Supplementary Table S2. SYRCLE risk-of-bias assessment

Domain	Amin et al., 2026	Golden et al., 2023	Sutkus et al., 2022	Fleming et al., 2020a	Fleming et al., 2020b	Wang et al., 2019	Obelitz-Ryom et al., 2019	Mudd et al., 2017
1. Random sequence generation	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
2. Baseline characteristics	Low	Low	Low	Low	Low	Low	Low	Low
3. Allocation concealment	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Unclear
4. Random housing	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Unclear
5. Blinding of caregivers/investigators	Low	Low	Low	Low	Low	Low	Low	Low
6. Random outcome assessment	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Unclear
7. Blinding of outcome assessors	Low	Low	Low	Low	Low	Low	Unclear	Low
8. Incomplete outcome data	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
9. Selective outcome reporting	Low	Unclear	Low	Low	Low	Low	Low	Low
10. Other sources of bias	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear

Judgments: Low = adequate methods supported by reporting; High = evidence of likely bias; Unclear = insufficient information to determine risk.

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Piglets were stratified by body weight and litter origin and then "randomly assigned" to the intervention groups; the method used to generate the random sequence was not specified.	p. 1201, Animal model and housing
D2	Baseline characteristics	Low	Weight/litter stratification and adjustment for initial body weight were reported.	pp. 1201, 1203, 1209; Animal model and housing; Statistical analysis; Discussion
D3	Allocation concealment	Low	Treatment groups were coded Blue, Yellow and Purple, and personnel were blinded	p. 1201, Animal model and housing
D4	Random housing	Low	Random assignment of pens and distribution of treatment groups across pen locations were reported.	pp. 1201–1202, Animal model and housing
D5	Blinding of caregivers/investigators	Low	The authors state explicitly that "All research personnel were blinded to treatment-group allocation" and describe colour-coded groups.	pp. 1201–1202, Animal model and housing
D6	Random outcome assessment	Low	MRS was conducted at PND 38–39 with a randomized scan order.	p. 1202, MRS data acquisitions
D7	Blinding of outcome assessors	Low	All research personnel were blinded supports assessor blinding; imaging was undertaken by a professional imaging scientist and spectra processed with TARQUIN.	pp. 1201–1203, Animal model and housing; MRS data acquisitions/analysis
D8	Incomplete outcome data	Unclear	Four of 48 piglets were lost early, but their potential influence on baseline comparability warrants caution.	pp. 1201, 1209; Animal model and housing; Discussion
D9	Selective outcome reporting	Low	The Methods describe automated quantification of 33 metabolites in the Results.	pp. 1202–1206, MRS analysis; Results and Figures 4–5
D10	Other potential sources of bias	Unclear	Multiple metabolite comparisons were analysed at $P < 0.05$, without an explicitly reported multiplicity correction. Piglets were housed in pairs, but pen/litter clustering was not described in the GLM.	pp. 1201–1203, Housing; Statistical analysis

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Reports random assignment to control, 3′-SL and 6′-SL diets, but does not specify the method of sequence generation.	p. 1 Abstract; p. 2, Animals, housing, and diet groups
D2	Baseline characteristics	Low	Initial body weight and litter origin were considered when allocating piglets to three diets; all animals were male and similarly aged.	p. 2, Animals, housing, and diet groups
D3	Allocation concealment	Low	All study personnel in direct contact with animals were blinded to treatment groups for the duration of the study and data analyses.	p. 2, Animals, housing, and diet groups
D4	Random housing	Unclear	Animals housed individually then in treatment-group pens; randomization of housing/pen location not reported.	p. 2, Animals, housing, and diet groups
D5	Blinding of caregivers/investigators	Low	The supplied methods explicitly establish that husbandry staff and investigators were blinded to dietary assignment.	p. 2, Animals, housing, and diet groups
D6	Random outcome assessment	Low	Longitudinal MRI pigs were described as randomly selected (11 per treatment), reducing selection concerns for follow-up scanning	p. 3, Magnetic resonance imaging (MRI)
D7	Blinding of outcome assessors	Low	Behavioral scoring and masking of MRI segmentation were blinded	p. 3, Behavioral testing, Magnetic resonance imaging (MRI)
D8	Incomplete outcome data	Unclear	Subsets underwent MRI across two timepoints and outliers were removed using a stated rule; complete outcome-specific retention and balance need verification.	pp. 3–5, Magnetic resonance imaging (MRI), Statistical analysis
D9	Selective outcome reporting	Unclear	Extensive outcomes were presented, image acquisition issue at PND 30 leads to small sample size.	pp. 5–7, Results
D10	Other potential sources of bias	Unclear	Different head-coil arrangements were used at PND 30 and PND 58 and housing changed with age	pp. 2-3, Animals, housing, and diet groups, Magnetic resonance imaging (MRI)

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Random assignment across four factorial groups and stratification by litter/body weight reported, without a specific random-sequence method.	p. 3, Animal Care and Housing
D2	Baseline characteristics	Low	Initial body weight and litter origin were considered, with male piglets from a controlled genetic source and cohort structure.	p. 3, Animal Care and Housing
D3	Allocation concealment	Unclear	The researchers were blinded to the treatment groups. No concealed-allocation method explicitly provided.	p. 3, Materials and Methods
D4	Random housing	Unclear	Individual housing was described; random allocation to cage positions was not stated.	p. 3, Animal Care and Housing
D5	Blinding of caregivers/investigators	Low	Authors explicitly state all personnel remained blinded to dietary treatment identity throughout study and analyses.	p. 3, Experimental Treatment Groups
D6	Random outcome assessment	Unclear	MRS/MRI scan order or random sampling of piglets for outcome assessment is not clearly described.	pp. 3–4, Magnetic Resonance Imaging
D7	Blinding of outcome assessors	Low	The reported blinding applies to personnel involved in study analyses; manual extraction procedures remain a consideration.	p. 3, Experimental Treatment Groups; p. 4, Magnetic Resonance Imaging
D8	Incomplete outcome data	Unclear	Four pigs were excluded from behavior/imaging for another experimental purpose; DTI further excluded three corrupted scans and two outliers, with group-specific counts supplied. Bias impact requires verification.	pp. 3–4, Materials and Methods
D9	Selective outcome reporting	Low	Multiple imaging outcomes are reported.	pp. 4–7, Results
D10	Other potential sources of bias	Unclear	DTI attrition and exclusion of outliers may influence estimates; study analysed factorial contrasts with cohort as a blocking factor, but no clear additional systematic bias established.	p. 4, Statistical Analysis

Fleming et al. (2020a), Nutrients. https://doi.org/10.3390/nu12072131

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Six independent cohorts; litter and starting body weight counterbalanced, but no random sequence method reported.	p.2, Animals and Housing
D2	Baseline characteristics	Low	Male pigs of the same genetic line; starting body weight and litter balanced within cohorts.	p.2, Animals and Housing
D3	Allocation concealment	Unclear	Allocation concealment before assigning pigs to groups not described.	p.2, Animals and Housing
D4	Random housing	Unclear	Individual cages and standardized housing described, but allocation to cage/level is not reported as random.	p.2, Animals and Housing
D5	Blinding of caregivers/investigators	Low	Researchers conducting the experiment remained blinded to dietary identity until final analyses.	p.3, Dietary Treatments
D6	Random outcome assessment	Unclear	All pigs underwent MRI, with scans clustered on the same day by cohort; scan order was not specified as random	p.5, Magnetic Resonance Imaging (MRI)
D7	Blinding of outcome assessors	Low	A second trained image reviewer was blinded; study researchers and behavioral scorers were also masked.	pp.3, 5, Dietary Treatments, Structural MRI
D8	Incomplete outcome data	Unclear	Data exclusions based on outliers and behavioral noncompliance were made, no isolated HMO group were reported.	p. 7, Statistical Analysis
D9	Selective outcome reporting	Low	Principal outcomes and nonsignificant results reportedly documented.	p. 8, Results, Supplementary tables
D10	Other potential sources of bias	Unclear	Use of different dose for each source of oligosaccharide may limit generalizability of results. Unquantified feed refusals may limit certainty about actual intake.	pp.3, 11, Dietary Treatments, Discussion

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Six cohorts counterbalanced for litter and initial weight; no random sequence method reported.	p.2, Animals and Housing
D2	Baseline characteristics	Low	Male pigs of the same genetic line; starting body weight and litter balanced within cohorts.	p.2, Animals and Housing
D3	Allocation concealment	Unclear	Allocation concealment before assigning pigs to groups not described.	p.2, Methods
D4	Random housing	Unclear	Individual cages and standardized housing described, but allocation to cage/level is not reported as random.	p.2, Animals and Housing
D5	Blinding of caregivers/investigators	Low	Researchers conducting study, acquiring and analyzing outcomes remained blinded until analyses ended.	p.2, Dietary Treatments
D6	Random outcome assessment	Unclear	All pigs were intended for MRI; random scan order is not described.	pp.4–5, Magnetic Resonance Imaging
D7	Blinding of outcome assessors	Low	The authors state that image acquisition and data analysis personnel were blinded to dietary identity.	p.2, Dietary Treatments
D8	Incomplete outcome data	Unclear	Two of 12 piglets in the BMOS + HMO group were removed, their potential influence on baseline comparability warrants caution.	p. 6, Statistical Analysis
D9	Selective outcome reporting	Low	Principal outcomes and nonsignificant results reportedly documented.	pp.6, 7, Statistical Analysis, Results
D10	Other potential sources of bias	Unclear	Total oligosaccharide doses differed markedly across intervention arms; this limits attribution of some comparisons to oligosaccharide type. Feed refusals not quantified.	p.3, Dietary Treatments

Wang et al. (2019), Nutritional Neuroscience, DOI: 10.1080/1028415X.2019.1691856.

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Piglets were stratified by body weight and litter origin and then "randomly assigned" to the intervention groups; the method used to generate the random sequence was not specified.	Abstract, p. 885; Animals, p. 886
D2	Baseline characteristics	Low	Initial body weight was reported to be similar across groups, and assignment considered weight and litter.	Animals, p. 886; Results, p. 888
D3	Allocation concealment	Low	Treatment groups were coded green, blue and red, and personnel were blinded	Animals, p. 886
D4	Random housing	Low	Piglets were housed in pairs; random allocation of pens or pen positions to treatments was reported.	Abstract, p. 885; Animals, p. 886
D5	Blinding of caregivers/investigators	Low	The authors explicitly state that all participating personnel were blinded to the SMOS dose level throughout the study; feeding personnel were included in the later description.	Animals, p. 886; MRS acquisitions, p. 887
D6	Random outcome assessment	Low	MRS was conducted at PND 38–39 with a randomized scan order.	MRS acquisitions, p. 887
D7	Blinding of outcome assessors	Low	The paper explicitly states that staff and students responsible for MRS acquisition and data analysis were blinded to treatment cohorts. Automated TARQUIN processing was additionally used.	MRS acquisitions/data analysis, p. 887
D8	Incomplete outcome data	Unclear	Two of 48 piglets died from pneumonia and E. coli septicaemia, leaving 46 analysed. Potential effects of missingness are not specified in the main article.	Animals, p. 886; Abstract, p. 885
D9	Selective outcome reporting	Low	The study describes 33 metabolites and presents many outcomes, including nonsignificant findings.	Statistical analysis, p. 888; Results, pp. 888–890
D10	Other potential sources of bias	Unclear	Piglets were housed in pairs, but the paper does not establish whether pen/litter clustering was handled in the analysis.	Animals, p. 886; Statistical analysis, p. 888

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Preterm piglets were stratified by litter and birth weight and allocated to two diets; random sequence generation was not described.	pp. 2–3, Experimental Design and Animal Procedures
D2	Baseline characteristics	Low	Stratification by litter/birth weight was reported, both male and female piglets were used.	pp. 2–3, Experimental Design and Animal Procedures
D3	Allocation concealment	Unclear	The researchers were blinded to the treatment groups. Allocation concealment is not described.	pp. 2–3, Experimental Design and Animal Procedures
D4	Random housing	Unclear	Incubator and rearing procedures described, but random housing placement across interventions is not established.	pp. 2–3, Experimental Design and Animal Procedures
D5	Blinding of caregivers/investigators	Low	The study explicitly states that researchers were blinded to preterm treatment groups.	p. 3, Experimental Design and Animal Procedures
D6	Random outcome assessment	Unclear	Random selection for ex vivo MRI assessment not documented; random ordering reported for some behavioral procedures does not establish imaging randomization.	pp. 3–5, Motor Acquisition, Home Cage Activity, Open Field Assessment, and Cognitive Performance, Ex Vivo Magnetic Resonance Imaging,
D7	Blinding of outcome assessors	Unclear	General researcher blinding reported, but whether MRI segmentation/ROI assessors remained blind is not explicitly established.	pp. 2–3, Experimental Design and Animal Procedures; pp. 4–5 Ex Vivo Magnetic Resonance Imaging
D8	Incomplete outcome data	Unclear	Six of 46 preterm piglets were euthanized owing to clinical complications; possible effects of unequal losses are not fully assessed.	pp. 2–3, Experimental Design and Animal Procedures
D9	Selective outcome reporting	Low	Principal outcomes and nonsignificant results reportedly documented.	pp. 7–14, Results
D10	Other potential sources of bias	Unclear	Ex vivo imaging of the right hemisphere of the brain were performed.	pp. 4–5 Ex Vivo Magnetic Resonance Imaging

Domain	SYRCLE item	Judgment	Published evidence and justification	Source location
D1	Sequence generation	Unclear	Random allocation reported, with litter and starting weight considered across four replicates; the sequence-generation procedure was not specified.	p. 2, Animal Care & Housing
D2	Baseline characteristics	Low	Initial body weight and litter origin were considered when allocating piglets to four diets; all animals were male and similarly aged.	p. 2, Animal Care & Housing
D3	Allocation concealment	Unclear	Methods do not describe a procedure preventing foreknowledge of subsequent diet allocation.	p. 2, Animal Care & Housing
D4	Random housing	Unclear	Individual rearing units and environmental conditions are described, but random assignment to cage positions is not reported.	pp. 2-3, Animal Care & Housing
D5	Blinding of caregivers/investigators	Low	The authors explicitly report that personnel conducting the study remained blinded to treatment identities through final analysis.	p. 6, Statistical Analysis
D6	Random outcome assessment	Unclear	All pigs underwent MRI; randomization or balancing of imaging/acquisition order is not specified.	p. 4, Magnetic Resonance Imaging
D7	Blinding of outcome assessors	Low	Personnel involved in acquisition and analysis were explicitly stated to be blinded to treatment identity.	p. 6, Statistical Analysis
D8	Incomplete outcome data	Unclear	Health-related removals of several piglets described but without complete group-specific attrition and its potential impact.	p. 2, Animal Care & Housing
D9	Selective outcome reporting	Low	Multiple MRI/DTI/MRS outcomes reported.	p. 1 Abstract; pp. 4–6 Methods
D10	Other potential sources of bias	Unclear	TBSS/VBM excluded control and HIGH groups, limiting full between-group interpretation; insufficient information to establish whether this produced systematic bias.	p. 7, Tract-Based Spatial Statistics