

List of papers

Paper I: Malla N, Nørgaard J.V, Lærke N.H, Heckmann L.H.L, Roos N. 2022. Some insect species are good-quality protein sources for children and adults: Digestible Indispensable AA Score (DIAAS) determined in growing pigs, *The Journal of Nutrition*, 152(4):1042–1051, <https://doi.org/10.1093/jn/nxac019>

Paper II: Malla N, Roos N, Van der Heide M.E, Nørgaard J.V. 2023. Meal of yellow and lesser mealworm and defatted black soldier fly larvae for weaned piglets and their effect on growth performance and gut health, Submitted to *Animal Feed Science and Technology*.

Paper III: Malla N, Nørgaard J.V, Roos N. 2023. Protein quality of edible insects in view of current assessment methods, *Animal Frontiers*, In Press, <https://doi.org/10.1093/af/vfad015>

Other publications

The following publication is not included in the thesis.

Malla N, Roos N. Are insects good quality protein for humans? 2023. Editorial, *Journal of Insects as Food and Feed*, In press.

English summary

Background: Insects have gained global recognition as a sustainable alternative to conventional protein sources, with the potential to address global food and nutrition insecurity as well as reduce the ecological footprint of animal production. While insects are part of traditional diets in many regions, the Western world is still unwilling to consume insects in their diets, mainly due to acquired distaste and a lack of information on their nutritional benefits. Studies demonstrating similar or higher digestibility and protein quality of insects compared with conventional proteins can aid their acceptance as viable food sources. Additionally, a great deal of interest has been placed on the biofunctional properties of insects owing to their ability to improve gut health, leading to good growth performance without any negative impact on animal health. These functional properties of insects can be valuable in animal production, particularly for overcoming the challenges associated with weaned piglets. Therefore, the overall aim of this thesis is to provide scientific evidence and add to the current knowledge on the suitability of insects as food and feed.

Methods: This thesis is based on two experimental studies and a review. **Paper I** reports the protein quality of five insect species (LMW, lesser mealworm; YMW, yellow mealworm; HC, house crickets; BC, banded crickets; and BSF, black soldier fly) based on the digestible indispensable amino acid score (DIAAS) recommended by the Food and Agriculture Organisation (FAO). A 6×6 Latin square design was used in this study. During the experiment, all six cannulated pigs were fed one of the six different diets (one nitrogen-free and five insect-based diets) every experimental week for six weeks so that all pigs received all diets by the end of the experiment; hence, each diet was assessed in six replicate pigs. An indigestible marker was used to assess digestibility. Individual insect, diet, and ileal digesta samples were collected to assess their chemical composition. **Paper II** aimed to evaluate the effects of three insect (LMW, YMW, and BSF) based diets (partially replaced with soybean protein concentrate) on growth performance, gut health, and immune response in weaned piglets compared with soybean protein (control). A total of 96 piglets were randomly assigned to one of the four diets (24 piglets per diet) for 14 days. All the animals were euthanised at the end of the experiment. All insect species and diet samples were collected to assess their chemical composition, blood for diamine oxidase and D-lactate concentration, stomach digesta for pH, small intestine and colon digesta for pH, microbial and volatile fatty acid analysis, and mucosal scraping of the small intestine for secretory IgA concentration. **Paper III** reviewed suitable methods for evaluating digestibility and protein quality, focusing on methods that have reported on the protein quality of insects. Additionally, the literature reviewed was based on the protein and AA digestibility of insects used in livestock feed (especially poultry and pigs) and their effect on growth performance.

Results: In **Paper I**, sulphur AAs (methionine + cysteine) were the limiting AAs in mealworm and cricket species for the age groups above six months old. For young children and those older than three

years, the DIAAS of both cricket species was higher than 75. Only one species of mealworm, LMW, had a DIAAS > 75 for individuals older than three years. The study in **Paper II** found that insect-based diets fed to weaned piglets did not affect growth performance significantly ($P > 0.05$), but were comparable to soybean-based diets. There was no significant difference ($P > 0.05$) in gut health and immune parameters between the diets. Only piglets fed the LMW and YMW diets had significantly ($P < 0.05$) lower diamine oxidase (DAO) concentrations (321 and 309 ng/ml, respectively) than those fed the control diet (382 ng/ml).

Conclusion: Crickets and mealworm species are good protein sources for older children, adolescents, and adults, with the cricket species having slightly better DIAAS than mealworms. While assessing the protein quality of insects using DIAAS, the standard nitrogen-to-protein conversion of 6.25 needs reconsideration as this conversion does not account for non-protein-nitrogen present in insects, thereby underestimating the DIAAS of insects. Additionally, the non-protein-nitrogen content of insects has challenged the direct comparison of insect protein quality with studies of other food sources. Sulphur AA was the first limiting amino acid in all four species of crickets and mealworms.

The inclusion of mealworms or BSF supported the growth and overall health of weaned piglets. The piglets fed insect-based diets and soybean-based diets performed equally in terms of growth performance and all related gut health parameters. Reduced plasma DAO concentrations in piglets fed with mealworms indicated protection of the intestinal mucosal barrier function.

Overall, these studies confirmed that insects are promising alternative protein sources for human food and animal feed, although further research is required to document and understand the properties and qualities of each of the relevant insect species. Their protein quality, comparable growth performance to that of conventional feed, and positive impact on gut health parameters highlight their suitability as viable alternatives. Additionally, mealworm-based diets contribute to improved gut health by protecting the intestinal mucosal barrier in weaned piglets. These findings expand our knowledge and pave the way for a more sustainable and innovative approach to food and feed production. A more comprehensive understanding of the potential use of insects for food and feed requires further research, with careful consideration of the methods applied.

Sammendrag (Danish summary)

Baggrund: Insekter har fået global anerkendelse som et bæredygtigt alternativ til konventionelle proteinkilder med potentiale for at håndtere global fødevarerforsyning, bedre ernæring samt reducere den miljømæssige belastning fra dyreproduktion. Mens insekter er en del af den traditionelle kost i mange regioner, er den vestlige verden stadig tøvende overfor insekter i kosten, primært på grund af usikkerhed og mangel på information om deres ernæringsmæssige fordele. Studier der viser en tilsvarende eller højere protein fordøjelighed og kvalitet af insekter i forhold til konventionelle proteiner, kan hjælpe med at øge insekternes accept som fødevarer. Derudover er der stor interesse for insekters biofunktionelle egenskaber på grund af deres evne til at forbedre tarmens sundhed, hvilket fører til god vækst og færre antibiotikakrævende behandlinger. Disse funktionelle egenskaber af insekter kan være værdifulde indenfor dyreproduktion, især til at overvinde udfordringerne forbundet med fravænnede pattegrise. Derfor er det overordnede mål med denne afhandling at levere videnskabelig evidens og bidrage til den nuværende viden om insekters egnethed som fødevarer og foder.

Metode: Denne afhandling er baseret på to eksperimentelle studier og en litteraturgennemgang. **Artikel I** rapporterer proteinkvaliteten af fem insektarter (LMW, lille melorm; YMW, almindelig melorm; HC, husfårekilling; BC, stribet fårekilling; og BSF, sort soldaterflue) baseret på metoden fordøjelig essentiel aminosyre score (DIAAS), som anbefales af FAO. Dette studie blev udført ved hjælp af et 6x6 romerkvadrat design. Under forsøget blev alle seks ileumfistulerede grise fodret med en af de seks forskellige diæter (en kvælstoffri diæt og fem insektbaserede diæter), i én uge, således at alle grise havde modtaget alle diæter ved afslutningen af forsøget efter seks uger; dermed blev hver diæt evalueret på seks grise. En ufordøjelig markør blev brugt til beregning af fordøjelighed. Insekter, diæter og tarmindehold blev indsamlet for at analysere deres kemiske sammensætning. Studiet i **Artikel II** havde til formål at evaluere effekten af tre insekt-baserede (LMW, YMW og BSF) diæter med delvist erstatning af sojaprotein koncentrat på vækst, tarmsundhed og immunrespons hos fravænnede grise sammenlignet med sojaprotein (kontrol). I alt blev 96 grise tilfældigt fordelt til en af fire diæter (24 grise per diæt) i 14 dage. Alle dyrene blev aflivet ved eksperimentets afslutning. Alle insektarter og diæter blev indsamlet for at vurdere deres kemiske sammensætning. Blodprøver blev analyseret for diaminoxidase og D-laktat koncentrationer, maveindhold for pH, tyndtarm og tyktarmindhold for pH og kortkædede fedtsyrer. Afskrabninger af slimhinden fra tyndtarmen blev analyseret for IgA. **Artikel III** er baseret på litteraturgennemgang, der undersøgte egnede metoder til at vurdere insekternes fordøjelighed og protein kvalitet. Derudover er litteratur gennemgået for protein- og aminosyrefordøjelighed af insekter, når de anvendes i foder (især til fjerkræ og grise), og deres effekt på vækst.

Resultater: I **Artikel I** var de svovlholdige aminosyrer (SAA, methionin + cystein) de begrænsende aminosyrer i melorm og fårekilling til mennesker i aldersgruppen over seks måneder. For småbørn og dem over tre år, var DIAAS for begge fårekillingearter højere end 75%. Kun en art af melorm, LMW,

havde en DIAAS > 75% for personer over tre år. Studiet i **Artikel II** fandt, at insektbaserede diæter givet til fravænnede grise ikke påvirkede væksten signifikant ($P > 0,05$), men var sammenlignelige med sojaproteinbaserede diæter. Der var ingen signifikant forskel ($P > 0,05$) i tarmsundhed og immunparametre mellem diæterne. Kun grisene, der fik LMW- og YMW-diæterne, havde signifikant ($P < 0,05$) lavere koncentrationer af diaminoxidase (DAO) (hhv. 321 og 309 ng/ml) end dem, der fik kontroldiæten (382 ng/ml).

Konklusioner: Fårekylinger og melormearterne er gode proteinkilder til ældre børn, unge og voksne, hvor fårekylingearterne har lidt bedre kilde til aminosyrer end melorme. Når man vurderer proteinkvaliteten af insekter ved hjælp af DIAAS, bør omregningsfaktoren på 6,25 fra kvælstof til råprotein tages op til fornyet overvejelse, da denne omregning ikke tager højde for ikke-protein kvælstof i insekter og derved undervurderer DIAAS for insekter. Derudover udfordrer ikke-protein kvælstof i insekter den direkte sammenligning af insektproteinkvalitet med undersøgelser af andre fødekilder der kan indeholde et andet niveau af ikke-protein kvælstof. Svovlholdige aminosyrer var de først begrænsende aminosyrer i alle fire arter af fårekylinger og melorme.

Inkludering af melorme eller sort soldaterflue understøttede vækst og generel sundhed hos fravænnede grise. De grise, der blev fodret med insekt-baseret foder og sojabønnebaseret foder, klarede sig ligeså godt med hensyn til vækst og relaterede tarmsundhedsparametre. Reduceret DAO-koncentration i plasma i smågrise fodret med melorme indikerede beskyttelse af tarmslimhindebarrierefunktionen.

Samlet set bekræftede undersøgelserne, at insekter er lovende alternative proteinkilder i fødevarer og foder, selvom der er behov for yderligere forskning for at dokumentere og forstå egenskaberne og kvaliteterne af hver af de relevante insektarter. Deres proteinkvalitet, sammenligningen af vækstpotentiale med konventionelt foder og positive indvirkning på tarmsundhedsparametre, fremhæver insekternes egnethed som realistiske alternativer. Derudover bidrager melorm-baserede diæter til forbedret tarmsundhed ved at beskytte tarmslimhinden hos fravænnede pattegrise. Disse resultater bidrager til at udvide vores viden og baner vejen for en mere bæredygtig og innovativ tilgang til fødevarer- og foderproduktion. En mere omfattende forståelse af potentialet ved at bruge insekter til fødevarer og foder kræver yderligere forskning med nøje overvejelse af de anvendte evalueringsmetoder.

Table of contents

PREFACE.....	2
LIST OF PAPERS	4
ENGLISH SUMMARY	5
SAMMENDRAG (DANISH SUMMARY)	7
ABBREVIATIONS	9
1 INTRODUCTION	15
1.1 Objectives	15
2 BACKGROUND.....	17
2.1 Brief history on scientific milestones on insect as food and feed	17
2.2 Sustainability aspect of insects.....	18
2.3 Nutritional properties of insects	19
2.3.1 Protein and amino acids	19
2.3.2 Indispensable amino acids	20
2.4 Biofunctional properties of insects	21
2.4.1 Antimicrobial peptides.....	21
2.4.2 Medium chain fatty acids	22
2.4.3 Chitin and chitosan	23
2.4.4 Relevance in weaned piglets	23
2.4.5 Relevance in environmental enteric dysfunction	24
2.5 Dietary protein.....	24
2.6 Protein digestibility.....	25
2.6.1 Digestibility.....	25
2.6.2 Total tract digestibility	25
2.6.3 Ileal digestibility	26

2.7 Protein and amino acid requirements and recommendations	28
2.8 Protein quality.....	29
2.8.1 Methods for determining protein quality	30
2.8.2 Amino acid score	30
2.8.3 Why DIAAS ?.....	30
2.8.4 Reference pattern	31
2.9 Insect as Food.....	32
2.9.1 Role of animal source food in human nutrition.....	32
2.9.2 Relevance of insect as food.....	33
2.9.3 Insect consumption	33
2.9.4 Protein quality of insects in relation to human requirement.....	35
2.10 Insect as pig feed	35
2.10.1 Relevance of insects as feed.....	35
2.10.2 Nutrient digestibility of insects when fed to pigs.....	36
2.10.3 Growth performance of pigs when feeding insects.....	37
2.10.4 Biofunctional properties of insects when fed to animals.....	39
3 METHODOLOGY	41
3.1 Ethical approvals	41
3.2 Insect products.....	41
3.3 Experimental Diets	41
3.4 Animals	43
3.5 Experimental design	43
3.6 Sampling and laboratory analysis	46
3.6.1 Bacteria and short-chain fatty acids	46
3.6.2 Gut permeability	47
3.6.3 Gut immunity.....	47
3.7 Data analysis	47

3.8 Paper III	48
4 RESULTS	49
4.1. Summary of Paper I	49
4.2 Summary of Paper II.....	49
4.3 Summary of Paper III	49
4.4 Paper I	51
4.5 Paper II.....	63
4.6 Paper III	97
5 DISCUSSION	131
5.1 Protein content.....	131
5.2 Protein quality of insects using DIAAS.....	131
5.2.1 Digestibility of insects	132
5.2.2 Nitrogen to protein conversion.....	134
5.3 Current recommendation of protein and IAA requirement	135
5.3.1 Implications of IAA requirements in DIAAS	136
5.3.2 Implication of EAR of protein in the DIAAS	136
5.4 Digestible indispensable AA.....	137
5.5 Insects in mixed diets.....	137
5.6 Insects for athletes	138
5.7 Animal performance.....	139
5.8 Biofunctional properties.....	140
5.9 Diamine oxidase as biomarker for EED	142
6 PERSPECTIVE AND FUTURE RESEARCH	144
6.1 Global practice to determine the protein quality	144
6.2 DIAAS as a protein quality measure.....	144

6.3 Relevance of insects in dietary transition	146
7 CONCLUSION.....	147
8 REFERENCES	149
9 APPENDICES.....	163
Appendix A – Conference presentations.....	163
Appendix B – Supplementary material to the Journal of Nutrition (Paper I)	164