Effect of lupines or quinolizidine alkaloids concentrations in porcine diets on the performance parameters of growing pigs: Protocol for a systematic review and meta-analysis.

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Amendments: This review is not an amendment of a previously published protocol.

The protocol is to be registered with Systematic Reviews for Animals & Food, syreaf.org. and deposited at OpenAgrar

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The structure of this protocol was inspired by an existing protocol of Higgs et al. (1)

Introduction:

The European livestock sector is highly dependent on the imports of plant proteins for animal feed, mainly soybeans from Argentina, Brazil and the United States (2). Importing soy increases the carbon footprint of livestock farming and soy production is a driver of deforestation in the Amazon rainforest (2, 3). Strategies to replace soya-based protein use other plant protein sources such as rapeseed or various legumes such as peas, beans or lupin seeds. (2). The three most commonly cultivated lupins are *Lupin albus, L angustifolius* and *L. luteus* (4). The seeds of these lupins are characterised by a high crude protein content (39.8 to 48.2% crude protein in dry matter) and high concentrations of essential amino acids (5). However, lupin seeds contain various antinutritive factors such alpha-galactosides, phytic acid, saponins, tannins and quinolizidine alkaloids (QA) (4). With the exception of sparteine, the toxicology of the various QA is only superficially characterised (4). The concentration of QAs in lupins has been reduced by different breeding strategies (6), but QA in lupins can still threaten the health of various species (4). After ingestion of certain levels, QA-intake is associated with a variety of symptoms in different species, including feed refusal, lethargy, intestinal stasis, reduced reproductive performance and even sporadic death (4). Especially pigs are sensitive to QA (4, 7). Nevertheless, numerous studies have investigated the varying levels of lupine and corresponding QA-levels in porcine diets reporting conflicting effects on porcine performance parameters. The proposed systematic review and meta-analysis will systematically explore the published literature on varying levels of lupines and corresponding QA-levels in porcine diets on porcine performance parameters. To the best of our knowledge, no systematic review or meta-analysis has been conducted to investigate the effect of lupines or corresponding QA-levels on porcine performance.

Objective:

The primary objective of this systematic review is to assess the effect of quinolizidine alkaloids (QA)-concentrations in porcine diet on health measures. The objective of the meta-analysis is to clarify at

which level in the porcine diet lupines or corresponding QA-concentrations influence the performance of growing pigs.

The review defines the methodology with the following PICO questions:

Population: Weaned Pigs not older than nine months.

Intervention/Exposure: Diets containing different levels of lupines

Comparator: Isonitrogenous and Isocaloric diets not containing lupines.

Outcome: Zootechnical data: (Feed Intake (FI), Average daily gain (ADG), Feed Conversion Ratio (FCR)) and Body weight (BW)/Live weight of pigs during the weaning and fattening period or data to calculate corresponding performance parameters.

Study questions: Is it possible to include lupins in porcine diets without affecting either performance or health parameters?

Systematic review:

Primary outcome: Performance parameters

Secondary: Health parameters

Meta-analysis:

1. At which levels can lupins be added to porcine diets without affecting the performance of growing pigs?

Methods:

Eligibility criteria

The following studies will be included:

- Original scientific reports
- All study designs except case reports
- Only Peer Reviewed Articles
- Studies either written in English or German
- Pigs that have undergone surgical modification of the gastrointestinal tract, including procedures such as ileorectal anastomosis, insertion of a T-canula into the ileum or similar procedures, are excluded.
- At least one of the diets studied must contain one of the following lupine species:
    - *L. albus*
    - *L. angustifolius*
    - *L. luteus*
- Reporting Zootechnical data: (Feed Intake (FI), Average Daily Gain (ADG), Feed Conversion Ratio (FCR)) of pigs during the weaning and fattening periods or data to calculate the corresponding performance parameters.

Information sources: A literature search using the following databases and search terms will be conducted by JBS and NG.

- Pubmed Central
- Scopus
- Web of Science
- EBESCO

Search terms

Pubmed Central:

((“Swine”[Mesh]) AND "Lupinus"[Mesh])

Scopus, Web of Science, EBESCO:

“Swine” OR “Pig” OR ”Suidae” OR AND "Lupine*" OR "Quinolizidine Alkaloids"

The “Mesh-terms” has undergone minor adaptations in order to facilitate the search on the other platforms, which restrict the search to domesticated pigs, thus excluding warthogs from the search.

In addition, the search term used by EFSA in its scientific opinion (4) was refined to meet the needs of the above research question, resulting in the following search term:

TOPIC: (lupanine OR lupinine OR isolupanine OR isoangustifoline OR sparteine OR cytisine OR methylcytisine OR albine OR angustifoline OR isosparteine OR anagyrine OR thermopsine OR multiflorine OR tetrahydrorhombifoline OR hydroxylupanine OR angeloyloxylupanine OR tigloyloxylupanine OR cinnamoyloxylupanine OR quinolizidine OR lupin*) AND TOPIC: (toxicity OR toxi* OR acute OR subacute OR subchronic piglet OR swine)

A backward and a forward search based on previously identified publications will also be performed to ensure that all relevant literature will be captured. Respective eligibility criteria, inclusion criteria and exclusion criteria need to be met as well. Identified studies will be managed with EndNote™ (Clairvate Analytics, Philadelphia, USA) and duplicates will be removed.

The number of hits with the name of the database and the date of running the search will be recorded.

Selection Process

We plan to apply a 2-level screen for study inclusion. Level 1 will evaluate the title and the abstract and will focus on the following questions:

- Was the study published in German or English?
- Was the study published in a Peer-Reviewed-Journal?
- Was the study conducted in pigs?
- Did one group of pigs receive a diet containing lupines?

Respective questions will be answered by JBS and only studies meeting the above-mentioned requirements will be considered for Level 2

In the second level the selected studies will be screened for the following inclusion and exclusion criteria by two reviewers BW and JBS. Respective questions will be answered with “yes”, “no” or “unclear”

- Are the fed diets isocaloric or isonitrogenous?
- Is the following zootechnical data presented or is it possible to calculate respective zootechnical data from presented data. Respective zootechnical data are:
    - Feed Intake (FI)
    - Average daily gain (ADG)
    - Feed conversion ratio (FCR)
    - Live weight at the beginning and the end of the trial

The study will only be included in the data extraction step if both questions are answered in the affirmative. If the reviewers disagree, the decision is discussed with a third independent person.

Data items

- Authors
- Publication year
- Study population
    - Age/ BW at beginning of the study
    - Breed
    - Sex
    - Group size
    - Duration of study
- Diet
    - Crude Protein Content in diet
    - Energy content in diet
    - Dry matter content
- Study group
    - Lupin species
    - Lupin subgenus
    - Level of Lupins in diet
    - Sum of quinolizidine alkaloids (QA) in diet
        - Portion of single QA
- Control group
    - Main protein source in the diet
- Performance data/ Outcome
    - Feed intake
        - Average daily intake
        - Intake during entire study
    - Weight gain
        - Average daily gain (ADG)
        - Weight gain during the entire study
    - Feed conversion ratio
    - Weight at the end of the study
    - Reports concerning the health of investigated pigs
- Biases/ confounding
    - Has the QA-concentration been measured in lupines or diet?
    - Did the bodyweight differ between groups at the beginning of the study?
    - Did the groups have the same sex-ratio?
    - Was group allocation randomized?
    - Were the researchers blinded?
    - Did diets contain the same lysine-quantities?
    - Funding

Outcome

The primary outcomes are the performance parameters feed intake, weight gain and feed conversion ratio. The outcome is evaluated separately according to the age of the pigs studied. Three different observation periods are considered. These were chosen on the basis of the age of the pigs and are defined as follows: weaners (weaned pig weighing around 8 kg, younger than 10 weeks), growers (growers between 10-18 weeks, weighing 25-50 kg), finishers (finishers 50-110 kg, older than 18 weeks). This classification is based on the publication by Wilson et al. (8) and was introduced because the age of the pigs has a decisive influence on the performance parameters mentioned above. If the

observation cannot be clearly assigned to the different observation periods, the period in which the pigs stayed the longest is chosen after discussion between the principal investigators BW and JBS.

Risk of Bias

Risk of bias will be evaluated based on the SYRCLE's risk of bias tool for animal studies (9). In addition, another set of items is added to the risk of bias assessment. This relates to the concentration of lysine in the diet, as lysine is the first limiting amino acid for pig growth (10) and variations in dietary lysine concentration may influence the outcome of the study. In addition, we included the question of whether the QA concentration was reported in the diet or in the lupines, as the QA intake could influence performance parameters.

Data synthesis

Data are extracted from the respective publications and summarised in a table. A study may report more than one observation, as a publication may examine different observation periods, different lupin species and different lupin levels. If it is possible to extract sufficient data, correlation analysis between performance parameters and dietary lupin content or corresponding QA-intake will be performed. An additional analysis will be performed based on the Handbook for Systematic Reviews of Interventions (11) using the appropriate R packages if sufficient data are available. Sufficient data is defined as follows: ten outcomes per age group and lupin species. If insufficient data are available, only descriptive statistics will be reported as performed by other researchers (12, 13). We plan to apply a "multilevel" Meta-Analysis as preliminary literature search showed that several studies investigated different concentration of lupines in feed and different lupin species (14). Additionally, we plan to use a "Random-Effects Model" as different studies were conducted under diverse conditions creating additional heterogeneity (14). Furthermore, we plan to perform meta regression if sufficient data is available to investigate whether there is a correlation between lupin concentration in diets or corresponding QA intake and performance parameters (14).

Discussion

Limitations:

In this meta-analysis, we plan to investigate previously published studies that have included lupines in porcine diets and documented the effect of lupines on the performance of growing pigs. The inclusion of lupines in the diet of pregnant sows may also be an interesting research topic, as it is known that the QAs such as sparteine interact with the smooth muscle cells of the uterus and should therefore be investigated separately (15). The goal is to provide evidence for save inclusion rates for lupines in porcine diets without excluding information based on the quality of selected experiments. Based on our previous experience in the field of animal nutrition, animal science and veterinary science, we expect that the chosen tool for assessing risk of bias may have shortcomings, as blinding and random allocation in particular play a subordinate role in study design in this field of research compared to other fields of research. At this point, we would like to emphasise that the chosen outcome measures are resistant to bias, as the outcome are based solely on simple measures (scales) and do not include subjective measures. We are also aware that varying levels of lupins in porcine diets may affect other parameters such as diet digestibility or gut function, which again should be evaluated in different systematic review and meta-studies. Furthermore, we expect that different breeds may react variably to the diets fed. It should also be noted that different units are used worldwide to measure the energy content of porcine diets. These units are not easily converted and may challenge the comparison of energy levels between studies difficult.

Conclusion:

This meta-analysis has got the aim to clarify at which level lupines can be added to a porcine diet to reduce the dependency of the animal husbandry sector of soy and to reduce carbon food print of pork production. Results can help to create a more sustainable pork production and might help to secure world rising demands of high-quality protein

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