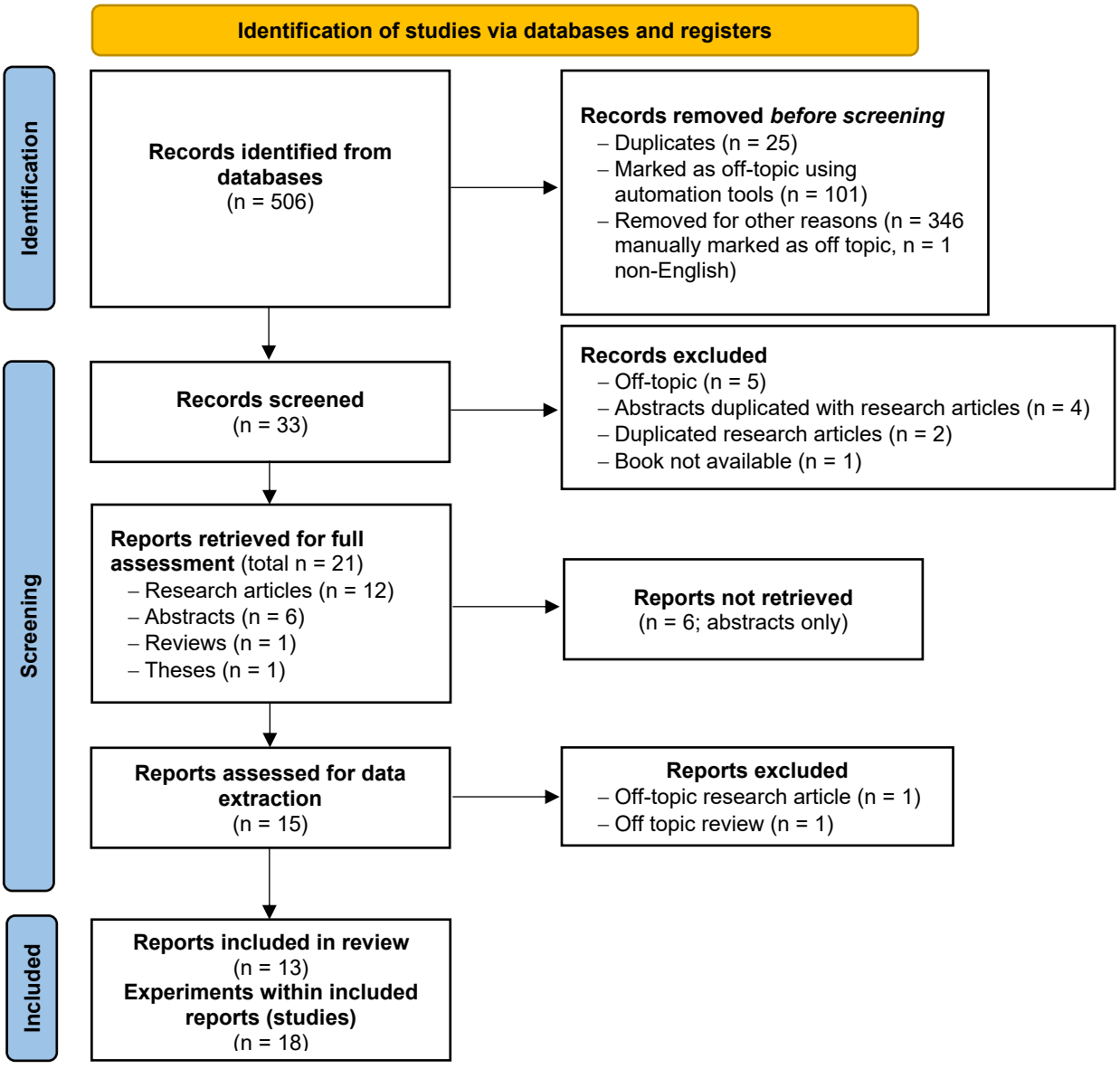
The searches were augmented with library searches of relevant journals, examination of cited literature in papers identified and review of citations in review papers. Papers were primarily screened on their citation title by two reviewers and secondarily screened by two reviewers based on the full text. Experiments were included in the analysis if they met the following inclusion criteria: were full manuscripts from peer-reviewed journals published in English language or were peer reviewed theses; experiments were *in vivo*; the experiments evaluated use of BAS in cattle; these were stated to be randomized; had appropriate analysis of data; contained sufficient data to determine the effect size for production outcomes (e.g., the number of cattle or pens in each treatment and control group); had a measure of effect so that the data were amenable to effect size (**ES**) analysis for continuous data as standardized mean difference (**SMD**) or ES; and had a measure of variance (SE or SD) for each effect estimate or a *P*-value. For dichotomized data, the number affected and not affected in treatment and control comparisons were required for analysis. Experiments that could not be adequately interpreted or used purposive and non-representative sampling methods were excluded. However, almost half the experiments (n = 6) were pseudo-replicated, and this effect was evaluated.

Fig. 1 depicts a PRISMA diagram (Page et al., 2021) of the flow of data collection for the meta-analysis. The search identified 506 records of which 473 were excluded before screening for the following reasons: the abstract was in English but the full article was not in English (n = 1); the record was duplicated (n = 25); the record was marked as being off-topic by automation tools (n = 101); or the record was identified as being off-topic by manual review of the title, that is did not use BAS or related pheromones (n = 346). Of the 33 records that reached screening, only 21 reports reached the second screening step of full report retrieval and assessment. One study (Osella et al., 2018) that evaluated BAS in lactating dairy cows had no consistent outcomes with other studies and was excluded. A total of 13 reports or studies with up to 18 experiment comparisons were included in the meta-analysis. A list of articles excluded with the reason is provided in Table A1. In the case of production variables reported over interim intervals, that is ADG, DMI, and G:F, only results for the entire experimental interval were evaluated. For the health data, mortality and diarrhea were clearly defined; however, for respiratory disease we used a definition from Colombo et al. (2020) based on cattle treated with antibiotics for the condition. In cases where metabolic measures that were used as indicators of stress were taken over multiple time-points, the most consistent times among experiments were used in the evaluation. Values presented only in figures were extracted using an image extraction software (https://automeris.io/WebPlotDigitizer/).

Data were extracted from each of the experiments that met the inclusion criteria and were audited by three reviewers. The descriptive data extracted included aspects of experimental design and details

about the cattle used. Design details include the number of experimental units per control and treatment, the experimental unit (individual animal or pen), whether the experiment was pseudo-replicated, the company from which the BAS treatment was sourced, the type of control, the stress planned to be moderated, the number of days on trial, the total dose of the treatment, and the delivery methods of the treatment (Table 1). Animal details included: breed, sex, age at start of the experiment, production system (dairy calf, pasture, or feedlot)(Table 1). Data was only extracted for outcomes that had n ≥3 studies.

Figure 1. PRISMA flow diagram (adapted from Page et al., 2021) of the systematic review from initial search and screening to final selection of publications to be included in the meta-analysis on bovine appeasing substance in cattle. The *n* refers to the number of records for identification and screening that includes experimental articles, abstracts, books, review papers, theses, patents, and other records.



3.2 Statistical analysis

Initial data exploration included production of basic statistics using Stata (Version 18, StataCorp. LP, College Station, TX) to examine the data for possible errors and to estimate the means and measures of dispersion. Normality of the data was examined by visual and statistical appraisal for continuous variables.

The following production outcomes were suitable for analysis: final BW, ADG, DMI, and G:F. Initial BW was also analyzed but is not considered as an outcome variable. The health metrics suitable were incidence of diarrhea, incidence of respiratory disease, and mortality incidence. The only sufficient stress markers were blood and hair cortisol, blood haptoglobin, and blood non-esterified fatty acid (**NEFA**) concentrations. Antibody levels against IBR-1, BVD, BRSV, and PI-3 were able to be evaluated.

Differences in continuous responses that were measured in identical units (production and stress outcomes) were evaluated by SMD [Stata *esize(mdiff*)] and by ES analysis when units measured varied (virus outcomes) using Stata *esize(cohend, holkinse)*. The SMD estimates were pooled initially using the DerSimonian and Laird (1986) random effects models (**D&L**). Only random effects models were used, as previous work concluded that when there was uncertainty in the evaluative units caused by clustering of observations, the random effects model was appropriate (White and Thomas, 2005). For the dichotomized health data (diarrhea, respiratory disease, and mortality), Stata *meta esize(rdiff)* was used to determine the risk difference between groups. When experiments were nested within studies, which occurred for all outcomes except NEFA and virus antibodies, Stata *meta multilevel* (Goldstein et al., 2000; Thompson et al., 2001) was used to provide a more correct measure of the SMD using the units appropriate to the single level analysis. The SMD estimate for NEFA and the ES estimates for the virus outcomes are provided as Cohen's d estimates from the D&L, in the case of antibodies against IBR-1 and PI-3 the D&L is calculated with a maximum likelihood estimation. The respective SMD, ES, or risk difference for each outcome are presented using Stata *meta forestplot*.

Variations among the experimental comparison level SMD were assessed using a chi-squared (**Q**) test of heterogeneity. Heterogeneity in experimental comparisons reflects underlying differences in clinical diversity of the research site and interventions, differences in experimental design and analytical methods, and statistical variation around responses. The clinical diversity of the site includes all the non-study design aspects of variation, such as facility design, environment, and cattle management that may be measured and controlled for in meta-analysis but are often not reported or measured. Identifying the presence and sources of heterogeneity improves understanding of the responses to the interventions used. An α level of 0.1 was used because of the relatively poor power of the χ^2 test to detect heterogeneity among small numbers of trials (Egger and Smith, 2001). Heterogeneity of results among the study and also experimental comparisons were quantified using the I^2 statistic (Higgins and Thompson, 2002) and the H^2 statistic (Lin et al., 2017) and were reported as the total estimates from the study and experiment levels. The I^2 provides an estimate of the proportion of the true variance of effects of the treatment, that is the true variance, tau-squared ($\boldsymbol{\tau^2}$) divided by the total variance observed in the comparison (Borenstein et al., 2017) that reflect measurement error. The H^2 provides an estimate of heterogeneity and where H is interpreted as the ratio of the SD of the estimated overall ES from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis in which an H^2 of 1 indicates no heterogeneity (Lin et al., 2017). The I^2 is reported for single level analysis for NEFA and the virus outcomes with the Cochrane's I^2 and for the multilevel (all other outcomes) using the total study I^2 Higgins-Thompson estimation using Stata *estat heterogeneity* based on methods developed by Nakagawa and Santos (2012). While *estat heterogeneity* provides estimates of heterogeneity at the study and experiment level, we report the total heterogeneity only. For these outcomes that were suitable for multilevel models we also assessed the univariate I^2 but these are not reported. Negative values of I^2 are assigned a value of 0, consequently the value I^2 lies between 0 and 100%. An I^2 value between 0 and 40% might not be

important, 30 to 60% may represent moderate heterogeneity, 50 to 90% might represent substantial heterogeneity, and 75 to 100% might represent considerable heterogeneity (Higgins and Green, 2011). We provide I^2, τ^2 and H^2 to allow readers the opportunity to evaluate those metrics. The dichotomized data were also evaluated for heterogeneity using L'abbe plots (Stata *meta labbeplot*)(data not shown).

A key focus of meta-analysis is to identify and understand the sources of heterogeneity or variation of response among comparisons. However, given the limited number of experiments available the only meta-regression analyses suitable were for study design to investigate the effects of pseudo-replication and to test the effect of days on trial and the combined effects of these. Statistical confounding was assessed as present if the co-efficient of the predictor variable changed by more than 25%. The effects of a pseudo-replicated experiment by Cappellozza et al. (2020) with a large increase in final BW on treatment effect estimates was investigated using the Stata *meta summarize leaveoneout* option and this experiment was consequently removed.

Data were evaluated for potential publication bias using *meta funnelplot* with the contours of significance identified. Funnel plots are a simple scatter plot of the intervention effect estimates from individual comparisons plotted against comparison precision. The name "*funnel plot*" arises because precision of the intervention effect increases as the size and precision of a comparison increases. Effect estimates from comparisons with a small number of animal units will scatter more widely at the bottom of the graph and the spread narrows for those with higher numbers of units. In the absence of bias, the plot should approximately resemble a symmetrical (inverted) funnel. A power calculation for a sample size was done using Stata *power* for a Chi-square test.

4. Results and discussion

The literature that was amenable to quantitative review on BAS use in cattle was reasonably limited with only 13 suitable studies (Fig. 1; Table 1) but substantial considering that BAS is an emerging technology with all experiments used being published after the year 2019. From these the analysis of 15 outcome variables and initial BW is sizable as it is not expected that each experiment would report the same outcomes, especially considering the range of cattle classes and stresses (Table 1). The experiments varied from using 50 to 954 total head and 10 experiments were conducted using pen as the unit of interest. There were six experiments that were pseudo-replicated, that is, had a single treatment and control group separately housed or pastured. Controlling for this study flaw was not significant ($P > 0.4$); however, some confounding was indicated by more than a 20% change in coefficient values when evaluated in the multilevel regression analysis (data not shown).

The initial BW varied from 40 to 638 kg (data not shown) indicating a considerable difference in weight and age of cattle evaluated. Cattle classes ranged from male, female, and mixed *Bos taurus* and *Bos indicus* breeds from dairy calves at 0 d of age to adult beef cattle at 810 d of age at experiment d-0, with 9 experiments not stating age at study commencement. Experiments were primarily conducted at weaning and at feedlot entry, with one evaluating transport stress, and three evaluating effects in pasture-fed cattle (Table 1).

Table 1. Summary of descriptive details of each experiment included in the meta-analysis on intervention with bovine appeasing hormone (BAS). Details include: authors, the number of units (individual animals or pens) in an experiment and their unit of measure, whether the experiment was pseudo-replicated, class of stock, that is breed, sex and age at d-0 of the experiment, treatment source, control applications, type of stress, diet type, duration of the study, total treatment dose, and application site or method.

Authors	n of units		Unit of measure	Pseudo[1]	Breed	Sex	Age at d 0 (d)	Treatment source[2]	Control	Stress	Diet type	DUR (d)	Total BAS dose (ml)	Delivery region
	BAS	**Control**												
Angeli et al. (2020)	70	70	Individual	N	Gir × Holstein	F	0	IRSEA	DEGEE	Neonatal & dehorning	Dairy calf	70	30[3]	Nuchal
Bringhenti et al. (2023)	205	205	Individual	N	Holstein	F	1	Fera D&B	None	Neonatal, weaning & group housing	Dairy calf	100	30[4]	Nuchal and muzzle
Cappellozza et al. (2020) -1	60	60	Individual	Y	Nelore	NS	219	Nutricorp	Water	Weaning	Pasture	45	5	Nuchal
Cappellozza et al. (2020) -2	422	413	Individual	Y	Nelore-inf	NS	NS	Nutricorp	Water	Transport	Feedlot	1	5	Nuchal
Colombo et al. (2020)	12	12	Pen	N	Angus-inf	M	NS	IRSEA	DEGEE	Feedlot entry & vaccination	Feedlot	46	5	Nuchal
Cooke et al. (2020) -1	94	92	Individual	Y	Nelore-inf	Mix	211	Nutricorp	Water	Weaning	Pasture	45	5	Nuchal
Cooke et al. (2020) -2	70	70	Individual	Y	Nelore-inf	M	810	Nutricorp	Water	Feedlot entry & vaccination	Feedlot	45	5	Nuchal
Fonseca et al. (2021) -1	3	3	Pen	N	Nelore	M	NS	IRSEA	DEGEE	Transport, vaccination, & feedlot entry	Feedlot	108	5[5]	Nuchal
Fonseca et al. (2021) -2	3	3	Pen	N	Nelore	M	NS	IRSEA	DEGEE	Transport vaccination, & feedlot entry	Feedlot	108	5[5]	Nuchal
Hervet et al. (2021)	159	106	Individual	Y	Charolais	M	317	IRSEA	DEGEE	Weaning, transport & feedlot entry	Feedlot	30	5	Forehead
Kimbrough (2024) -1	2	2	Pen	N	Beef NS	F	NS	Fera D&B	None	Feedlot entry & vaccination	Feedlot	63	20[6]	Nuchal and rostral
Kimbrough (2024) -2	2	2	Pen	N	Beef NS	F	NS	Fera D&B	Tulathromycin	Feedlot entry & vaccination	Feedlot	63	20[6]	Nuchal and rostral
Kvamme et al. (2024)	9	9	Pen	N	Angus × Hereford	Mix	160	IRSEA	DEGEE	Weaning, vaccination., transport & feedlot entry	Feedlot	90	20[7]	Nuchal
Mackey et al. (2024) -1	6	6	Pen	N	Angus-inf	M	NS	Fera D&B	None	Feedlot	Feedlot	64	120[8]	Oiler
Mackey et al. (2024) -2	8	8	Pen	N	Angus-inf	M	NS	Fera D&B	Mineral oil	Feedlot	Feedlot	7	120[8]	Oiler
Pickett et al. (2024)	5	5	Pen	N	Angus-inf	M	NS	Fera D&B	Mineral oil	Feedlot entry & vaccination	Feedlot	60	20[9]	Nuchal and muzzle
Schubach et al. (2020)	4	4	Pen	N	British × Nelore	Mix	233	IRSEA	DEGEE	Weaning, vaccination & feedlot entry	Feedlot	42	5	Nuchal

Vieira et al. (2023)	43	43	Individual	Y	Nelore	Mix	240	IRSEA	Saline solution	Weaning & vaccination	Pasture	100	5	Nuchal

inf = influenced; n = No; Y = yes; F = female; M = male; NS Not stated; DEGEE = Diethylene glycol monoethyl ether; DUR = duration of experiment

[1]Pseudo = evaluated as pseudo-replicated.

[2]Company that BAS treatment was sourced. IRSEA = Institut De Recherche En Sémiochimie Et Éthologie Appliquée (Quartier Salignan, France); Nutricorp (Araras, SP, Brazil); Fera D&B = Fera Diagnostics and Biologicals (College Station, TX).

[3]Six treatments were applied at 14-d intervals at a 5 ml dose rate.

[4]Six treatments in total, the first 5 at 14-d intervals with 2.5 ml of treatment applied to the nuchal area and 2.5 ml applied to the muzzle at each application.

[5]Half the cattle also received a second dose as the study was a 2x2 factorial with BAS vs Control at loading and feedlot entry.

[6]Delivered at d 0 and d 14 of the experiment, with 5 ml delivered to the nuchal and 5 ml to the rostral region at each application.

[7]Four treatments were applied at 5 ml doses at 14-d intervals.

[8]Dose was delivered over a 7-d period.

[9]Delivered at d 0 and d 14 of the experiment, with 5 ml delivered to the nuchal and 5 ml to the muzzle at each application.

Table 2. Raw means±SD and numbers of experimental units of measure for production outcomes including initial BW, final BW, ADG, DMI, and G:F for bovine appeasing substance (BAS) treated and control cattle.

Author	n		Initial BW (kg)		Final BW (kg)		ADG (kg)		DMI (kg)		G:F (kg/kg)	
	BAS	Control	BAS	Control	BAS	Control	BAS	Control	BAS	Control	BAS	Control
Angeli et al. (2020)	70	70	41±10	40±10	95±10	91±10	0.78±1.72	0.73±1.72				
Bringhenti et al. (2023)	39	39			132±20	127±20						
Cappellozza et al. (2020) -1	60	60	191±26	192±26	Not included			1.45±0.40				
Cappellozza et al. (2020) -2	422	413										
Colombo et al. (2020)	12	12	261±9	262±9	295±9	291±9	1.01±0.17	0.86± 0.17	5.0±0.73	4.9±0.73	0.17±0.03	0.14±0.03
Cooke et al. (2020) -1	94	92	235±20	235±20	251±9	249±9	0.36±0.19	0.29± 0.19				
Cooke et al. (2020) -2	70	70	333±16	333±16	400±16	404±16	1.50±0.33	1.58±0.33				
Fonseca et al. (2021) -1	3	3	341±5	341±5	471±8	457±8			9.3±0.22	9.0±0.22	0.17±0.06	0.16±0.06
Fonseca et al. (2021) -2	3	3	341±5	341±5	467±8	462±8			9.4±0.22	9.0±0.22	0.16±0.06	0.17±0.06
Hervet et al. (2001)	159	106	368±26	372±36			Not included					
Kimbrough (2024) -1	2	2	187±5	188±5	250±6	247± 6	1.00±0.07	0.98± 0.07	4.2±0.34	4.7±0.34	0.24±0.01	0.21±0.01
Kimbrough (2024) -2	2	2	186±5	188±5	255±6	258± 6	1.09±0.07	1.13±0.07	4.9±0.34	4.9±0.34	0.22±0.01	0.23±0.01
Kvamme et al. (2024)	9	9	217±8	217±8	271±11	271±11	1.27±0.21	1.28±0.21	6.2±0.51	6.4±0.51	0.20±0.01	0.20±0.01
Mackey et al. (2024) -1	6	6	635±20	638±20	646±17	654±17	0.83±0.61	1.07± 0.61				
Mackey et al. (2024) -2	8	8	599±20	601±20	600±17	602±17	0.27±0.60	0.32±0.60				
Pickett et al. (2024)	5	5	200±4	199±4	254±9	248± 9	0.88±0.12	0.94±0.12	4.8±0.21	4.8±0.21	0.17±0.02	0.18±0.02
Schubach et al. (2020)	4	4	185±7	185±7	231±8	228± 8	1.08±0.10	1.04± 0.10	6.5±0.20	6.4±0.20	0.17±0.01	0.16±0.01
Vieira et al. (2023)	43	43	198±6	198±6	196±6	195±6	-0.13±0.46	-0.15±0.46				

Table 3. Summary of standardized mean difference and heterogeneity estimates for production data for cattle treated with bovine appeasing substance.

Outcome	n of studies	n of experiments	Standardized mean difference[1] (95% CI)	I^2 estimated heterogeneity (%)[2]	Tau-squared[3]	H^2 estimate[4]	*P*-value
Initial BW, kg	12	16	-0.358 (-1.244, 1.539)	0	0	1.0	0.434
Final BW, kg	11	15	1.313 (-1.303, 3.930)	84.0	18.9	6.3	0.325
ADG, kg/d	10	13	0.043 (-0.108, 0.194)	0	0	1.0	0.578
DMI, kg	6	8	0.058 (-0.255, 0.371)	0	0	1.0	0.717
G:F, kg/kg	6	8	0.008 (-0.069, 0.085)	0	0	1.0	0.837

[1]Effect size is a standardized mean difference to statistically compare bovine appeasing substance vs. control differences among experiments. Effect size estimates are provided from the multilevel method that accounts for the nesting of multiple experiment comparisons within studies using the effect size from the DerSimonian and Laird (D&L) model.

[2]I^2 is a measure of variation beyond chance among studies and among experiments included in the meta-analysis. The I^2 reported is calculated from the multilevel model using the total I^2 Higgins-Thompson estimation at the study and experiment levels and is a value between 0 and 100%.

[3]Tau-squared is an estimate of between-study and between-experiment variance and is calculated from the multilevel model. The tau-squared reported is the total of both the between-study and between-experiment estimates.

[4]The H^2 provides an estimate of heterogeneity. The *H* estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an $H^2 = 1$ is homogenous.

Table 4. Summary of risk difference and heterogeneity estimates for diarrhea, respiratory disease, and mortality for cattle treated with bovine appeasing substance.

Outcome	n of studies	N of experiments	Risk Difference (95% CI)[1]	I^2 estimated heterogeneity (%)[2]	Tau-squared[3]	H^2 estimate[4]	*P*-value
Diarrhea	3	4	-0.029 (-0.100, 0.042)	0	0	1.0	0.427
Respiratory disease	5	6	0.027 (-0.040, 0.093)	0	0	1.0	0.434
Mortality	5	6	-0.016 (-0.062, 0.030)	0	0	1.0	0.502

[1]Estimated risk difference to compare disease incidence for bovine appeasing substance vs. control differences among experiments. Risk difference estimates are provided from the multilevel method that accounts for the nesting of multiple experiment comparisons within studies.

[2]I^2 is a measure of variation beyond chance among studies and among experiments included in the meta-analysis. The I^2 reported is calculated from the multilevel model using the total I^2 Higgins-Thompson estimation at the study and experiment levels and is a value between 0 and 100%.

[3]Tau-squared is an estimate of between-study and between-experiment variance and is calculated from the multilevel model. The tau-squared reported is the total of both the between-study and between-experiment estimates.

[4]The H^2 provides an estimate of heterogeneity. The *H* estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an $H^2 = 1$ is homogenous.

Table 5. Summary of standardized mean differences and heterogeneity estimates for markers of stress for cattle treated with bovine appeasing substance.

Outcome	Model level	n of studies	n of experiments	Standardized mean difference (95% CI)[1]	I^2 estimated heterogeneity (%)[2]	Tau-squared[3]	H^2 estimate[4]	*P*-value
Blood cortisol, ng/ml	Multilevel	6	8	-3.845 (-7.600, -0.090)	93.0	20.5	14.4	0.045
Hair cortisol, pg/mg of hair	Multilevel	4	6	-0.038 (-0.364, 0.288)	0	0	1.0	0.821
Blood haptoglobin, mg/ml	Multilevel	7	9	-0.048 (-0.212, 0.115)	0	0	1.0	0.563
Blood non-esterified fatty acids, µEq/L	Univariate	3	3	0.056 (-0.038, 0.150)	95.5	0.007	22.3	0.240

[1]Effect size is a standardized mean difference to statistically compare bovine appeasing substance vs. control differences among studies or experiments. For non-esterified fatty acids, the effect size estimates are provided at the univariate level as there are no experiments nested within studies. For haptoglobin and cortisol outcomes the effect size estimates are from the multilevel method that accounts for the nesting of multiple experiment comparisons within studies using the effect size from the DerSimonian and Laird (D&L) model.

[2]I^2 is a measure of variation beyond chance among studies and among experiments included in the meta-analysis. For non-esterified fatty acids, Cochrane's I^2 is reported from a univariate model. For haptoglobin and cortisol outcomes the I^2 reported is calculated from the multilevel model using the total I^2 Higgins-Thompson estimation at the study and experiment levels and is a value between 0 and 100%.

[3]Tau-squared is an estimate of between-study and between-experiment variance. For haptoglobin and cortisol outcomes the tau-squared reported is the total of both the between-study and between-experiment estimates calculated from the multilevel model. For non-esterified fatty acids, it is reported at the experiment level.

[4]The H^2 provides an estimate of heterogeneity. The *H* estimated from the multilevel model for haptoglobin and cortisol outcomes and is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an $H^2 = 1$ is homogenous. For non-esterified fatty acids, it is reported at the experiment level.

Table 6. Summary of effect size and heterogeneity estimates for antibodies for disease for cattle treated with bovine appeasing substance.

Outcome	n of studies and experiments	Effect size (95% CI)	I^2 estimated heterogeneity, %[3]	Tau-squared[4]	H^2 estimate[5]	*P*-value
Infectious bovine rhinotracheitis-1	4	0.047 (-0.300, 0.394)[1]	0	0	1.00	0.790
Bovine viral diarrhea	6	0.295 (-0.269, 0.860)[2]	46.7	0.21	1.88	0.305
Bovine respiratory syncytial virus	3	0.034 (-1.055, 1.123)[2]	68.3	0.62	3.16	0.951
Parainfluenza virus-3	4	0.048 (-0.916, 1.011)[1]	79.5	0.72	4.89	0.923

[1]Effect size is the effect size (z-score) to statistically compare bovine appeasing substance vs. control differences among studies where units of measurement are not consistently reported. Effect size estimates are provided as Cohen's d using the DerSimonian and Laird (D&L) method calculated with a maximum likelihood estimation as there are no experiments nested within studies.

[2]Effect size is the effect size (z-score) to statistically compare bovine appeasing substance vs. control differences among studies where units of measurement are not consistently reported. Effect size estimates are provided as Cohen's d using the D&L method as there are no experiments nested within studies.

[3]I^2 is a measure of variation beyond chance among experiments included in the meta-analysis. Cochrane's I^2 is reported from univariate D&L models and is a value between 0 and 100%.

[4]Tau-squared is an estimate of between-experiment variance.

[5]The H^2 provides an estimate of heterogeneity. The *H* estimated from the DerSimonian and Laird (1986) model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an $H^2 = 1$ is homogenous.

Choice of control substances varied and included: none (n = 3), diethylene glycol monoethyl ether (DEGEE; n = 7) which is the carrier for BAS, mineral oil (n = 2), water (n = 4), saline solution (n = 1), and tulathromycin (n = 1). Treatment was sourced from one of three manufacturers, Institut De Recherche En Sémiochimie Et Éthologie Appliquée (**IRSEA**; Quartier Salignan, France; n = 8), Nutricorp (Araras, SP, Brazil; n = 4), or Fera Diagnostics and Biologicals (College Station, TX; n = 6). All but the two experiments by Mackey et al. (2024) administered the BAS topically to the skin. Mackey et al. (2024) allowed the cattle to self-administer the BAS with an oiler. In general, when the BAS was sourced from IRSEA or Nutricorp a 5 ml dose was applied to the nuchal region only, either once or at 14-d intervals. Whereas from Fera Diagnostics and Biologicals the BAS was applied at 2.5- or 5-ml doses to both the nuchal and the muzzle or rostral regions and more than once. Application of the BAS more than once by 8 of the 18 experiments resulted in between 5 and 120 ml of total BAS being applied over the duration of the respective experiments. However, there were not enough different total dose rates over consistent durations to evaluate this effect. It is also likely to have been confounded by BW. The spread of duration of the experiments which ranged from 1 to 108 d was amenable to exploration through meta-regression but had no effect ($P > 0.2$); however, the transport stress experiment by Cappellozza et al. (2020) experiment 2 that was only for one day was influential on results.

The number of experimental units contributing from the treatment and control groups and their respective means±SD for each experiment that contributed production variables are reported in Table 2 and those for markers of stress, in Table A2. The raw incidence data for the health metrics is in Table A3 and virus results in Table A4. We report the results of multilevel analysis unless noted as follows: initial BW and production (Table 3), health (Table 4), stress (Table 5), and viruses (Table 6). Figures for the forest plots and funnel plots not reported in the body of this paper are provided in the Appendix (Fig. A1-A11). An evaluation of contour enhanced funnel plots did not identify evidence of publication bias for outcomes (Fig. 2 and Fig. A11). There was no significant difference in initial BW ($P = 0.434$; Table 3), indicating no initial allocation bias.

Final BW was not increased for treated cattle on multilevel analysis ($P = 0.325$; Table 3 and Fig. 3). The final model did not include an experiment by Cappellozza et al. (2020), that had a large increase in final BW, as it was identified as an influential pseudo-replicated experiment. Individual experiments by Angeli et al. (2020), Cooke et al. (2020) experiment 1, and Fonseca et al. (2021) experiment 1 showed positive final BW responses to treatment (Fig. 3) and the I^2 was 84.0%, suggesting BAS intervention may be beneficial for final BW if more consistent experiments were conducted. Examination at the univariate level, which is less robust than at the multilevel showed treatment produced cattle 1.72 kg heavier (95% CI 0.06, 3.39; $P = 0.043$; Figure 3) in final BW, further supporting a benefit, albeit small, in intervention and a need for larger replicated experiments.

The ADG results were less frequently reported than those of final BW (n = 13 experiments from 10 studies) and were not significant but consistent ($P = 0.578$; $I^2 = 0\%$; Table 3 and Fig. 4). However, Cappellozza et al. (2020) experiment 1 in calves at weaning on pasture, Colombo et al. (2020) studying feedlot performance, Cooke et al. (2020) experiment 1 in beef bulls at weaning found significant increases in ADG with treatment (Fig. 4). Although evaluation over the entire experimental periods showed no overall improvement in production outcomes Angeli et al. (2020) reported improved ADG between experiment days 42 and 56 and Cooke et al. (2020) between 0 and 15 days after feedlot entry which could suggest time of measurement may influence outcomes. Data were insufficient to evaluate this but the I^2 indicates good consistency in results despite the varied designs.

There was no significant overall effect on DMI in the limited evaluation provided by 8 experiments in 6 studies ($P = 0.717$; Table 3). Only the 2 experiments from the study by Fonseca et al. (2021) found a significant increase in DMI in Nelore bulls fed in a feedlot. Similarly for G:F that also only evaluated 8 experiments in 6 studies, overall treatment did not increase G:F in the multilevel model ($P = 0.837$; Table 3 and Fig. 5) but the SMD approached significance in the univariate model (SMD = 0.008; 95% CI -0.003, 0.019; $P = 0.148$; Fig. 5). Colombo et al. (2020) found a significant increase in G:F in Angus

steers fed in a feedlot as did one of the two experiments by Kimbrough (2024) in beef heifers in a feedlot.

Stress has immuno-metabolic effects in cattle which can lead to an increased risk of health disorders (Chen et al., 2015). There were limited studies and experiments that evaluated disease and mortality with a maximum of 6 experiment comparisons and 5 studies (Table 4). Overall evaluations of the effects of treatment on diarrhea, respiratory disease, and mortality (Fig. 6) did not identify any significant treatment effects ($P > 0.3$; Table 4). Experiments by both Bringhenti et al. (2023) and Pickett et al. (2024) showed decreased mortality with treatment (Fig. 6). Bringhenti et al. (2023) also saw a reduction in diarrhea with treatment. To detect a reduction in mortality or other health outcomes with an ES of 40% with a study power of 0.6, an α of 0.05 pen studies on 8 lots, each with 6 randomised pens containing 80 head would be required. The study power is driven by number of lots or pens per lot rather than the number of cattle in the pen. Consequently, we consider that more experiments are needed given that 33.3% of experiments in this small dataset with a combined univariate weighting of 28.9% saw reductions in mortality and 66.7% with a combined univariate weighting of 63.9% had positive point directions for mortality (Fig. 6). Respiratory disease increased with treatment as defined by receiving one antimicrobial treatment for this disease in the experiment by Colombo et al. (2020) and Pickett et al. (2024). Colombo et al. (2020) speculated that earlier detection of BRD signs in BAS treated cattle may have occurred based on the BAS having a calming effect on cattle and lowering their natural defence behaviours of disguising abnormal behaviours during illness (Weary et al., 2009). In the case of Colombo et al. (2020) a lower number of BAS cattle required a second dose of antibiotics. These considerations highlight the need to explore and assess more than a single morbidity metric when trying to mitigate respiratory disease, especially when the mode of action of the intervention is not precisely known. Ethical and cost considerations are crucial, unfortunately the data were insufficient to assess these. Numerical reductions in total cost of pharmaceuticals in USD for treating respiratory disease occurred for intervention with BAS (Angeli et al., 2020; Pickett et al., 2024), supporting the need for more work in this field.

Blood cortisol was reduced by treatment in the 8 experiments from 6 studies reporting this ($P = 0.045$; $I^2 = 93.0\%$; Table 5; Fig. 7). The results had substantial heterogeneity even when the effects of experiments within study were evaluated ($I^2 = 93.0\%$; Table 5; Fig. 7). Although the exact mode of action of BAS is not known (Vieira et al., 2023) the target organs involved in pheromone perception include the main olfactory epithelium and vomeronasal organ (Kekan et al., 2017). The vomeronasal organ is located between the mouth and nose of mammals and is related to pheromone recognition creating a neuroendocrine cascade stimulating the hypothalamus to produce a neuro-endrocrine response that reduces the perception of a threat (Cappellozza and Cooke, 2022), putatively having a calming effect on the individual as the adrenocortical response is lowered.

The high heterogeneity in blood cortisol may reflect rapid increases in circulating blood cortisol that can confound results elicited by handling for blood sampling (Schubach et al., 2017). Cortisol secretion in cattle is also pulsatile with an ultradian rhythm with mean pulse intervals of approximately 120 min, so single blood samples may not be sufficient for evaluation of interventions (Lefcourt et al., 1993). Hair cortisol is an integrated measure of stress over a period of time as cortisol is gradually accumulated in emerging hair (Schubach et al., 2017) and has recently been carefully evaluated for diagnostic value in cattle by determining whether concentrations reflected repeated challenge with adrenocorticotrophic hormone injections and correlated with cortisol levels in saliva (Heimbürge et al., 2020). Hair samples were reliable indicators of stress, ideally when taken within four weeks of the end of the stress (Heimbürge et al., 2020), while correlations of the area under the curve with saliva were high, correlations with blood cortisol were not evaluated. The 6 experiments in 4 studies did not find a significant reduction in hair cortisol concentrations ($P = 0.821$; $I^2 = 0\%$; Table 5). Not each of the experiments that reported hair cortisol concentration reported blood cortisol and vice versa, thus consistency in results might not be expected given the sample sizes.

Increased adrenocortical function has been positively associated with circulating haptoglobin concentrations in cattle (Cooke and Bohnert, 2011; Cooke et al., 2012). Thus, we hypothesized blood haptoglobin concentrations would be decreased if the blood cortisol was decreased; this did not occur, and results were homogeneous (P = 0.538; Table 5; Fig. 8). The overlap in experiments that reported both circulating haptoglobin and cortisol was high but not identical. Variability in the time of sampling may limit the potential to detect positive changes as cortisol traditionally triggers an inflammatory cascade between 48 to 72 h after the cortisol peak (Cappellozza and Cooke, 2022). Not all experiments blood sampled within this period and the cascade can be transient and temporary (Cappellozza and Cooke, 2022). For experiments such as Cooke et al. (2020) that sampled at more than one time point we used the sample that was most consistent with other experiments, d 15 in this case.

Elevated circulating cortisol concentrations leads to among other metabolic responses, tissue mobilization (Chen et al., 2015), resulting in the release of NEFA. Despite a positive effect on blood cortisol with treatment, treatment had no effect on blood NEFA (Table 5) but was only reported in 3 experiments. The haptoglobin and NEFA responses suggest that inflammatory responses in the cattle were not altered by treatment.

Stress-induced metabolic and inflammatory challenges not only predispose cattle to BVD directly but also reduce vaccine efficacy (Cooke, 2017). Antibodies for IBR-1 (4 studies), BVD (6 studies), BRSV (3 studies), and PI-3 (4 studies) were evaluated in treated and control cattle (Table 6). For IBR-1 results were not significant (P = 0.790) and consistent (I^2 <0.01%). No study identified a significant effect of treatment on IBR-1 antibodies; however, Vieira et al. (2023) found a significant increase in BVD antibodies in treated cattle on d 51 after treatment. The antibodies for BVD were not significantly altered by treatment (P = 0.305) and were moderately heterogenous (I^2 = 46.7%). With only 3 studies, antibodies for BRSV were not influenced by treatment (P = 0.951) and were substantially heterogenous (I^2 = 68.3%). Kvamme et al. (2024) found significantly increased antibodies in the control cattle to BRSV on d 64 and 90 and to PI-3 on d 42, 64, and 90 of their study. Antibodies for overall PI-3 were not influenced by treatment (P = 0.923) and were substantially heterogenous (I^2 = 79.5%; Table 6).

One of the most challenging aspects of this paper was the matter of pseudo-replicated experiments. We speculate that the use of pseudo-replication in 6 experiments was attributable to the potential for airborne cross-contamination of treatment groups due to the test product. The impact of pseudo-replication on the validity of research is a matter for some debate (Hurlbert, 1984; Oksanen, 2001; St-Pierre, 2007; Schank and Koehnle, 2009). While we consider that pseudo-replication is undesirable and advise against this (Lean and Lean, 2010), we have accepted these papers and used the number of cattle noted in the paper, rather than assigning a nominal value of n = 1 to the treatment and control observations. This is a limitation of the study; however, the approach used provides the most favorable opportunity to assess an emerging technology. The latter consideration was informed by the lack of significance of study design when evaluated as a covariable. The confounding effect of pseudo-replication may reflect a real effect indicating a less rigorous study design with implications resulting from the different environment of the cattle groups or be a result of the limited number of experiments available for most variables and statistical method limitations for sparse data. The influence of a single pseudo-replicated experiment is evident in the difference in results for final BW with the exclusion of Cappellozza et al. (2020) experiment 1 and supports the need to be very cautious in the use and evaluation of pseudo-replicated experiments.

Responses of cattle to different stresses are complex and multifaceted with unique phenotypic outcomes (Chen et al., 2015). There were several outcome variables such as meat quality, exit velocity from a treatment chute, days to disease detection, and costs of pre-weaning veterinary treatments that could not be evaluated due to the lack of sufficient studies (n ≤3) and inconsistent reporting. Similarly, there were several potential sources of variation that could not be assessed due to the lack of sufficient experiments and may have driven the hypothesis towards the null. For example, the

responses may be different between stress events, *Bos indicus* and *Bos taurus* cattle, number of doses, dosage time relative to stress events, cattle classes, and management systems. Examination of heterogeneity metrics at both the univariate and multivariate level showed a decrease in heterogeneity when the effect of experiment within study (multilevel models) is accounted for indicating that variation at the study level is influential and needs further exploration.

Figure 2. Contour-enhanced funnel plots for (A) final BW (kg), (B) ADG (kg/d), (C) blood cortisol concentrations (ng/ml), and (D) blood haptoglobin concentrations (mg/ml) for cattle treated with bovine appeasing substance. The effect estimates of the experiments are graphed against the standard error of the experiments. Levels of significance for experiments and within the broken lines are 0.01, 0.05, and 0.10.

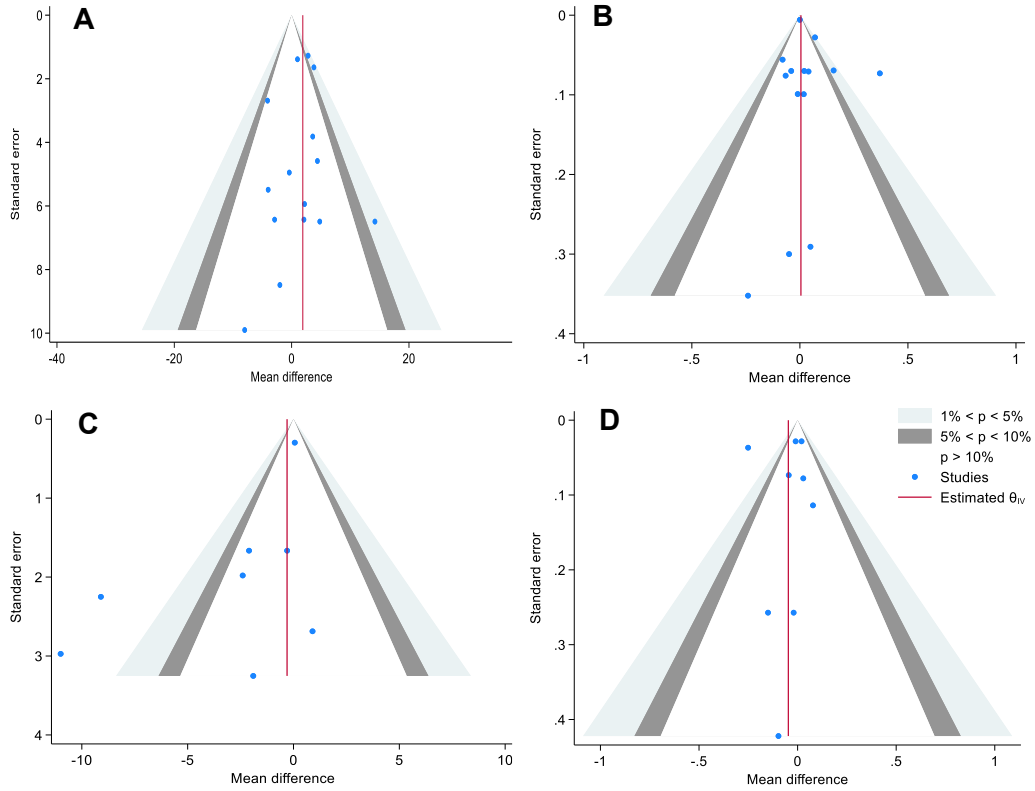
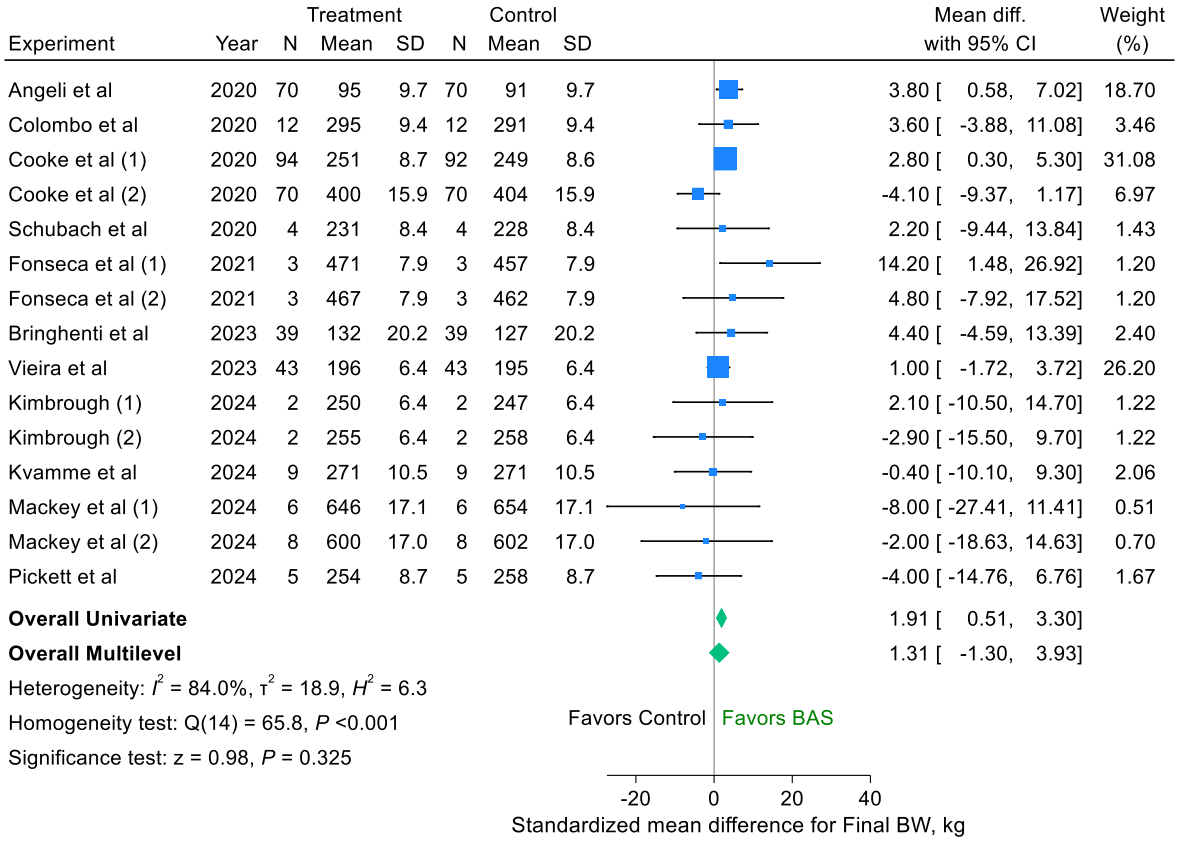
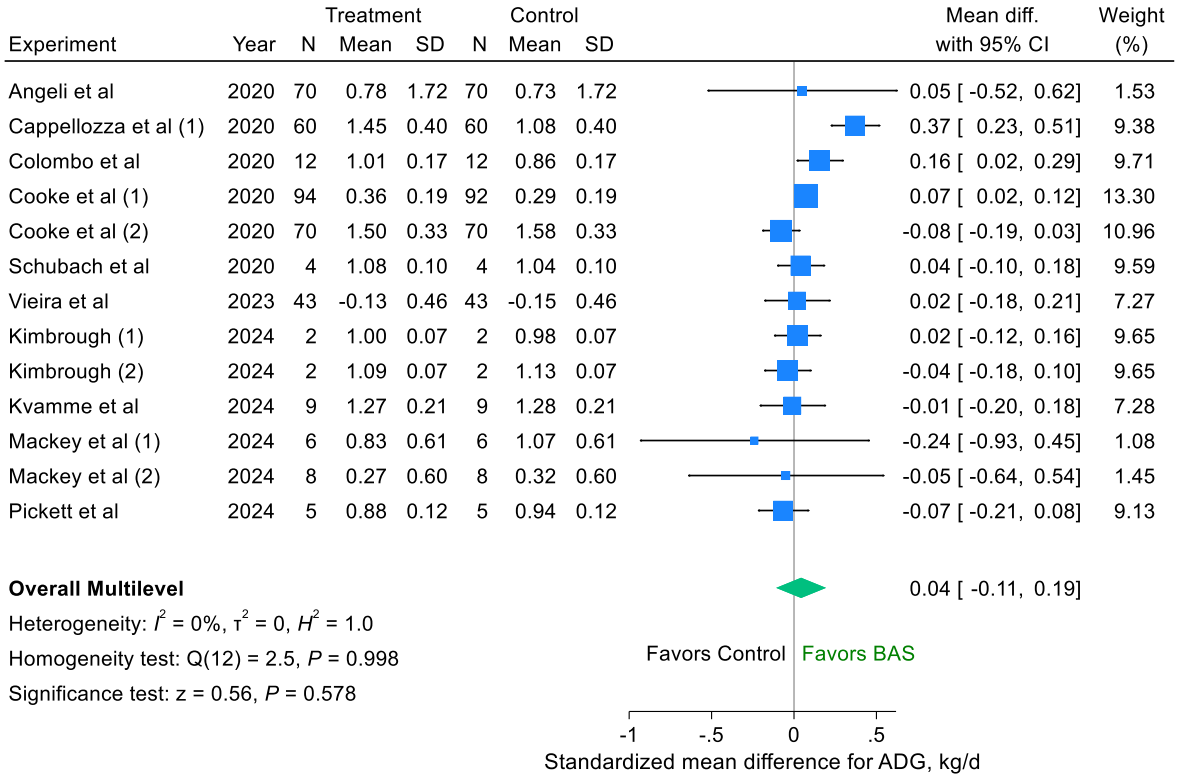


Figure 3. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on the final BW of cattle.



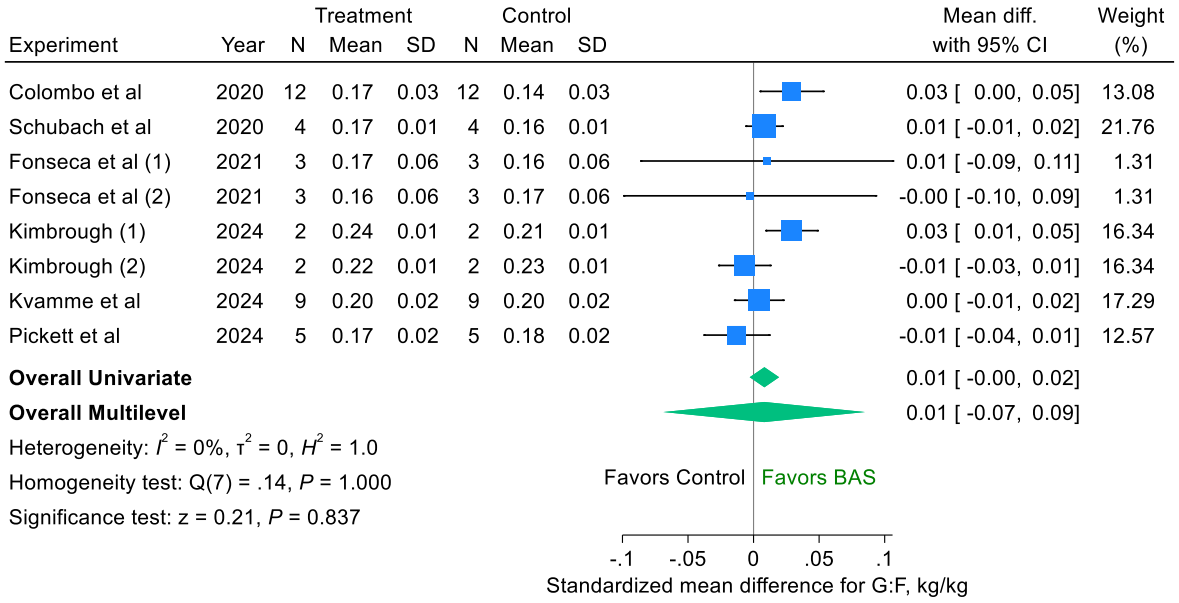
In figure 3, the solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in final BW, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the DerSimonian and Laird (1986) and multilevel meta-analytical random effects models (Goldstein et al., 2000) are indicated by the univariate and multilevel respective diamonds at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.325). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The final BW was considerably heterogeneous as indicated by the overall I^2 of 84.0% estimated from the multilevel model. The τ^2 is the true variance between the total of studies and experiments and is high. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is heterogenous.

Figure 4. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on the ADG of cattle.



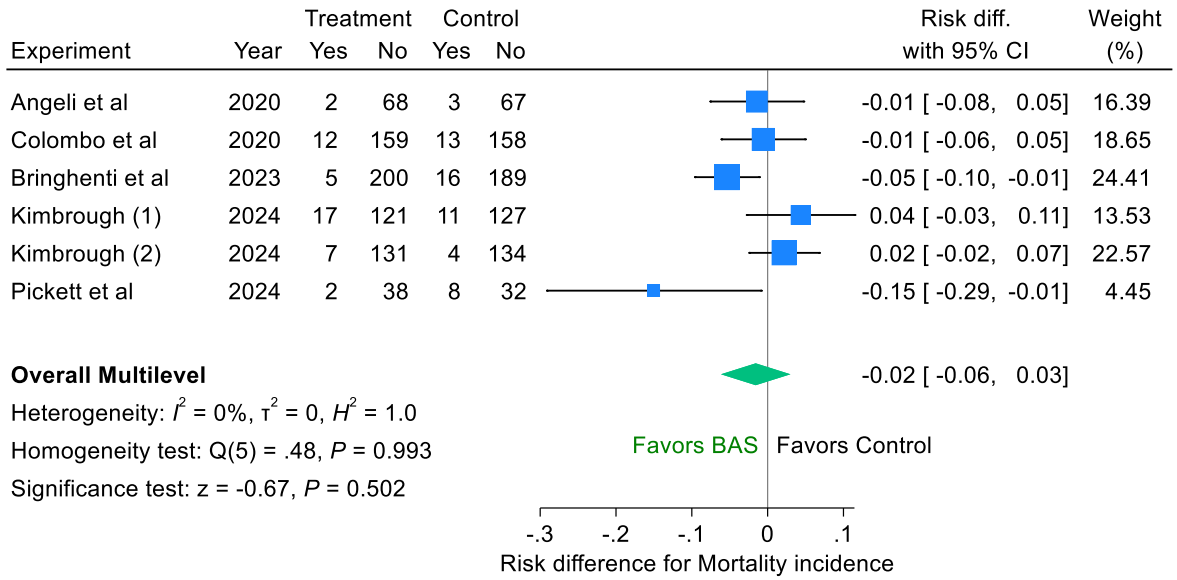
In figure 4, the solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in ADG, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the multilevel meta-analytical random effects model (Goldstein et al., 2000) are indicated by the multilevel diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.578). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The ADG was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure 5. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on the G:F of cattle.



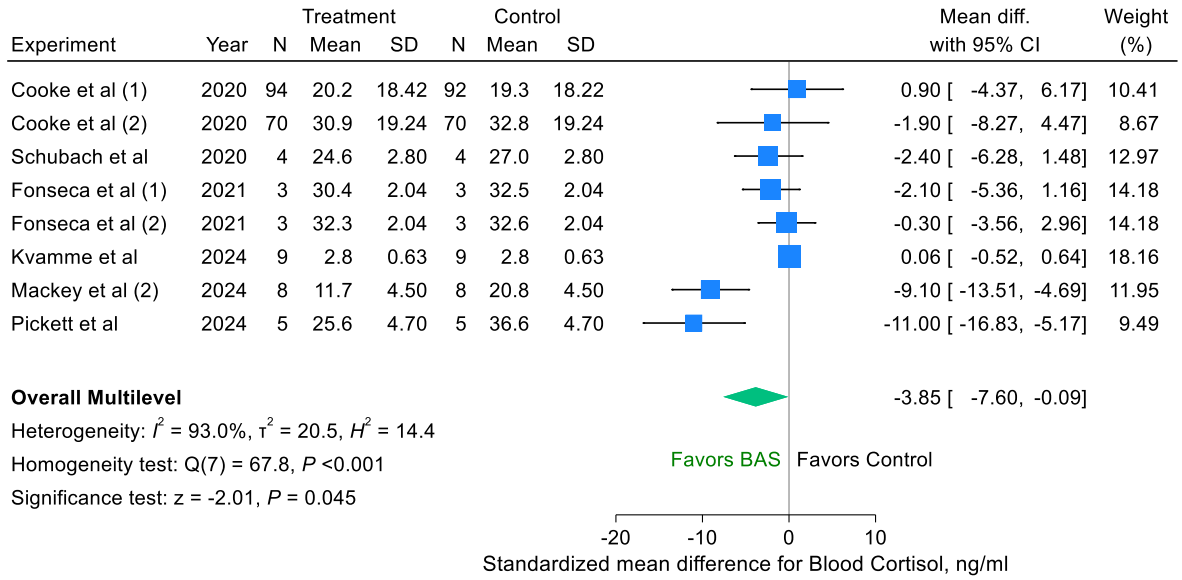
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in G:F, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the DerSimonian and Laird (1986) and multilevel meta-analytical random effects models (Goldstein et al., 2000) are indicated by the univariate and multilevel respective diamonds at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.837). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The G:F was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure 6. Forest plot of the Risk Difference and 95% CI of the effect of bovine appeasing substance intervention on the mortality of cattle.



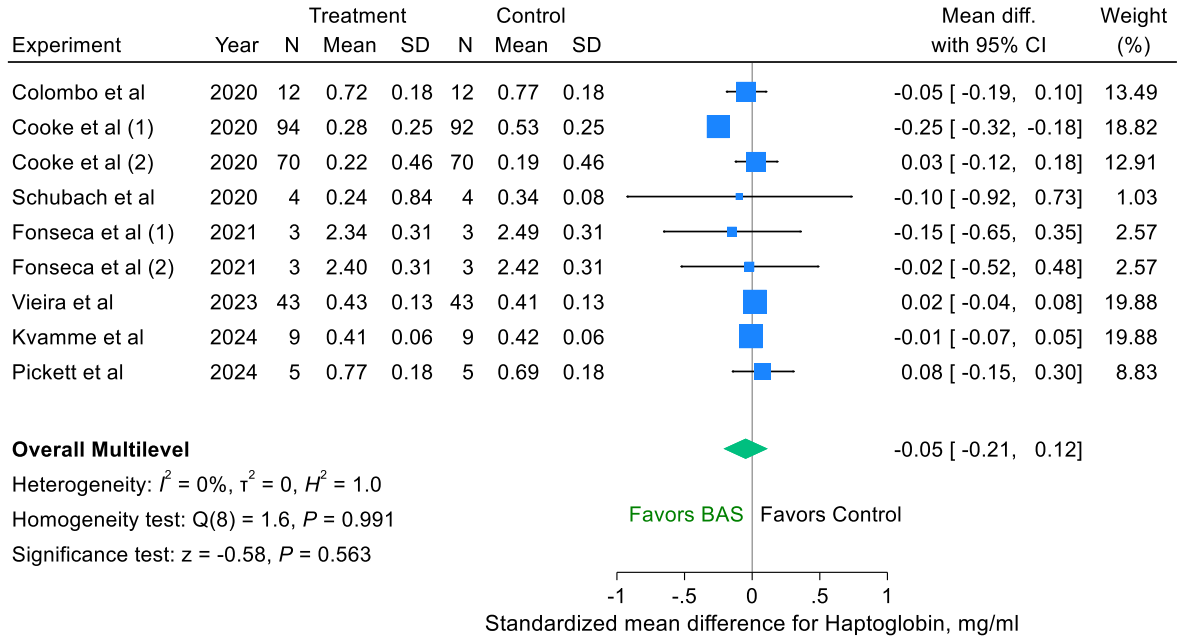
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in risk difference, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall risk difference estimate. The larger the box, the greater the comparison contribution to the overall risk difference estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the risk difference. The overall pooled risk difference and 95% CI pooled using a multilevel meta-analytical random effects model (Thompson et al., 2001) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.502). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The mortality was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure 7. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on blood cortisol of cattle.



The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in blood cortisol, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the multilevel meta-analytical random effects model (Goldstein et al., 2000) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a significant effect (P = 0.045). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The blood cortisol was considerably heterogeneous as indicated by the overall I^2 of 93.0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is high. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is heterogenous.

Figure 8. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on blood haptoglobin of cattle.



The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in haptoglobin, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison and reflects the relative weighting of the comparison at the univariate level to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the multilevel meta-analytical random effects model (Goldstein et al., 2000) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.563). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The haptoglobin was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

5. Conclusion

The reduction in blood cortisol which supports the biological mode of action of BAS and the positive significant responses of individual experiments including for final BW, mortality, and antibodies indicate that intervention with BAS has some potential to mitigate the effects of routine husbandry stresses such as weaning and feedlot entry on cattle; thus, partially supporting our hypothesis. Further some individual experiments had significant responses at intervals shorter than the entire experimental interval that we evaluated for consistency. There may also be ethical considerations in mediating the impacts of routine husbandry stress on cattle if BAS is effective in that context. There was little evidence that BAS modified responses to inflammation as indicated by a lack of effect on haptoglobin or NEFA. The limited number of experiments and large variation in study design such as cattle type and total volume of BAS applied over certain timeframes prevented extensive exploration of potential sources of heterogeneity. Use of multilevel models to account for the effects of experiment within study is important as variation at the study level was influential and needs further exploration. The implications of this study are that more experiments are needed to more fully understand responses to BAS treatment and those experiments should utilize the information obtained from the existing experiments on timing of sampling to detect treatment effects. At present the only effect that can be ascribed to BAS treatment is a reduction in blood cortisol concentrations. We recommend against the use of pseudo-replicated experiments based on the findings of this study.

5.1 Key findings

- Only blood cortisol was significantly reduced by treatment.
- While some individual studies demonstrated significant outcomes, overall, the findings were not significant for production outcomes or health.
- There was enough evidence to support further field studies.
- Study designs need to be carefully considered to avoid pseudo-replication as the risks of this were evident.
- The most obvious areas to investigate are those associated with production or health outcomes including body weight gain, ADG, and mortality or morbidity.
- As noted above in the sample size calculations, pen studies on the outcomes require large numbers of pens and lots to evaluate quite large (40%) effect size differences in outcomes. (To detect a reduction in mortality or other health outcomes and/or production with an effect size of 40% with a study power of 0.6, and an α of 0.05 pen studies on 8 feedlots, each with 6 randomised pens containing 80 head would be required). The study power is driven by the number of lots or pens per lot rather than the number of cattle in the pen. Given the need to provide pen separation, these studies will be quite challenging to execute.

5.2 Benefits to industry

- Blood cortisol concentrations were significantly reduced by treatment indicating the potential for BAS to mitigate the effects of routine animal husbandry stress on cattle.
- However, despite positive experimental results in some studies, overall, no other results of the meta-analysis were significant.

- At present, there are no benefits for industry, but some promise of potential benefit based on positive results on individual studies, positive point directions, and significance at the univariate level for final BW.

6. Future research and recommendations

It should be possible to work with the developers and producers of BAS to conduct further experiments using the results of this meta-analysis to design further field studies as:

- Positive point directions for final BW and mortality suggest the need for more studies to determine whether the intervention can be justified on a cost-efficacy or ethical basis.
- These studies should utilise existing information on timing of sampling to detect treatment effects.
- Suggested areas for funding would be castration, weaning, feedlot entry, and pre-slaughter transport interventions which could all be addressed in a single well replicated study under Australian conditions and standard management practices.
- To detect a reduction in mortality or other health outcomes, and/or an improvement in production with an effect size of 40% with a study power of 0.6, an α of 0.05 pen studies on 8 feedlots, each with 6 randomised pens containing 80 head would be required. The study power is driven by number of lots or pens per lot rather than the number of cattle in the pen.

7. References

Angeli, B., B. Cappellozza, J. L. Moraes Vasconcelos, and R. F. Cooke. 2020. Administering an appeasing substance to gir × Holstein female dairy calves on pre-weaning performance and disease incidence. Animals (Basel) 10. doi:10.3390/ani10111961

Borenstein, M., J. P. T. Higgins, L. V. Hedges, and H. R. Rothstein. 2017. Basics of meta-analysis: I^2 is not an absolute measure of heterogeneity. Res. Synth. Methods 8:5-18. doi:https://doi.org/10.1002/jrsm.1230

Bringhenti, L., E. Colombo, M. X. Rodrigues, and R. F. Cooke. 2023. Effect of maternal bovine appeasing substance on health and performance of preweaned dairy calves. The Bovine Practitioner 27:1-4.

Cappellozza, B. I., J. P. Bastos, and R. F. Cooke. 2020. Short communication: Administration of an appeasing substance to Bos indicus-influenced beef cattle improves performance after weaning and carcass ph. Livest. Prod. 238. doi:10.1016/j.livsci.2020.104067

Colombo, E. A., R. F. Cooke, A. P. Brandão, J. B. Wiegand, K. M. Schubach, G. C. Duff et al.,. 2020. Administering an appeasing substance to optimize performance and health responses in feedlot receiving cattle. J Anim Sci 98. doi:10.1093/jas/skaa339

Cooke, R. F., A. Millican, A. P. Brandão, T. F. Schumaher, O. A. de Sousa, T. Castro et al.,. 2020. Short communication: Administering an appeasing substance to Bos indicus-influenced beef cattle at weaning and feedlot entry. Animal 14:566-569. doi:10.1017/s1751731119002490

DerSimonian, R., and N. Laird. 1986. Meta-analysis in clinical trials. Control. Clin. Trials 7:177-188. doi:https://doi.org/10.1016/0197-2456(86)90046-2

Egger, M., and G. D. Smith. 2001. Principles of and procedures for systematic reviews. In: M. Egger, G. D. Smith and A. D. G. (eds.) Systematic reviews in health care meta-analysis in context No. 23-42. British medical journal books, London.

Fonseca, V. G. L., B. I. Cappellozza, O. A. de Sousa, M. Sagawa, B. Rett, M. L. Chizzotti et al.,. 2021. Strategic administration of an appeasing substance to improve performance and physiological responses of Bos indicus feedlot cattle. J Anim Sci 99. doi:10.1093/jas/skab322

Glass, G. V. 1976. Primary, secondary, and meta-analysis of research. Educational Researcher 5:3-8. doi:10.3102/0013189x005010003

Goldstein, H., M. Yang, R. Omar, R. Turner, and S. Thompson. 2000. Meta-analysis using multilevel models with an application to the study of class size effects. J. Roy. Stat. Soc. Ser. C. (Appl. Stat.) 49:399-412. doi:https://doi.org/10.1111/1467-9876.00200

Hervet, C., J. Boullier, M. Guiadeur, L. Michel, L. Brun-Lafleur, A. Aupiais et al.,. 2021. Appeasing pheromones against bovine respiratory complex and modulation of immune transcript expressions. Animals (Basel) 11. doi:10.3390/ani11061545

Higgins, J. P. T., and S. Green. 2011. Cochrane handbook for systematic reviews of interventions version 5.1.0 [updated march 2011]. The Cochrane Collaboration.

Higgins, J. P. T., and S. G. Thompson. 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21:1539-1558. doi:https://doi.org/10.1002/sim.1186

Kimbrough, M. M. 2024. Effects of a maternal bovine appeasing substance and/or metaphlyaxis with tulathromycin in high-risk, newly received feedlot heifers.

Kvamme, K., R. S. Marques, V. Alves Cruz, A. Limede Cintra, M. A. Ogg, S. R. McCoski et al.,. 2024. Multiple administrations of bovine-appeasing substance during a 42-d preconditioning program followed by feedlot receiving and its effects on physiologic, health, and performance responses of feeder cattle. J Anim Sci 102. doi:10.1093/jas/skae151

Lean, I. J., A. R. Rabiee, T. F. Duffield, and I. R. Dohoo. 2009. Invited review: Use of meta-analysis in animal health and reproduction: Methods and applications. J. Dairy Sci. 92:3545-3565. doi:https://doi.org/10.3168/jds.2009-2140

Lin, L., H. Chu, and J. S. Hodges. 2017. Alternative measures of between-study heterogeneity in meta-analysis: Reducing the impact of outlying studies. Biometrics 73:156-166.

Mackey, S. J., R. F. Cooke, and A. T. Pickett. 2024. Administering the maternal appeasing substance before slaughter to improve carcass characteristics of finishing cattle. Transl Anim Sci 8:txae048. doi:10.1093/tas/txae048

Nakagawa, S., and E. S. A. Santos. 2012. Methodological issues and advances in biological meta-analysis. Evol. Ecol. 26:1253-1274. doi:10.1007/s10682-012-9555-5

Osella, M. C., A. Cozzi, C. Spegis, G. Turille, A. Barmaz, C. L. Lecuelle et al.,. 2018. The effects of a synthetic analogue of the bovine appeasing pheromone on milk yield and composition in valdostana dairy cows during the move from winter housing to confined lowland pastures. J. Dairy Res. 85:174-177. doi:10.1017/S0022029918000262

Page, M. J., J. E. McKenzie, P. M. Bossuyt, I. Boutron, T. C. Hoffmann, C. D. Mulrow et al.,. 2021. The prisma 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372:n71. doi:10.1136/bmj.n71

Pageat, P. 2000. Pig appeasing pheromones to decrease stress, anxiety and aggressiveness. Google Patents.

Pickett, A. T., R. F. Cooke, I. S. de Souza, and S. J. Mackey. 2024. Administering the maternal bovine appeasing substance improves overall productivity and health in high-risk cattle during a 60-d feedlot receiving period. J Anim Sci 102. doi:10.1093/jas/skae221

Sargeant, J. M., and A. M. O'Connor. 2020. Scoping reviews, systematic reviews, and meta-analysis: Applications in veterinary medicine. Front. Vet. Sci. 7. doi:10.3389/fvets.2020.00011

Schubach, K. M., R. F. Cooke, C. L. Daigle, A. P. Brandão, B. Rett, V. S. M. Ferreira et al.,. 2020. Administering an appeasing substance to beef calves at weaning to optimize productive and health responses during a 42-d preconditioning program. J Anim Sci 98:1-10. doi:10.1093/jas/skaa269

Tempelman, R. 2025. Invited review: A review of some commonly used meta-analysis methods in dairy science research. J. Dairy Sci.

Thompson, S. G., R. M. Turner, and D. E. Warn. 2001. Multilevel models for meta-analysis, and their application to absolute risk differences. Statistical methods in medical research 10:375-392. doi:https://doi.org/10.1177/096228020101000060

Vieira, D. G., M. Vedovatto, H. J. Fernandes, E. A. Lima, M. C. D'Oliveira, U. A. Curcio et al.,. 2023. Effects of an appeasing substance application at weaning on growth, stress, behavior, and response to vaccination of Bos indicus calves. Animals (Basel) 13. doi:10.3390/ani13193033

White, I. R., and J. Thomas. 2005. Standardized mean differences in individually-randomized and cluster-randomized trials, with applications to meta-analysis. Clinical Trials 2:141-151. doi:https://doi.org/10.1191/1740774505cn081oa

8. Appendix

Table A1. Author and reason for exclusion

Author	Reason for exclusion
Archunan et al. (2014)	Book not available
Brandao et al. (2019)	Abstract duplicated with research article
Cappellozza and Cooke (2022)	Off-topic review
Colombo (2023)	Off-topic thesis
Colombo et al. (2020)	Duplicated research article
Cooke et al. (2024)	Abstract duplicated with research article
Coria-Avila et al. (2022)	Off-topic Review
de Lima et al. (2024)	Abstract only
Dias et al. (2022)	Abstract only
Johnston et al. (2024)	Abstract only
Mallette (2023)	Off-topic thesis
Marques et al. (2022)	Abstract duplicated with research article
McCartor (1959)	Off-topic thesis
Millican et al. (2019)	Abstract duplicated with research article
Osella et al. (2018)	Off-topic research article
Rekwot et al. (2001)	Off-topic review
Schubach et al. (2020a)	Abstract only
Schubach et al. (2020b)	Duplicated research article
Viera et al. (2021a)	Abstract only
Viera et al. (2021b)	Abstract only

Archunan, G., S. Rajanarayanan, and K. Karthikeyan. 2014. Cattle pheromones. Pages 461-488 in Neurobiology of chemical communication. C. Mucignat-Caretta, ed. CRC Press/Taylor & Francis, Boca Raton (FL).

Brandao, A., R. F. Cooke, K. Schubach, T. Schumaher, O. Souza, T. Castro, and B. Cappellozza. 2019. Administering a synthetic appeasing pheromone to *Bos indicus* influenced beef cattle at weaning and feedlot entry. J. Anim. Sci. 97:52-53. doi:10.1093/jas/skz053.118.

Cappellozza, B. I. and R. F. Cooke. 2022. Administering an appeasing substance to improve performance, neuroendocrine stress response, and health of ruminants. Animals (Basel) 12. doi:10.3390/ani12182432.

Colombo, E. A. 2023. Management of feedlot cattle under stressful conditions. Phd thesis. Vol. PhD. Texas A&M University.

Colombo, E. A., R. F. Cooke, A. P. Brandão, J. B. Wiegand, K. M. Schubach, C. Sowers, G. C. Duff, V. N. Gouvêa, and B. I. Cappellozza. 2020. Administering an appeasing substance to optimize welfare and performance of receiving cattle. Transl. Anim. Sci. 4:S1-s5. doi:10.1093/tas/txaa086.

Cooke, R. F., N. C. Kertz, A. Pickett, and S. Mackey. 2024. PSV-24 administering the maternal bovine appeasing substance before slaughter to improve carcass characteristics of finishing cattle. J. Anim. Sci. 102(Suppl 3):560-561. doi:https://doi.org/10.1093/jas/skae234.628.

Coria-Avila, G. A., J. G. Pfaus, A. Orihuela, A. Domínguez-Oliva, N. José-Pérez, L. A. Hernández, and D. Mota-Rojas. 2022. The neurobiology of behavior and its applicability for animal welfare: A review. Animals 12:928. doi:https://doi.org/10.3390/ani12070928.

de Lima, E. A., M. Vedovatto, F. Farias, J. Ranches, M. Ferreira, B. I. Cappellozza, O. Souza, and G. L. L. Franco. 2024. Effect of an appeasing substance application on growth, stress, handling quality and reproduction of *Bos indicus* beef heifers synchronized to fixed-time AI. J. Anim. Sci. 102(Suppl 1):62-63.

Dias, N. W. W., S. Pancini, G. Goncherenko, J. Currin, S. Clark, J. L. Stewart, R. Cooke, and V. R. G. Mercadante. 2022. Administering a bovine appeasing substance to beef heifers during an estrous synchronization protocol and its impacts on temperament and pregnancy rate. J. Anim. Sci. 100(Suppl 3):149-149. doi:10.1093/jas/skac247.276.

Johnston, E. P., E. B. Kegley, J. G. Powell, C. Pennicott, J. L. Reynolds, R. A. Cheek, and S. Weimer. 2024. Effectiveness of maternal bovine appeasing substance on growth, physiological, and behavioral responses of high-risk stocker cattle. J. Anim. Sci. 102(Suppl 3):548-548. doi:10.1093/jas/skae234.616.

Mallette, R. 2023. Evaluation of a nutritional calming supplement for stress management in beef cattle. Masters thesis. Vol. Masters. Louisiana State University and Agricultural & Mechanical College.

Marques, R. S., K. Kvamme, V. Cruz, E. Colombo, and R. Cooke. 2022. Effects of multiple bovine appeasing substance administration during a 42-day preconditioning program on physiologic, health, and performance responses of feeder cattle. J. Anim. Sci. 100(Suppl 4):14-16. doi:https://doi.org/10.1093/jas/skac313.020.

McCartor, M. M. 1959. Tranquilizers or tranquilizing type compounds in beef cattle wintering and fattening rations. Masters thesis. in Department of Animal Husbandry. Vol. Masters Thesis. K-State University.

Millican, A., R. F. Cooke, A. Brandao, T. Schumaher, O. Souza, T. Castro, and B. Cappellozza. 2019. Administering an appeasing substance to *Bos indicus* influenced beef cattle at weaning and feedlot entry. J. Anim. Sci. 97(Suppl 3):149-149. doi: 10.1093/jas/skz258.305.

Osella, M. C., A. Cozzi, C. Spegis, G. Turille, A. Barmaz, C. L. Lecuelle, E. Teruel, C. Bienboire-Frosini, C. Chabaud, L. Bougrat, and P. Pageat. 2018. The effects of a synthetic analogue of the bovine appeasing pheromone on milk yield and composition in Valdostana dairy cows during the move from winter housing to confined lowland pastures. J. Dairy Res. 85:174-177. doi:10.1017/S0022029918000262.

Rekwot, P. I., D. Ogwu, E. O. Oyedipe, and V. O. Sekoni. 2001. The role of pheromones and biostimulation in animal reproduction. Anim. Reprod. Sci. 65:157-170. doi:10.1016/s0378-4320(00)00223-2.

Schubach, K., B. I. Cappellozza, M. R. Burim, A. Velasco, F. Sagrado, J. P. Bastos, and R. F. Cooke. 2020a. Administration of an appeasing substance at castration improves performance of pre-conditioned beef crossbred steers. J. Anim. Sci. 98(Suppl 4):347-348. doi:https://doi.org/10.1093/jas/skaa278.612.

Schubach, K. M., R. F. Cooke, A. P. Brandão, B. Rett, V. Ferreira, G. Scatolin, E. A. Colombo, C. L. Daigle, K. G. Pohler, and B. I. Cappellozza. 2020b. Administering an appeasing substance to beef calves at weaning to optimize welfare and productivity. Transl. Anim. Sci. 4(Suppl 1):S74-s78. doi:10.1093/tas/txaa101.

Viera, D. G., J. Ranches, B. I. Cappellozza, H. J. Fernandes, M. D'Oliveira, R. Rocha, L. Valerio, U. Curcio, and G. L. Franco. 2021a. Appeasing substance application at weaning enhanced growth and temperament of *Bos indicus*-influenced weaned calves in grazing system. J. Anim. Sci. 99(Suppl 3):220-220. doi:https://doi.org/10.1093/jas/skab235.400.

Viera, D. G., J. Ranches, M. Vedovatto, G. G. Pirota, B. I. Cappellozza, N. Canutoce, T. G. Pereira, L. F. Yshiayam, T. Henrique, and H. J. Fernandes. 2021b. Effects of a bovine appeasing substance application at weaning on temperament and growth of *Bos indicus* influenced heifers. J. Anim. Sci. 99(Suppl 3):3-3. doi:10.1093/jas/skab235.005.

Table A2. The number of cattle that contributed to the health dataset to determine the incidence of health disorders and mortality for bovine appeasing substance (BAS) treated and control cattle

Authors	Diarrhea						Respiratory disease						Mortality					
	Treatment			Control			Treatment			Control			Treatment			Control		
	n	Yes	No	n	Yes	No	n	Yes	No	n	Yes	No	n	Yes	No	n	Yes	No
Angeli et al. (2020)	70	37	33	70	32	38	70	10	60	70	13	57	70	2	68	70	3	67
Bringhenti et al. (2023)	205	120	85	205	145	60							205	5	200	205	16	189
Colombo et al. (2020)[1]							171	101	70	171	81	90	171	12	159	171	13	158
Hervet et al. (2021)							100	32	107	100	23	77						
Kimbrough (2024) -1	138	28	110	138	28	110	138	28	110	138	28	110	138	17	121	138	11	127
Kimbrough (2024) -2	138	11	127	138	14	124	138	11	127	138	14	124	138	7	131	138	4	134
Pickett et al. (2024) [1]							40	28	12	40	19	21	40	2	38	40	8	32

Yes = the number of cattle that had the health disorder or died; No = the number of cattle that did not have the health disorder or did not die.
[1]Respiratory disease incidence was based on receiving one antibiotic treatment.

Table A3. Raw means±SD and numbers of cattle for stress markers for bovine appeasing substance (BAS) treated and control cattle.

Authors	n		Blood cortisol (ng/ml)		Hair cortisol (pg/mg of hair)		Blood haptoglobin (mg/ml)		Blood NEFA (µEq/L)	
	BAS	Control	BAS	Control	BAS	Control	BAS	Control	BAS	Control
Colombo et al. (2020)	12	12			4.19±0.62	4.17±0.62	0.72±0.18	0.77±0.18	0.29±0.06	0.27±0.06
Cooke et al. (2020) -1	94	92	20.2±18.4	19.3±18.2	2.51±2.91	2.47±2.88	0.28±0.25	0.53±0.25		
Cooke et al. (2020) -2	70	70	30.9±19.2	32.8±19.2	1.80±1.25	1.94±1.25	0.22±0.46	0.19±0.46		
Fonseca et al. (2021) -1	3	3	30.4±2.04	32.5±2.04	2.38±0.11	2.44±0.11	2.34±0.31	2.49±0.31		
Fonseca et al. (2021) -2	3	3	32.3±2.04	32.6±2.04	2.48±0.11	2.34±0.11	2.40±0.31	2.42±0.31		
Kvamme et al. (2024)	9	9	2.8±0.63	2.8±0.63			0.41±0.06	0.42±0.06	0.36±0.03	0.22±0.03
Mackey et al. (2024)	8	8	11.7±4.5	20.8±4.5						
Pickett et al. (2024)	5	5	25.6±4.7	36.6±4.7			0.77±0.18	0.69±0.18		
Schubach et al. (2020)	4	4	24.6±2.8	27.0±2.8	3.68±0.39	4.16±0.39	0.24±0.84	0.34±0.08	0.18±0.02	0.17±0.02
Vieira et al. (2023)	43	43					0.43±0.13	0.41±0.13		

NEFA = non-esterified fatty acids

Table A4. Raw means±SD and numbers of cattle for antibodies against viruses for bovine appeasing substance (BAS) treated and control cattle.

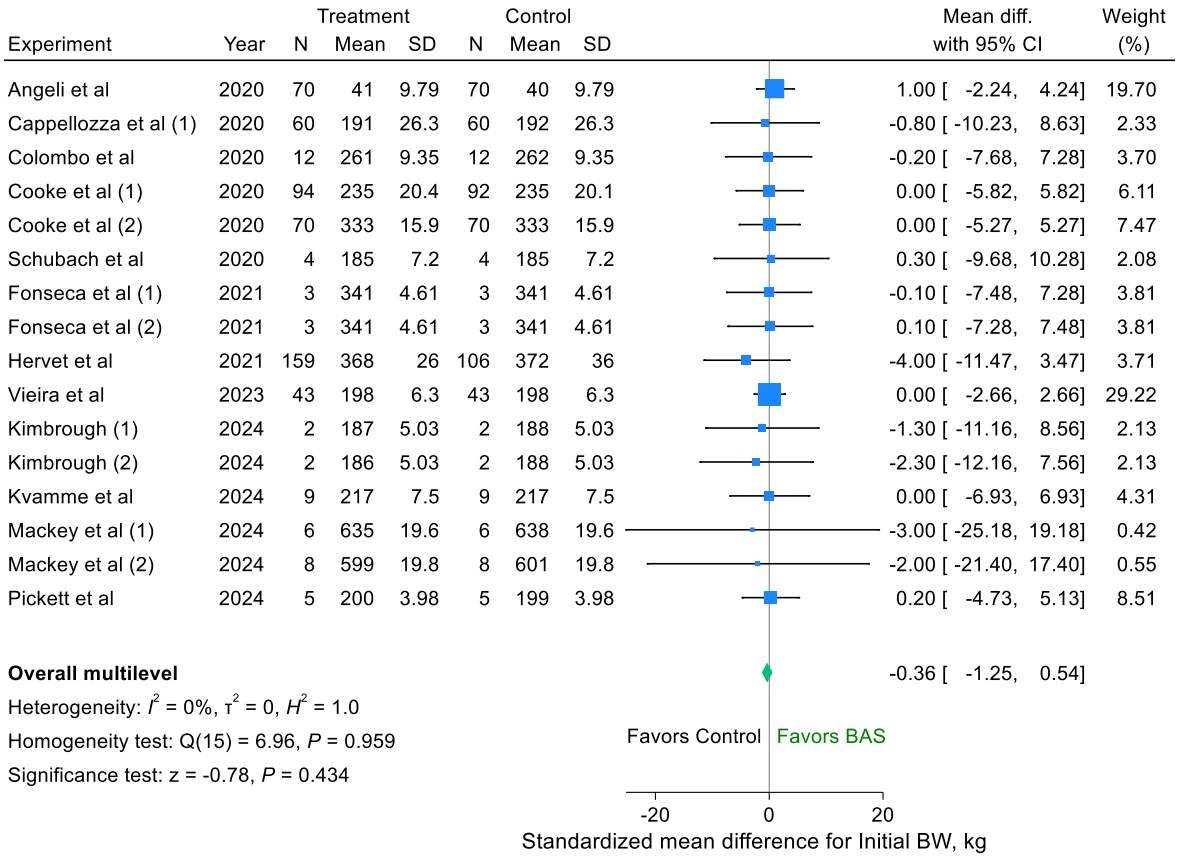
	n		Infectious bovine rhinotracheitis		Bovine viral diarrhea[1]		Bovine respiratory syncytial virus		Parainfluenza-3 virus	
Authors	BAS	Control	BAS	Control	BAS	Control	BAS	Control	BAS	Control
Colombo et al. (2020)[2]	12	12	205±57.16	206±57.16	0.98±0.33	1.09±0.33	111±36.4	98.8±36.4	79.1±22.52	68.2±22.52
Kimbrough (2024) -1[3]	2	2			3.18±0.13	3.52±0.21				
Kvamme et al. (2024)	9	9			0.97±0.18	0.84±0.18	40.3±10.8	51.1±10.8	20.8±6.0	31.4±6.0
Pickett et al. (2024)[2]	5	5	2.3±0.23	2.3±0.23	1.50±0.16	1.51±0.16				
Schubach et al. (2020)[2]	4	4	2.6±0.10	2.6±0.1	1.31±0.10	1.15±0.10	1.49±0.08	1.41±0.08	0.67±0.06	0.60±0.06
Vieira et al. (2023)[3]	43	43	3.1±3.48	2.9±3.48	6.40±4.0	4.26±4.0			6.15±3.48	4.82±3.48

[1]All include responses for both Bovine viral diarrhea Type I and II, except Vieira et al. (2023) and Kimbrough (2024) where values are for Type I only

[2]Disease data are reported as a sample-to-positive control ratio from Enzyme-linked immunosorbent assays.

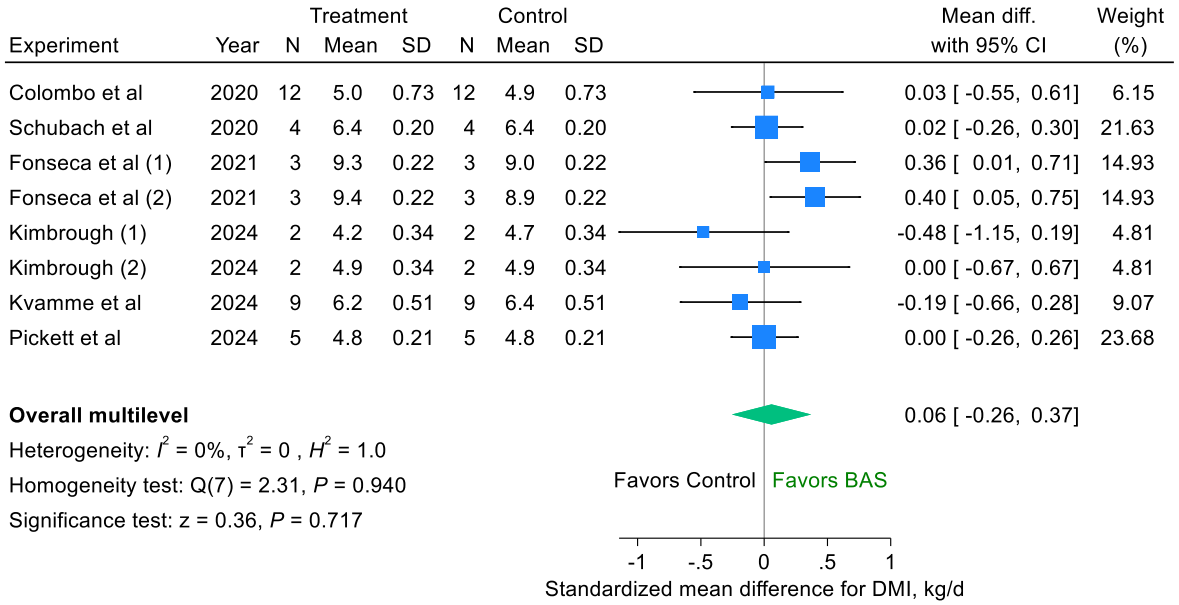
[3]Disease data are reported as $\log_2$ titers from serum neutralization tests.

Figure A1. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on the initial BW of cattle.



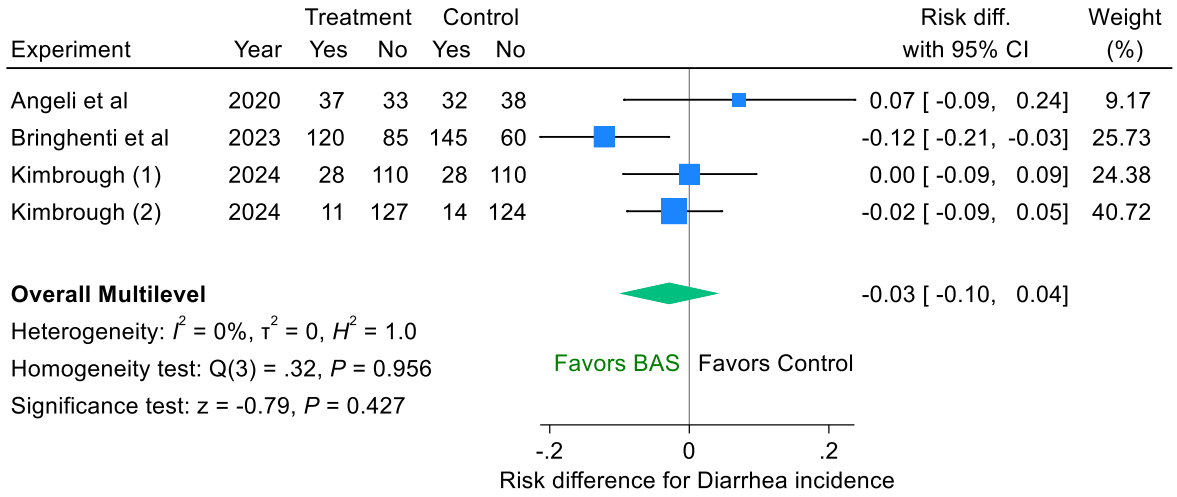
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in initial BW, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the multilevel meta-analytical random effects model (Goldstein et al., 2000) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.434). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The initial BW was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure A2. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on the DMI of cattle.



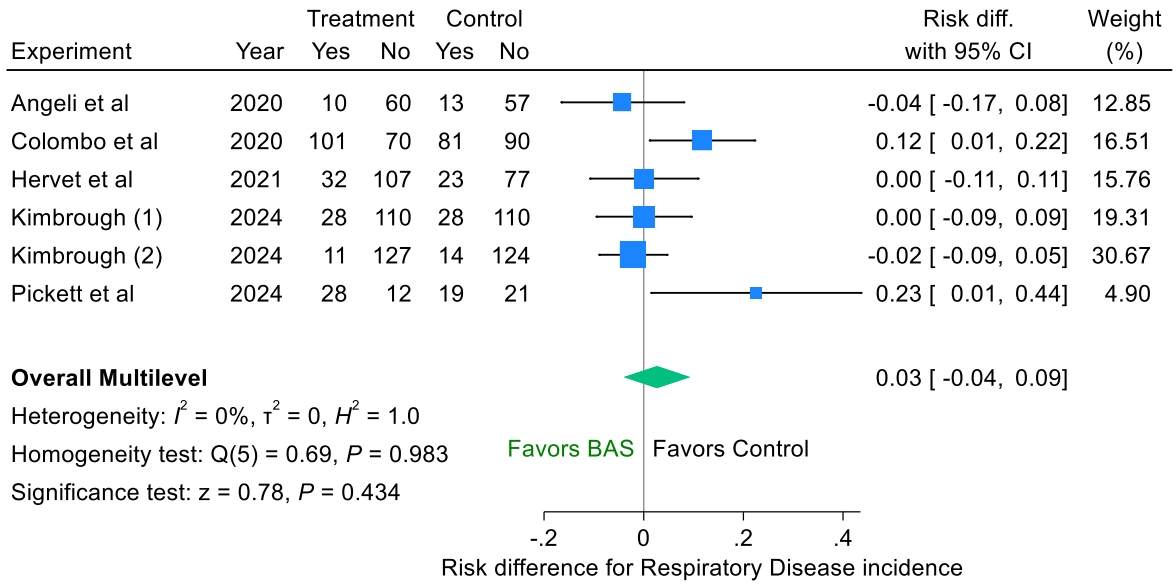
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in DMI, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the multilevel meta-analytical random effects model (Goldstein et al., 2000) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.717). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The DMI was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure A3. Forest plot of the Risk Difference and 95% CI of the effect of bovine appeasing substance intervention on incidence of diarrhea of cattle.



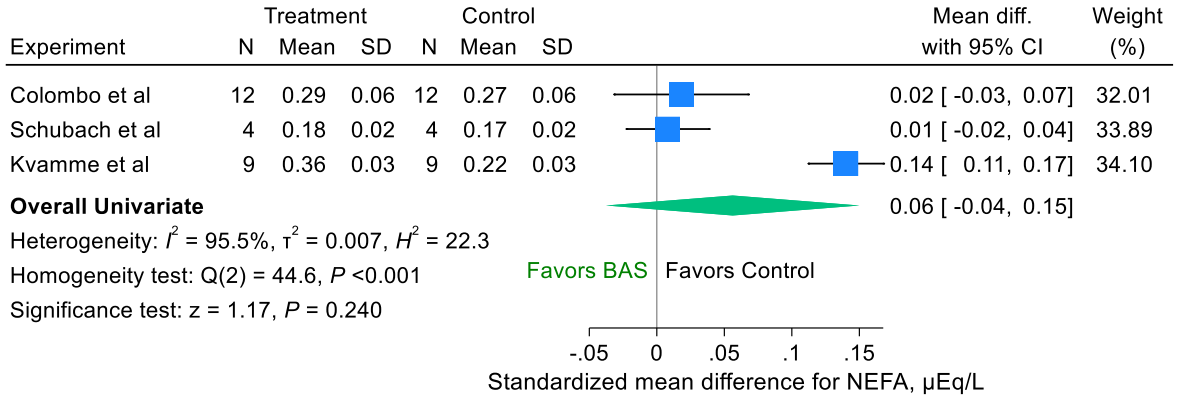
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in risk difference, while points to the right of the line indicate an increase. Each square around the point effect represents the mean risk difference for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall risk difference estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the risk difference. The overall pooled risk difference and 95% CI pooled using the multilevel meta-analytical random effects model (Thompson et al., 2001) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.427). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. Diarrhea incidence was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure A4. Forest plot of the Risk Difference and 95% CI of the effect of bovine appeasing substance intervention on incidence of respiratory disease of cattle.



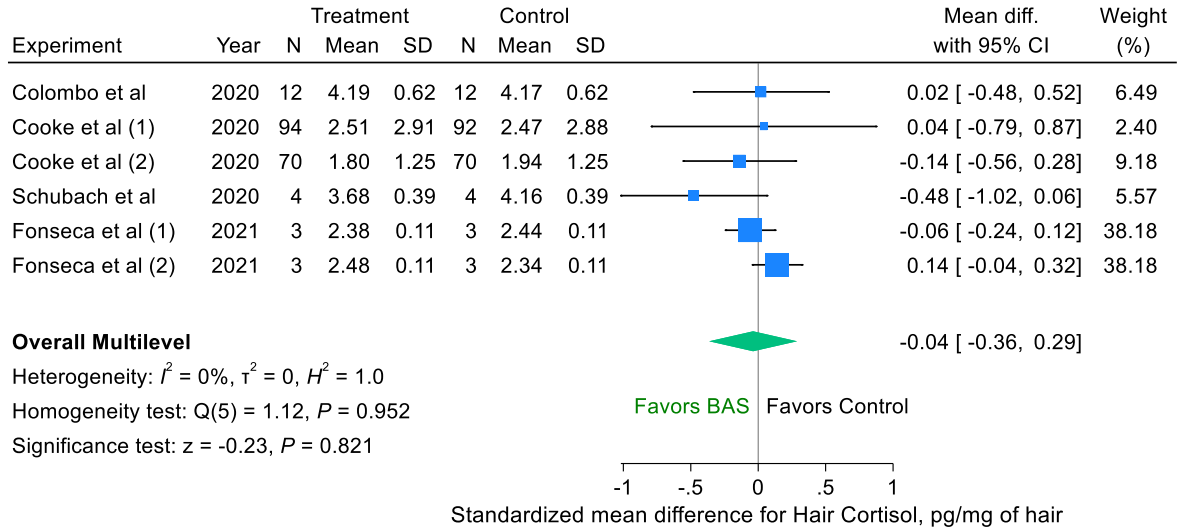
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in risk difference, while points to the right of the line indicate an increase. Each square around the point effect represents the mean risk difference for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall risk difference estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the risk difference. The overall pooled risk difference and 95% CI pooled using the multilevel meta-analytical random effects model (Thompson et al., 2001) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.434). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. Respiratory disease was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure A5. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on blood non-esterified fatty acids (NEFA) of cattle.



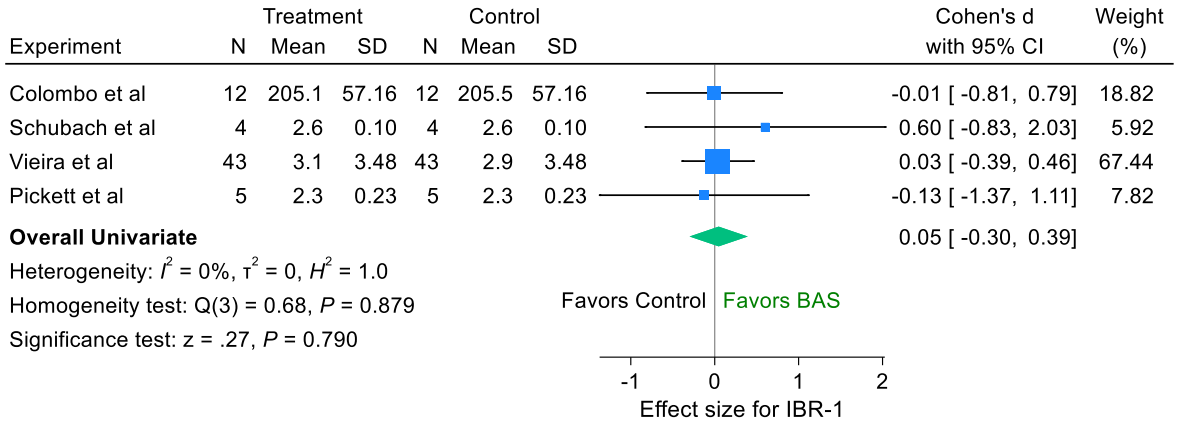
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in blood non-esterified fatty acids, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall SMD estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the DerSimonian and Laird (1986) model are indicated by the diamond at the bottom. The estimate of the overall effect size θ is reported as the significance test and indicates a non-significant effect (P = 0.240). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis estimated from the DerSimonian and Laird (1986) model. The blood NEFA were considerable heterogeneous as indicated by the overall I^2 of 95.5%. The τ^2 is the true variance between the experiments and is very low in. The H^2 also provides an estimate of heterogeneity. The H estimated from the DerSimonian and Laird (1986) model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is heterogenous.

Figure A6. Forest plot of the Standardized Mean Difference (SMD) and 95% CI of the effect of bovine appeasing substance intervention on hair cortisol of cattle.



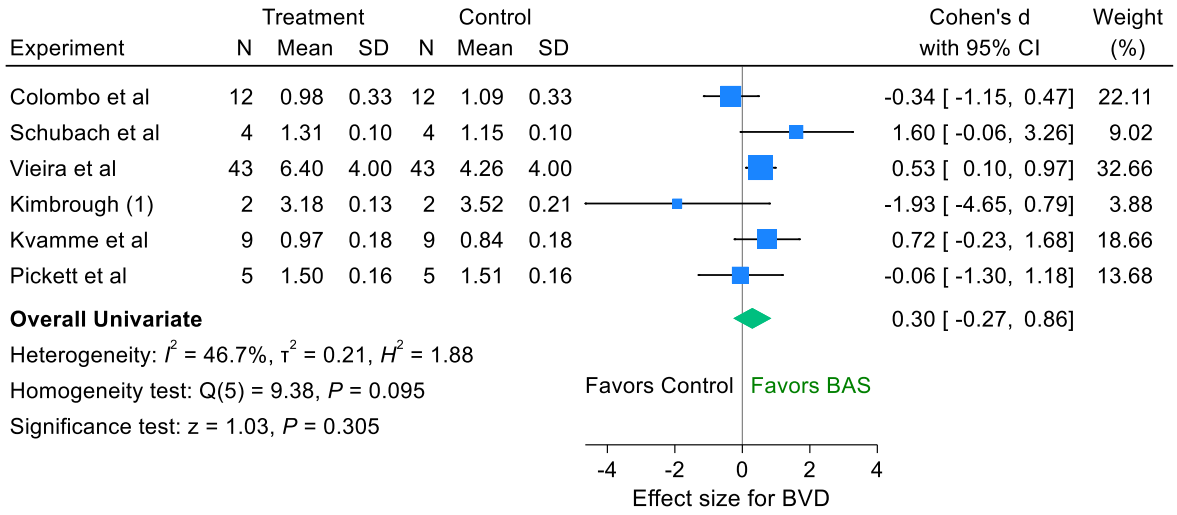
The solid vertical line represents a mean difference of zero or no effect. Points to the left of the line represent a reduction in hair cortisol, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall effect size estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the SMD. The overall pooled SMD and 95% CI pooled using the multilevel meta-analytical random effects model (Goldstein et al., 2000) are indicated by the diamond at the bottom. The estimate of the overall effect size θ for the multilevel model is reported as the significance test and indicates a non-significant effect (P = 0.821). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis. The hair cortisol was not heterogeneous as indicated by the overall I^2 of 0% estimated from the multilevel model. The τ^2 estimated from the multilevel model is the true variance between the total of studies and experiments and is very low. The H^2 also provides an estimate of heterogeneity. The H estimated from the multilevel model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure A7. Forest plot of the effect size (z-score) and 95% CI of the effect of bovine appeasing substance intervention on blood antibodies for Infectious bovine rhinotracheitis-1 (IBR-1) in cattle.



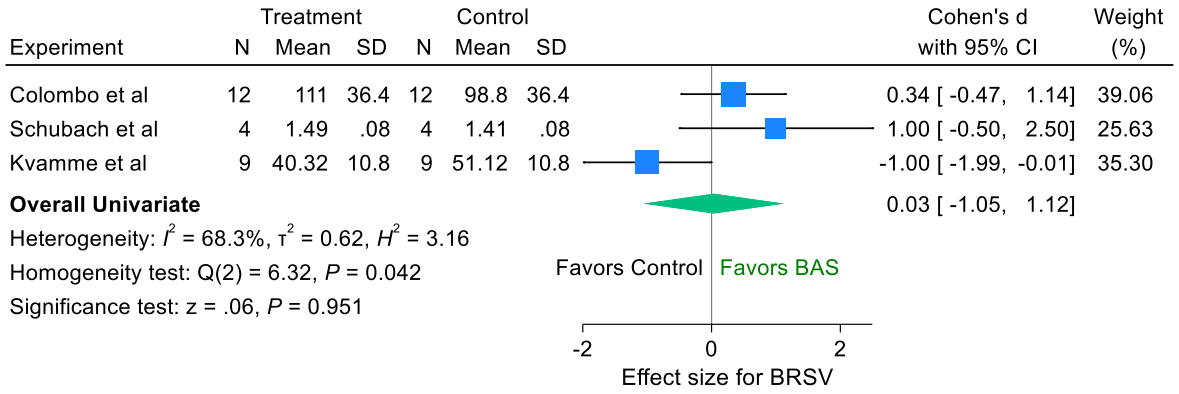
The solid vertical line represents a mean Cohen's d effect size difference of zero or no effect. Points to the left of the line represent a reduction in blood antibodies for IBR-1, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall effect size estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effects size. The overall pooled effect size and 95% CI pooled using the DerSimonian and Laird (1986) model calculated with a maximum likelihood estimation is indicated by the diamond at the bottom. The estimate of the overall effect size θ is reported as the significance test and indicates a non-significant effect (P = 0.790). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis estimated from the DerSimonian and Laird (1986) model. The blood antibodies for IBR-1 were not heterogeneous as indicated by the overall I^2 of 0%. The τ^2 is the true variance between experiments and is very low in the DerSimonian and Laird (1986) model. The H^2 also provides an estimate of heterogeneity. The H estimated from the DerSimonian and Laird (1986) model is interpreted as the ratio of the SD of the estimated overall ES from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is homogenous.

Figure A8. Forest plot of the effect size (z-score) and 95% CI of the effect of bovine appeasing substance intervention on blood antibodies for Bovine viral diarrhea (BVD) in cattle.



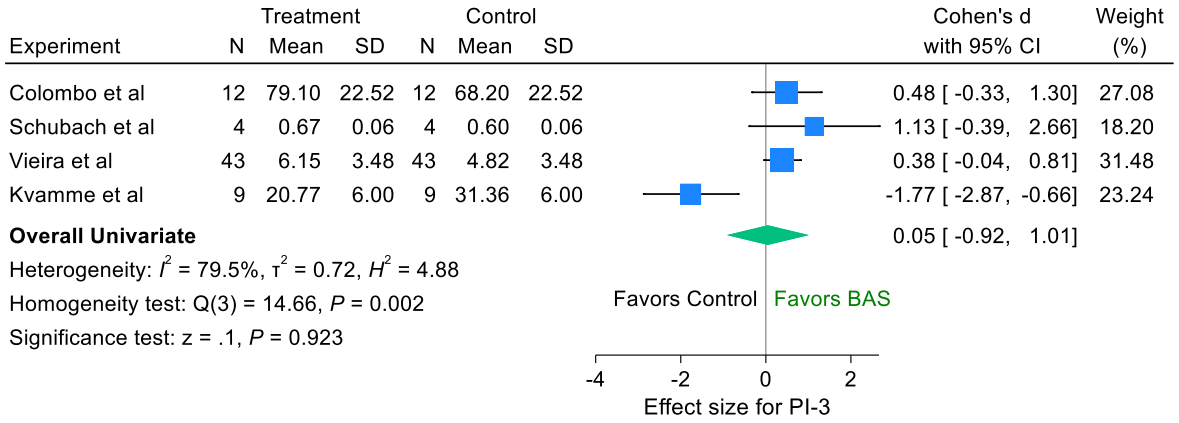
The solid vertical line represents a mean Cohen's d effect size difference of zero or no effect. Points to the left of the line represent a reduction in blood antibodies for Bovine viral diarrhea, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall effect size estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effects size. The overall pooled effect size and 95% CI pooled using the DerSimonian and Laird (1986) model is indicated by the diamond at the bottom. The estimate of the overall effect size θ is reported as the significance test and indicates a non-significant effect (P = 0.305). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis estimated from the DerSimonian and Laird (1986) model. The blood antibodies for BVD were moderately heterogeneous as indicated by the overall I^2 of 46.7%. The τ^2 is the true variance between experiments and is reasonably low in the DerSimonian and Laird (1986) model. The H^2 also provides an estimate of heterogeneity. The H estimated from the DerSimonian and Laird (1986) model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is moderately homogenous.

Figure A9. Forest plot of the effect size (z-score) and 95% CI of the effect of bovine appeasing substance intervention on blood antibodies for Bovine respiratory syncytial virus (BRSV) in cattle.



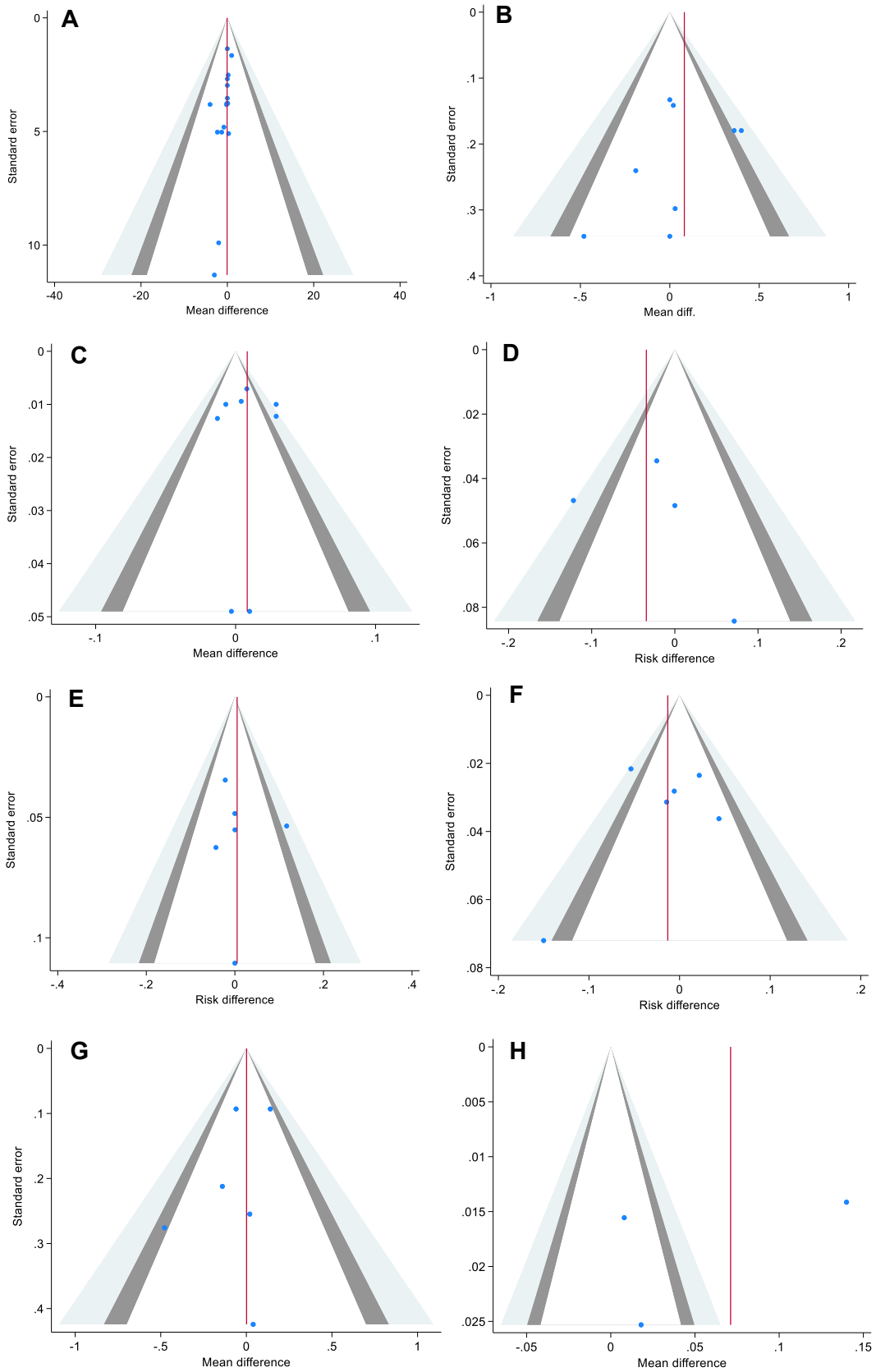
The solid vertical line represents a mean Cohen's d effect size difference of zero or no effect. Points to the left of the line represent a reduction in blood antibodies for Bovine respiratory syncytial virus, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall effect size estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effects size. The overall pooled effect size and 95% CI pooled using the DerSimonian and Laird (1986) model is indicated by the diamond at the bottom. The estimate of the overall effect size θ is reported as the significance test and indicates a non-significant effect (P = 0.951). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis estimated from the DerSimonian and Laird (1986) model. The blood antibodies for BRSV were substantially heterogeneous as indicated by the overall I^2 of 68.3%. The τ^2 is the true variance between experiments and is moderate in the DerSimonian and Laird (1986) model. The H^2 also provides an estimate of heterogeneity. The H estimated from the DerSimonian and Laird (1986) model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is heterogenous.

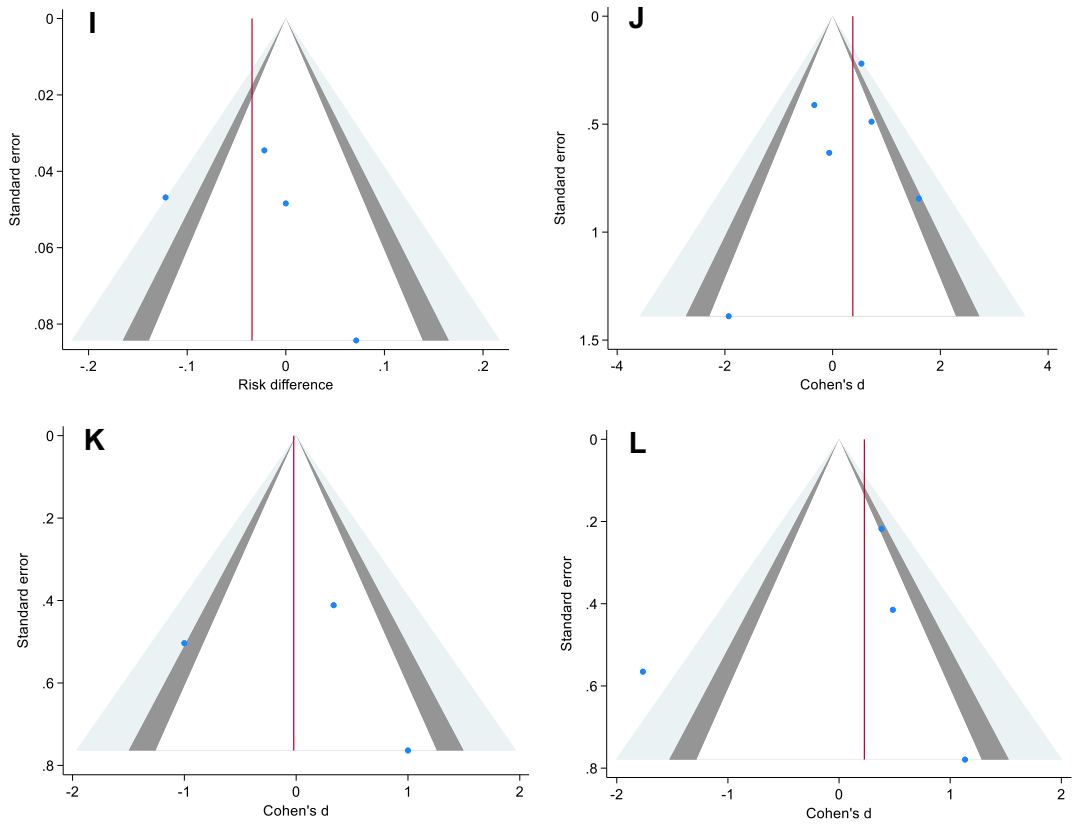
Figure A10. Forest plot of the effect size (z-score) and 95% CI of the effect of bovine appeasing substance intervention on blood antibodies for Parainfluenza virus-3 (PI-3) in cattle



The solid vertical line represents a mean Cohen's d effect size difference of zero or no effect. Points to the left of the line represent a reduction in blood antibodies for Parainfluenza virus-3, while points to the right of the line indicate an increase. Each square around the point effect represents the mean effect size for that comparison at the univariate level and reflects the relative weighting of the comparison to the overall effect size estimate. The larger the box, the greater the comparison contribution to the overall effect size estimate. The weights that each comparison contributed at the univariate level are in the right-hand column. The upper and lower limit of the line connected to the square represents the upper and lower 95% CI for the effects size. The overall pooled effect size and 95% CI pooled using the DerSimonian and Laird (1986) model calculated with a maximum likelihood estimation is indicated by the diamond at the bottom. The estimate of the overall effect size θ is reported as the significance test and indicates a non-significant effect (P = 0.923). The heterogeneity measure, I^2 is a measure of residual variation among experiments included in the meta-analysis estimated from the DerSimonian and Laird (1986) model. The blood antibodies for PI-3 were considerably heterogeneous as indicated by the overall I^2 of 79.5%. The τ^2 is the true variance between experiments and is moderate in the DerSimonian and Laird (1986) model. The H^2 also provides an estimate of heterogeneity. The H estimated from the DerSimonian and Laird (1986) model is interpreted as the ratio of the SD of the estimated overall effect size from a random effects meta-analysis compared to the SD from a fixed effects meta-analysis, where an H^2 = 1 is homogenous. In this case the H^2 is heterogenous.

Figure A11. Contour-enhanced funnel plots for (A) initial BW (kg), (B) DMI (kg/d), (C) G:F (kg/kg), (D) diarrhea, (E) respiratory disease, (F) mortality, (G) hair cortisol (pg/mg of hair), (H) blood non-esterified fatty acids, (I) Infectious bovine rhinotracheitis-1, (J) Bovine viral diarrhea, (K) Bovine respiratory syncytial virus, and (L) Parainfluenza virus-3. for cattle treated with bovine appeasing substance. The effect estimates of the experiments are graphed against the standard error of the experiments. Levels of significance for experiments and within the broken lines are 0.01, 0.05, and 0.10.




I
Standard error
0
.02
.04
.06
.08
-.2
-.1
0
.1
.2
Risk difference
J
Standard error
0
.5
1
1.5
-4
-2
0
2
4
Cohen's d
K
Standard error
0
.2
.4
.6
.8
-2
-1
0
1
2
Cohen's d
L
Standard error
0
.2
.4
.6
.8
-2
-1
0
1
2
Cohen's d