

S1 - ANIMAL ETHICS

The ARRIVE Essential 10: Compliance Questionnaire

Use this questionnaire to evaluate how well a manuscript complies with the ARRIVE Essential 10. It can be applied to any manuscript describing comparative experiments in living animals, by assessors such as journal staff, editors, or peer reviewers.

Item	Question(s)	Answers
1 Study Design	Are all experimental and control groups clearly identified?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
	Is the experimental unit (e.g. an animal, litter or cage of animals) clearly identified?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
2 Sample Size	Is the exact number of experimental units in each group at the start of the study provided (e.g. in the format 'n=')?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
	Is the method by which the sample size was chosen explained?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
3 Inclusion & Exclusion Criteria	Are the criteria used for including and excluding animals, experimental units, or data points provided?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
	Are any exclusions of animals, experimental units, or data points reported, or is there a statement indicating that there were no exclusions?	<input checked="" type="checkbox"/> Yes, for at least one analysis <input type="checkbox"/> No
4 Randomisation	Is the method by which experimental units were allocated to control and treatment groups described?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
5 Blinding	Is it clear whether researchers were aware of, or blinded to, the group allocation at any stage of the experiment or data analysis?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
6 Outcome Measures	For all experimental outcomes presented, are details provided of exactly what parameter was measured?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
7 Statistical Methods	Is the statistical approach used to analyse each outcome detailed?	<input checked="" type="checkbox"/> Yes, for at least one analysis <input type="checkbox"/> No
	Is there a description of any methods used to assess whether data met statistical assumptions?	<input checked="" type="checkbox"/> Yes, for at least one analysis <input type="checkbox"/> No <input type="checkbox"/> Not applicable
8 Experimental Animals	Are all species of animal used specified?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
	Is the sex of the animals specified?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No <input type="checkbox"/> Not applicable to species
	Is at least one of age, weight or developmental stage of the animals specified?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
9 Experimental Procedures	Are both the timing and frequency with which procedures took place specified?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
	Are details of acclimatisation periods to experimental locations provided?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No
10 Results	Are descriptive statistics for each experimental group provided, with a measure of variability (e.g. mean and SD, or median and range)?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No <input type="checkbox"/> Not applicable to the type of data collected
	Is the effect size and confidence interval provided?	<input checked="" type="checkbox"/> Yes, for at least one experiment <input type="checkbox"/> No <input type="checkbox"/> Not applicable to the type of analysis used

Animal Handling

One week prior to conducting the study, the pigs arrived at the farm to acclimatise and were evaluated for signs of distress or suffering several times by professional staff. No immune or genetic modifications were used, and the animals followed the Danish Specific Pathogen Free Program. The pigs were fed a standard diet, had free access to water and were housed two-by-two in concrete floor pens. Prior to transportation to the laboratory facilities the pigs were sedated on the farm by intramuscular injection of a commonly used veterinarian anaesthetic mix (*Zoletil 50 Vet, Virbac, Denmark*) before transportation to the laboratory facility to minimise animal stress and increase refinement. The study complied with the PREPARE and ARRIVE 2.0 guidelines.

Risk of bias reduction

Regarding the potential for interference between protocols and the risk of bias, we took several measures to mitigate these concerns. First, we conducted a pilot study concurrently with the original protocol pilot study to assess the hemodynamic effects of altering positive end-expiratory pressure (PEEP) levels. This approach assured the reversibility and feasibility of short-term PEEP alterations, providing confidence in its implementation in our multiprotocol design (i.e., prior to onset of the original study protocol).

Furthermore, we ensured a proper inter-study washout period between protocols to mitigate any residual effects. Additionally, the non-pharmacological intervention employed in the current protocol involved rapid washout and steady-state conditions, minimizing the risk of bias in subsequent measurements. Finally, the subsequent original protocol involved maintaining a steady-state PEEP level of 5 cmH₂O, further reducing the potential for bias.