Step 1: Identify Areas to Potentially Implement Alternatives

When planning an alternatives literature search, review the protocol (a proposed research study) and identify areas of the protocol that could incorporate the 3Rs (replacement, reduction, and refinement of animal use). Has the Principal Investigator (PI) considered methods/techniques that could reduce the number of animals used, minimize pain and distress, or in some cases, eliminate the use of animals altogether? These questions will provide the groundwork for your search strategy.

Below is a sample protocol for a study on necrotic enteritis (NE) in broiler chickens.

NOTE: The highlighted text and numbers in parenthetic statements at the end of each section, correspond with the list of the 3Rs examples directly below the protocol.

Effect of varying nutrients in the diet on necrotic enteritis (NE) in broiler chickens

A microbiologist is studying the effects of necrotic enteritis in broiler chickens (*Gallus gallus domesticus*). Broilers are affected by necrotic enteritis (a disease of the gut), which is caused by two pathogens, *Clostridium perfringens* and *Eimeria maxima*. Agricultural regulations restrict antibiotic use in chickens. The Principal Investigator (PI) is investigating alternative ways of preventing and mitigating the disease by studying the birds’ mucosal immune response in the gut. A possible alternative treatment to antibiotics for necrotic enteritis involves adding nutrients to the birds’ diets to increase their immune response. The *in vivo* chicken model is required for this study because the PI advises that there is currently *no in vitro model to study mucosal host immune response to poultry pathogens*. (1)

Materials and Methods

1. **2,000 birds over eight trials during a three-year period** (2)
2. *Eimeria maxima* and *Clostridium perfringens*

Methods

- The birds will be housed in starter and finisher unit cages inside a barn. The size of the cages and number of birds housed in each cage will vary according to the age/size of the birds. (For example, birds that are 3-5 weeks old will live in finisher unit cages of 24 x 24 inches with a maximum of five birds per cage). (3 and 4)
- Challenge: Birds will be challenged (infected) by *oral gavage* with the pathogens (*Eimeria maxima* and *Clostridium perfringens*) at two weeks of age. (5)
- Treatment: For each trial, groups of 40 birds will receive one of 18 nutrients or medications in their food.
- Monitoring health: Although infected birds will not receive any analgesics (6), the attending veterinarian and animal care staff will monitor the health of the birds for signs of pain and illness. Any bird that shows signs of extreme lethargy, as determined by the veterinarian, will be euthanized. (7) Ten birds from each group during each trial will be sacrificed at different time points and their intestinal tissue and blood will be collected for examination. (8) All remaining birds will be euthanized by cervical dislocation at 5 weeks of age and blood will be collected via cardiac puncture. (9)

Each of the following questions address at least one of the 3Rs:

(1) **Replacement—Models:** Can this study be conducted in a non-animal model? The PI advises there isn't a non-animal model available, but is this justified in the literature?

(2) **Reduction—Study Design:** Does this protocol duplicate published research? Is the number of animals proposed reasonable for this type of study? Can the researcher reduce the number of animals used, but still obtain statistical significance?

(3) **Refinement—Housing:** Are the cages and stocking density of the birds appropriate for the species and age of the birds? What refinements can be made to the cages (e.g., bedding, size, temperature, etc.) to reduce stress?

(4) **Refinement—Enrichment:** The protocol makes no mention of any enrichments that allow animals to express natural behaviors. What environmental enrichments could be added to their housing?

(5) **Refinement—Challenge Method:** Broilers are challenged (infected) with the pathogen using oral gavage, which is an invasive and stressful procedure. Are there less-invasive methods to infect the birds?

(6) **Refinement—Analgesia:** Some scientists are reluctant to use analgesics (pain relievers) due to the concern that they could skew the results. Is this concern justified, or are there other similar studies that have used analgesics without compromising the results?

(7) **Refinement—Monitoring of Animal Well-Being:** Are the scientists using a species-appropriate and proven method of monitoring animals for signs of pain and distress?

(8) **Reduction/Refinement—Imaging and Telemetry:** Can non-lethal methods and technologies be used (such as telemetry, imaging technology, or blood sampling) to study disease progression versus euthanizing birds at various stages of the disease? This could reduce the number of animals being sacrificed.
(9) **Refinement—Euthanasia:** Is cervical dislocation an acceptable method of euthanasia for this species? [Check the literature and the American Veterinary Medical Association’s [euthanasia guidelines](#)].

**Step 2: Finding Databases and Grey Literature**

One database does not cover 100% of the literature on a particular subject area or all types of materials (journals, books, meetings, conferences, etc.). Therefore, it is important to search multiples databases to capture all/most of the information needed. When choosing which databases to search, think about your academic discipline, the subject matter of your protocol, and what types of materials you want to find. You can also use other resources such as grey literature (e.g. conference abstracts, theses/dissertations, reports, newsletters, etc.), books, the National Agricultural Library (NAL) and Animal Welfare Information Center (AWIC) webpages, other 3Rs websites, or Google Scholar.

For this search, we will use the following bibliographic databases: PubMed, Web of Science, and EBSCO.

**Step 3-4: Developing a Search String**

We will use **Question 4** from the protocol to build our search string:

**Refinement—Enrichment:** The protocol makes no mention of any enrichments that allow animals to express natural behavior. What environmental enrichments could be added to their housing?

Use your question to come up with the different keywords/concepts that express the main topics and ideas you are searching for (see 1 – 2 below). Then brainstorm keywords and synonymous terms for that concept:

1. Animal or species keywords (chickens, broilers, broiler chickens, *Gallus gallus domesticus*, *G. domesticus*)
2. 3Rs keywords (environmental enrichment, enrichment, physical enrichment, sensory enrichment, nutritional enrichment, occupational enrichment)

*Note: When including 3Rs keywords, do not limit yourself to words such as, "alternatives" or the 3Rs (refinement, reduction, replacement). Include additional terms that are examples of these concepts. In this example, we've included keywords such "environmental enrichment" and "physical enrichment" since we're interested in enrichments that could improve the chickens' housing and encourage natural behaviors.*
Then, construct your search string by using syntax tools (Boolean operators, parentheses, truncation, etc.) to link your keywords, their synonyms, and concepts together.

Building a Search Using the PubMed Advanced Search Builder:

1. Go to PubMed.
2. Select Advanced below the search box. This will take you to PubMed's Advanced Search Builder.
3. Below Add terms to the query box, select Title/Abstract from the drop-down menu.
4. Add your animal/species concept keywords to the search box:
   a. In the Enter a search term box add, chicken* OR broiler* OR "broiler chicken*" OR "Gallus gallus domesticus" OR "G. domesticus". Select the blue ADD button. The keywords should populate in the "Query box".
5. Then, add your 3Rs concept keywords to the search box:
   a. In the Enter a search term box add, "environmental enrichment*" OR enrichment* OR "physical enrichment*" OR "sensory enrichment*" OR "nutritional enrichment*" OR "occupational enrichment*" and select the blue AND button. The keywords should populate in the "Query box".
6. Now that both of your concept groups are added into the query box, select the blue Search button to execute the search. View the search results.

Here is the full search string:

(chicken*[Title/Abstract] OR broiler*[Title/Abstract] OR "broiler chicken*"[Title/Abstract] OR "Gallus gallus domesticus"[Title/Abstract] OR "G. domesticus"[Title/Abstract]) AND ("environmental enrichment*"[Title/Abstract] OR enrichment*[Title/Abstract] OR "physical enrichment*"[Title/Abstract] OR "sensory enrichment*"[Title/Abstract] OR "nutritional enrichment*"[Title/Abstract] OR "occupational enrichment*"[Title/Abstract])

Step 5: Modifying a Search String

Conducting a literature search is a trial-and-error process. If you are finding a lot of irrelevant results, you will need to modify your search string. There are several options for modifying a search string, so choose one or more modifications based on the results. Also, there is no "right way" to modify a search string, so how you choose to modify won't necessarily be the same every time.

For this example search, we browsed through the titles and abstracts on the first few pages of results looking for relevant citations. We noticed that there were many irrelevant citations, and any relevant citations were using one of the animal keywords in the title. This suggests that most of the literature we are looking for will state in the title that the research is about chickens. One way to modify a search string is to limit the field you are searching, so for this
search, we will search our animal species keywords in the Title field rather than the Title/Abstract field.

After making this modification, we get the following search string and **results:**

(chicken*[Title] OR broiler*[Title] OR "broiler chicken*"[Title] OR "Gallus gallus domesticus"[Title] OR "G. domesticus"[Title]) AND ("environmental enrichment*"[Title/Abstract] OR enrichment*[Title/Abstract] OR "physical enrichment*"[Title/Abstract] OR "sensory enrichment*"[Title/Abstract] OR "nutritional enrichment*"[Title/Abstract] OR "occupational enrichment*"[Title/Abstract] OR "enriched environment*"[Title] OR "environmental complexity"[Title])

Then, we browsed through the first few pages of titles and abstracts in the results again. We still have over 700 results, and many of them are irrelevant. Therefore, we need to make additional changes to our search strings. As we skimmed the results, we noticed that "enriched environment" and "environmental complexity" were terms used to refer to environmental enrichment, so we can add these keywords to our 3Rs search string. Adding terms to your search string is another way to modify a search strategy. Also, like the animal species keywords, we saw that when the citation was relevant, our 3Rs keywords were used in the title of the citation. Thus, we can also limit our 3Rs search string from searching Title/Abstract to just Title to limit the number of irrelevant citations.

After making these two changes to our search string, we get the following search string and **results:**

(chicken*[Title] OR broiler*[Title] OR "broiler chicken*"[Title] OR "Gallus gallus domesticus"[Title] OR "G. domesticus"[Title]) AND ("environmental enrichment*"[Title] OR enrichment*[Title] OR "physical enrichment*"[Title] OR "sensory enrichment*"[Title] OR "nutritional enrichment*"[Title] OR "occupational enrichment*"[Title] OR "enriched environment*"[Title] OR "environmental complexity"[Title])

Now, we are seeing more citations that are relevant for our search. However, we are still getting quite a few citations that are talking about enrichment in a different context than what we are looking for. They discuss enrichment in the context of meat, carcasses, and culturing bacteria. We can eliminate these citations by adding another search string using the Boolean operator, NOT. For our NOT search string, we will search "All Fields" instead of "Title/Abstract".

After making this refinement to the search strategy, we get the following search string and **results:**

((chicken*[Title] OR broiler*[Title] OR "broiler chicken*"[Title] OR "Gallus gallus domesticus"[Title] OR "G. domesticus"[Title]) AND ("environmental enrichment*"[Title] OR enrichment*[Title] OR "physical enrichment*"[Title] OR "sensory enrichment*"[Title] OR "nutritional enrichment*"[Title] OR "occupational enrichment*"[Title] OR "enriched environment*"[Title] OR "environmental complexity"[Title])

After making these two changes to our search string, we get the following search string and **results:**
"nutritional enrichment*"[Title] OR "occupational enrichment*"[Title] OR "enriched environment*"[Title] OR "environmental complexity"[Title]) NOT (meat* OR carcass* OR bacteria OR culture*)

Now we have minimized the number of irrelevant results and found the information we are looking for, so this completes our search. Notice how we had to refine the search strategy multiple times to retrieve the information we needed.

Below are 5 out of several relevant citations discussing environmental enrichments for chickens. While most environmental enrichment research for chickens is conducted in a barn setting rather than a cage setting, we may still be able to modify some of these findings to fit a cage setting and meet the animals' needs.


**Step 6: Records and Citation Management**

Keep a record of your keywords, search strings, databases searched, and publication years searched. This allows you to simply update your search when needed in the future, rather than regenerating a list of keywords and building new search strings every time.

You can also use citation management software (CMS) such as EndNote or Zotero to store, organize and utilize the relevant citations you find. CMS helps with generating reference lists,
bibliographies, and footnotes. Contact your institution’s librarian to find out which citation managers are available to you. However, if you are a United States Department of Agriculture (USDA) employee and need assistance with using CMS, contact the National Agricultural Library.