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Raw or technologically treated proteaginous seeds as alternatives to soybean meal for dairy cows: comparative evaluation by meta-analysis of *in situ* and *in vivo* digestive parameters, nitrogen partition and dairy performance

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45 Abstract

46 The objectives of this study were to quantify the effects on nitrogen metabolism and dairy
47 performance of substituting soybean meal for proteaginous seeds or replacing raw with
48 treated proteaginous seeds in dairy cow diets. This study was focused on three proteaginous
49 seeds: faba bean, lupin and pea. Two databases were created, which gathered information on
50 *in vivo* and *in situ* results, respectively. These were then used to analyze nitrogen ruminal
51 degradability, nitrogen intestinal true digestibility, ruminal parameters, nitrogen partitioning
52 and milk production and composition. A total of 32 and 36 articles were analyzed from the *in*
53 *vivo* and *in situ* databases, respectively. Statistical analyses assessed the effects of substitution
54 for each measured or calculated variable; t-tests were applied to compare the difference
55 between the tested feed (i.e. raw or treated proteaginous seed, or treated proteaginous seed)
56 and the control feed (i.e. soybean meal, or raw proteaginous seed, respectively). The
57 proteaginous seeds contained less crude protein than soybean meal (on average -199 g/kg
58 DM, $P < 0.001$). The use of raw proteaginous seeds instead of soybean meal led to higher
59 nitrogen ruminal degradability (on average +16 g/100 g, $P < 0.005$), but treatment of these
60 seeds led to a decrease in nitrogen ruminal degradability (-13 g/100 g on average) compared
61 to raw seeds. Replacing soybean meal by raw faba bean, lupin or pea in the iso-crude protein
62 diets of dairy cows led to an increase in NH_3 in ruminal fluid (+20 mg/L, $P < 0.040$) and
63 tended to decrease milk protein content. However, when those seeds were treated and their
64 values compared to those of raw seeds, nitrogen in milk tended to increase, but milk fat
65 content was decreased. This quantitative review has allowed some general trends to be
66 highlighted, despite a limited amount of available data for some variables (in particular for
67 pea). Moreover, treatment of seeds are variable (use of different processes, pressures and
68 temperatures) and feeding practices of dairy cows are diverse (e.g. maize silage or grass silage
69 as main forage, forage:concentrate ratio ranging from 84:16 to 40:60), which can dilute the

70 effects of the addition of proteaginous seeds (added at up to 30 g/100 g of dietary dry matter)
71 to the dairy cow diets.

72 *Keywords:* proteaginous seed, soybean meal, dairy cows, nitrogen

73 *Abbreviations:* BC-VFA, branched-chain volatile fatty acid; dr_N, nitrogen intestinal true
74 digestibility; ED₆_N, nitrogen ruminal effective degradability assuming a particle outflow
75 from the rumen of 0.06 h⁻¹; MFC, milk fat content; MPC, milk protein content; NANM N,
76 non-ammoniacal non-microbial nitrogen; PDIA₆, dietary protein truly digestible in the
77 intestine assuming a particle outflow from the rumen of 0.06 h⁻¹; PIA₆, dietary protein
78 entering the intestine assuming a particle outflow from the rumen of 0.06 h⁻¹; PS,
79 proteaginous seeds; SBM, soybean meal

80 **1. Introduction**

81 Replacing soybean meal (SBM) by proteaginous seeds (PS) in dairy cow diets has been
82 studied for many years in different parts of the world (e.g. Ingalls and McKirdy, 1974; Yu et
83 al., 1999; Puhakka et al., 2016). Compared to soybean meal, PS contain less crude protein
84 (CP) (between 213 and 380 g/kg dry matter (DM) for PS versus 526 g/kg DM for SBM,
85 INRA, 2018). In addition, proteins of raw PS are more degradable in the rumen (nitrogen
86 ruminal degradability calculated with a particle outflow from the rumen of 0.06 h⁻¹ (ED₆_N)
87 between 0.70 and 0.81 g/g versus 0.63 g/g for SBM, INRA 2018), decreasing their nutritive
88 value for ruminants. To counteract this, heat-treatments of PS have been developed and have
89 become a well-known solution to reduce the rumen degradability of PS proteins (Cros et al.,
90 1991; Benchaar et al., 1992) and thus to improve their nutritive value.

91 Our first hypothesis was that raw seeds, compared to SBM, would lead to higher ruminal
92 degradation of proteins and lower dairy performance. Our second hypothesis was that treated
93 PS compared to raw seeds would lead to lower ruminal degradation of proteins and higher
94 dairy performance. To validate these hypotheses, the literature was searched for published
95 data on the digestion of raw or treated PS in dairy cow diets and a meta-analysis performed to

96 highlight the trends and quantify their effects on nitrogen ruminal degradability, nitrogen
97 intestinal true digestibility, ruminal parameters, nitrogen partitioning and milk production and
98 composition.

99

100 **2. Materials and methods**

101 *2.1 Selection of articles*

102 Publications of interest were identified by searching Google Scholar, Science Direct, Web
103 of Science and PubMed using the key words “ruminants AND (digestibility OR degradability
104 OR milk production) AND (faba bean OR lupin OR pea)”. Two databases were created, the
105 first gathered *in vivo* results about the addition of raw or heat-treated PS in ruminant diets,
106 and the second collected *in situ* results concerning seeds ruminal degradability and intestinal
107 true digestibility. All these results were published between 1974 and 2018 (except studies of
108 Mendowski et al., 2019 and 2020). Two major axes were studied: comparisons between SBM
109 and PS on the one hand, and between raw and treated PS on the other hand.

110 To be included in the *in vivo* database, publications were required to describe studies
111 carried out on dairy cows, focused on replacement of all or part of the concentrate protein
112 source by PS (faba bean, lupin or pea), and contain results on milk performance, nitrogen
113 digestion (in the rumen or in the intestine measured using duodenal and fecal flows), or
114 nitrogen partition between milk, feces and urines. To be included in the *in situ* database,
115 publications had to contain results on nitrogen degradability in the rumen and/or nitrogen true
116 digestibility in the intestine (measured using nylon bag *in situ* incubations) for faba bean,
117 lupin or pea. In all cases, nitrogen was determined using Dumas or Kjeldahl methods. The
118 steps involved in the selection of the publications are summarized in the Prisma flow diagram
119 (Figure 1).

120 *2.2 Creation of databases*

121 Two file templates were created with Microsoft Excel software (Microsoft Corporation,
122 Redmond, WA, USA, version 2016), for the *in vivo* and *in situ* experiments, respectively, as
123 methodologies and results differed between the two types of publications. For each
124 publication, an individual sub-file, which gathered all the information contained in the
125 publication, was completed using the corresponding template (*in vivo* and/or *in situ*) to be as
126 exhaustive as possible. If one publication contained both *in vivo* and *in situ* results, two
127 individual sub-files (one per template) were completed for this publication. Once all
128 individual sub-files were completed, both databases (*in vivo* and *in situ*) were created by
129 compiling their corresponding individual sub-files. For each database, a homogenization of
130 qualitative terms used for some data was necessary before coding, and some units were
131 converted when required. Moreover, additional calculations useful for interpretation were
132 performed when not directly reported in the publication. In the *in vivo* database, this
133 concerned mainly the proportion of dietary CP provided by the tested experimental feed (i.e.
134 the proportion of this feed in the diet \times CP content of this feed/CP content of the diet). As the
135 calculation of N in milk differed between publications, this variable was systematically
136 recalculated as (milk yield \times milk protein content / 6.38 / 0.95), assuming 6.38 g of N in 100 g
137 of milk proteins and 95 g of protein N in 100 g of total milk N (DePeters and Cant, 1992;
138 Spanghero and Kowalski, 1997). Nitrogen balance was systematically recalculated as N
139 intake – (N in milk + N in feces + N in urine), using N in milk calculated with the above-
140 described formula. In the *in situ* database, the effective degradability of N (ED₆_N) was
141 calculated using an outflow rate of particles from the rumen fixed at 0.06 h⁻¹, to make all
142 values from the different publications comparable. The ED₆_N (g/g) was calculated according
143 to Ørskov and McDonald (1979) as: $ED_6_N = a + (b \times c) / (c + 0.06)$, with a the soluble
144 fraction (g/g), b the degradable fraction (g/g) and c the rate of degradation of b (h⁻¹); or by the
145 “step by step method” from the different points of N degradation kinetics (Kristensen et al.,
146 1982). The content of dietary proteins entering the intestine (PIA₆) and of dietary proteins

147 truly digestible in the intestine (PDIA₆) was calculated, respectively, as PIA₆ = CP content of
148 the seed × (1 - ED_{6_N}) and PDIA₆ = PIA₆ × dr_N, with dr_N the intestinal true digestibility of
149 N (in g/g) assimilated to the proportion of N apparently disappearing from mobile bags in the
150 intestines (Vérité et al., 1987; INRA, 2018).

151 Finally, coding was carried out using an exhaustive inventory of all the experimental
152 factors, taking into account the PS studied, and the possible technological treatments applied
153 to the seed (e.g. extrusion, toasting...).

154 2.3 Treatment comparisons

155 For both *in vivo* and *in situ* data analyses, the PS studied were faba bean (*Vicia faba*),
156 lupin (mainly *Lupinus albus*, and *Lupinus angustifolius*, considered without distinction
157 because the two experiments with *Lupinus angustifolius* gave similar results to those with
158 *Lupinus albus*) and pea (*Pisum sativum*). Only the following comparisons were considered in
159 the present work (tested versus control): raw PS versus SBM; treated PS versus SBM; and
160 treated versus raw for the same PS. This led to a selection from the *in vivo* and *in situ*
161 databases, respectively, of 32 (representing 41 experiments) and 36 articles plus a non-
162 published study, corresponding to the *in situ* measurements on the 11 experimental
163 concentrates used in Mendowski et al. (2019 and 2020) by Chapoutot et al. (personal
164 communication): 1 soybean meal, 7 faba bean-linseed blends (90:10) (raw, extruded at low or
165 high temperature, with or without reducing sugars or an enzymatic cocktail), and 3 lupin-
166 linseed blends (90:10) (raw or extruded at low or high temperature. The ED_{6_N} and the dr_N
167 of these feeds were determined according to Michalet-Doreau et al. (1987) and Theodoridou
168 et al. (2010), respectively, with N content in the residues measured using Dumas method for
169 ED_{6_N} and Kjeldahl method for dr_N. Main results of this study were that extrusion of faba
170 bean and lupin blends reduced ED_{6_N} by 16 to 23 points, and increased dr_N by between 20
171 and 30 points (depending on extrusion modalities) compared to raw blends. Extruded faba

172 bean had similar ED₆_N to SBM, and dr_N of SBM was intermediate between raw and
173 extruded PS.

174 Treatments applied to PS were categorized according to their characteristics in order to
175 compare technological effects on the seeds. The three categories were: heat treatments, i.e.
176 heat but no pressure (which included cooking, i.e. boiling the seeds; steam cooking, i.e.
177 heating the seeds with steam; toasting and roasting, i.e. heating the seeds by conduction with
178 or without steam, respectively); thermo-mechanical treatments, i.e. heat and pressure (which
179 included flaking, i.e. steam cooking combined with flattening; expansion, i.e. heating the
180 seeds with hot air under high pressure; extrusion, i.e. forcing the seeds to pass through a die to
181 induce self-heating and destructuring of the seed cells; autoclaving, i.e. cooking the seeds
182 under high pressure); and other treatments (tanning, i.e. binding the amino function of
183 proteins). Within the “heat treatment” category, roasting and toasting have been gathered as
184 their description did not allow a proper distinction and their measured effects appeared
185 similar. Seeds treated in this manner are described as “toasted/roasted” seeds in the rest of this
186 article and analyzed together to increase the number of data within each category.

187 *2.4 Calculations and statistical analyses*

188 Statistical analyses were carried out using Minitab 17 software (Minitab Inc., USA, 2017).
189 For each comparison, effects of treatments were assessed using t-tests applied to the
190 difference between the tested versus control treatments (as described above), for each
191 measured or calculated variable of interest (listed in Table 3). The differences were
192 considered significant when below the threshold *P*-value of 0.05 and a trend was considered
193 at $0.05 < P\text{-value} < 0.10$. When only one comparison was found, the *P*-value reported in the
194 associated publication - when available - was indicated in the text.

195

196 **3. Results**

197 *3.1 In situ degradation and protein values*

198 The mean characteristics of all variables of interest from *in situ* database are summarized
199 in Table 1. Lupin was the main PS represented, followed by faba bean, and pea. Direct
200 comparisons between PS and SBM were scarce. Only six experiments from five publications
201 simultaneously reported results for CP, ED₆_N and dr_N required for PDIA₆ calculation.

202 3.1.1 Raw or treated proteaginous seeds versus soybean meal

203 Results of *in situ* degradation of raw or treated PS compared to SBM are summarized in
204 Table 4, and results on ED₆_N are represented in Figure 2. The PS contained less CP than
205 SBM ($P < 0.001$), with differences averaging -203, -147 and -248 g/kg DM for faba bean,
206 lupin and pea, respectively. All the raw PS had higher ED₆_N than SBM (+0.158, +0.181 and
207 +0.151 g/g for faba bean, lupin and pea, respectively, $P < 0.004$, Figure 2). This led to lower
208 PIA₆ values (-121, -123 and -130 g/kg DM for faba bean, lupin and pea, respectively, $P <$
209 0.001). Compared to SBM, dr_N tended to decrease with raw faba bean (-0.273 g/g, $P =$
210 0.096), but not with lupin. Only one comparison was found for raw pea (Solanas et al., 2005),
211 which concluded that PS were associated with significantly lower dr_N than SBM (-0.102
212 g/g, $P < 0.050$).

213 *In situ* data reporting direct comparisons of treated PS vs. SBM were scarce and variable
214 between studies (Solanas et al., 2005; Aguilera et al., 1992; Chapoutot et al., unpublished
215 data). Briefly, extrusion of faba bean led to significantly lower ED₆_N than SBM (-0.009 g/g,
216 $P < 0.001$), and extrusion of PS led to lower PIA₆, similar dr_N and lower PDIA₆ than SBM
217 (on average, -70 g/kg DM, -0.03 g/g and -62 g/kg DM for all PS) mainly due to the lower CP
218 content of PS. Autoclaving faba bean led to similar ED₆_N as SBM, but autoclaving lupin
219 tended to lower ED₆_N (-0.093 g/g, $P = 0.066$). The only comparison found for autoclaving
220 pea led to a lower ED₆_N (-0.153 g/g, Aguilera et al., 1992). Finally, roasting PS led to
221 similar ED₆_N and PIA₆ as SBM.

222 3.1.2 Treated versus raw proteaginous seeds

223 The *in situ* degradation results for treated faba bean, lupin and pea compared to the same
224 raw seeds are summarized in Table 5, and results for ED₆_N are represented in Figure 3.
225 Treatments did not affect the CP content of PS, except for extrusion of faba bean and lupin
226 (+15 and +10 g/kg DM, $P = 0.009$ and $P = 0.020$, $n = 6$ from 3 experiments and $n = 3$ from 2
227 experiment, respectively), which was an unexpected finding.

228 Extrusion of faba bean decreased ED₆_N (-0.111 g/g, $P = 0.001$) and consequently
229 increased PIA₆ (+34 g/kg DM, $P = 0.002$), increased dr_N (+0.179 g/g, $P = 0.002$) and PDIA₆
230 (+62 g/kg DM, $P < 0.001$). When faba bean was toasted or roasted, the ED₆_N decreased by
231 0.093 g/g compared with raw faba bean ($P = 0.005$). No effect was observed on PIA₆ with
232 toasted/roasted faba bean, whereas dr_N was increased by 0.051 g/g ($P < 0.001$), and PDIA₆
233 tended to be increased by 98.7 g/kg DM ($P = 0.083$, Table 5). Only one study comparing
234 autoclaved to raw faba bean was found, which showed that ED₆_N decreased by 0.224 g/g,
235 and PIA₆ consequently increased by 76.2 g/kg DM after autoclaving (Aguilera et al., 1992).

236 The ED₆_N was lower in extruded than raw lupin (-0.155 g/g, $P < 0.001$), whereas PIA₆,
237 dr_N and PDIA₆ were all higher (+55 g/kg DM, $P < 0.001$, +0.324 g/g, $P = 0.001$ and +92
238 g/kg DM, $P = 0.020$, respectively). Toasting or roasting lupin reduced ED₆_N (-0.161 g/g, $P <$
239 0.001), and increased PIA₆ (+52.0 g/kg DM, $P < 0.001$) and dr_N (+0.018 g/g, $P < 0.001$),
240 leading to an increase in PDIA₆ (+93.1 g/kg DM, $P = 0.040$). Publications about the effects of
241 other treatments on lupin were scarce. Aguilera et al. (1992) showed that autoclaving lupin
242 reduced ED₆_N by 0.313 g/g, thus increasing PIA₆ by 128 g/kg DM. Rodehutschord et al.
243 (1999) observed that cooking or tanning lupin reduced ED₆_N by 0.035 and 0.071 g/g, while
244 PIA₆ was increased by 10.8 and 18.9 g/kg DM, respectively.

245 When pea was treated by extrusion, the decrease in ED₆_N was not significant compared
246 to raw pea, but PIA₆ was higher (+47 g/kg DM, $P = 0.010$). Solanas et al. (2005) observed an
247 increase of 0.121 g/g in dr_N ($P < 0.050$) with extruded pea. Toasting pea reduced ED₆_N (-
248 0.190 g/g, $P < 0.001$) and increased PIA₆ (+60 g/kg DM, $P = 0.017$) and dr_N (+0.033 g/g, P

249 < 0.001, Table 5). The only comparison between autoclaved and raw pea concluded that
250 autoclaving reduced ED₆_N by 0.323 g/g and consequently increased PIA₆ by 86 g/kg DM
251 (Aguilera et al., 1992).

252 3.2. In vivo responses

253 Characteristics of cow milk production (days in milk and initial milk yield) and diets
254 (nature and quantities of dietary forage, dry matter intake (DMI)) included in the *in vivo*
255 database are summarized in Tables 2 and 3. Among PS, faba bean and lupin were the most
256 represented, particularly in comparisons with SBM. Out of the 112 diets included in the
257 database, 72 were distributed *ad libitum*, 12 were distributed *sub ad libitum* (i.e. at more than
258 90% of previously measured *ad libitum* DM intake, so that changes in intake reflect changes
259 in choice feed between treatments), 11 were distributed in a restricted manner, and 17 were
260 distributed in an unspecified manner. Only diets distributed *ad libitum* or *sub ad libitum* were
261 considered for DMI analyses. Most of the diets were based on maize silage. None of the
262 publications comparing SBM and PS, or raw and treated PS, except Pereira et al. (2017) and
263 Mendowski et al. (2019 and 2020), indicated whether a methionine supplementation was
264 applied, or provided the digestible methionine content of the diet.

265 3.2.1 Raw and treated proteaginous seeds versus soybean meal

266 Results of the *in vivo* effects of feeding raw or treated PS (faba bean, lupin or pea) instead
267 of SBM are summarized in Tables 6 to 8. Replacement of SBM by raw or treated PS was
268 made in iso-nitrogenous conditions ($P > 0.100$ for all seeds). Only one exception has been
269 observed for comparison between SBM and extruded lupin: the CP content of the diet was 5
270 g/kg DM higher with extruded lupin than with SBM ($P = 0.030$). Moreover, in most cases, the
271 proportion of dietary CP provided by the experimental concentrate (PS or SBM) did not differ
272 between tested and control treatments, except for extruded faba bean ($P = 0.039$, Table 6) and
273 extruded lupin ($P = 0.060$, Table 7) diets, which provided more dietary CP than SBM.
274 Replacement of SBM by PS in the diet did not affect DMI (measured on *ad libitum* or *sub ad*

275 *libitum* diets), except for extruded lupin, which tended to decrease DMI by 1.4 kg/d compared
276 to SBM ($P = 0.093$, Table 7).

277 Compared to SBM, including raw faba bean in dairy cow diets (Table 6) increased NH_3
278 content (+16.7 mg/L, $P = 0.039$; Figure 4), decreased total volatile fatty acids (VFA) (-6.8
279 mmol/L, $P = 0.036$), and tended to increase acetate/propionate ratio and branched-chain VFA
280 (BC-VFA) in ruminal fluid (+0.2 mol/mol, $P = 0.052$ and +0.2 mol/100 mol total VFA, $P =$
281 0.079, respectively). Raw faba bean also tended to decrease milk yield (-0.5 kg/d, $P = 0.079$),
282 decrease milk protein content (MPC; -0.7 g/kg, $P = 0.018$), N secreted in milk (-4.8 g/d, $P =$
283 0.001), and the N in milk/N intake ratio (from 28.6 to 28.0 g/100 g, $P = 0.026$), but also
284 decreased milk urea content (-30.0 mg/L, $P = 0.084$). With extruded faba bean, the N in
285 milk/N intake ratio decreased (from 29.3 to 28.5 g/100 g, $P = 0.047$) and milk urea tended to
286 decrease (-48.0 mg/L, $P = 0.079$) compared to SBM (Table 6). Few data were available with
287 steam cooked faba bean, but MPC tended to decrease (-0.7 g/kg, $P = 0.090$, Table 6)
288 compared to SBM.

289 Including raw lupin in the diet instead of SBM increased NH_3 in ruminal fluid (+21.1
290 mg/L, $P = 0.032$; Figure 4) and decreased MPC (-1.2 g/kg, $P < 0.001$, Table 7). When
291 extruded lupin was used instead of SBM, BC-VFA and MPC decreased (-0.2 mol/100 mol
292 total VFA, $P = 0.051$ and -2.2 g/kg, $P < 0.001$, respectively), and milk urea tended to decrease
293 (-67.0 mg/L, $P = 0.085$) (Table 7). Few data were available with roasted lupin, and no
294 difference compared with SBM was observed on ruminal parameters, milk yield, milk fat and
295 protein contents (Table 7). The only publication reporting replacement of SBM by tanned
296 lupin concluded that milk yield, neither milk fat and protein contents nor milk N secretion
297 were modified (Emile et al., 1991).

298 Replacing SBM by raw pea led to an increase in NH_3 in ruminal fluid (+25.7 mg/L, $P =$
299 0.009; Figure 4), and tended to increase BC-VFA in the rumen (+0.2 mol/100 mol VFA) and
300 to decrease N in milk (-6.1 g/d, $P = 0.092$, Table 8). Only one comparison was found about

301 duodenal flow, and it showed that non-NH₃ N and microbial N were numerically increased (P
302 > 0.050 , Khorasani et al., 2000), whereas non-NH₃ non-microbial N (dietary + endogenous)
303 was numerically decreased ($P > 0.050$, Khorasani et al., 2000). When pea was extruded, milk
304 fat content (MFC) tended to decrease (-1.4 g/kg, $P = 0.070$), but N in milk tended to increase
305 (+5.8 g/d, $P = 0.090$, Table 8 and Figure 6) compared to SBM. Petit et al. (1997) found that
306 extrusion increased N excreted in urine and N balance ($P < 0.050$). When pea was flaked,
307 milk urea was increased (+21.9 mg/L, $P = 0.016$, Table 8) compared to SBM. When pea was
308 expanded and compared to SBM, no difference was observed on ruminal NH₃, milk yield,
309 MFC and MPC (Masoero et al., 2006).

310 3.2.2 Treated versus raw proteaginous seeds

311 The *in vivo* effects of replacing raw by treated faba bean, lupin and pea are summarized in
312 Tables 9 to 11. Except for raw versus extruded lupin diets (+2.3 g CP/kg DM, $P = 0.014$,
313 Table 10), comparisons between raw versus treated seeds were made in iso-nitrogenous
314 conditions. Likewise, raw and treated seeds contributed to the same proportion of CP dietary
315 content. Whatever the PS or the applied treatment, DMI was not changed between raw versus
316 treated seeds (even when diets were distributed *ad libitum* and *sub ad libitum*), except for
317 toasted or roasted faba bean (-0.1 kg/d, $P = 0.092$, Table 9).

318 For faba bean, BC-VFA were lower (-0.5 mol/100 mol total VFA, $P = 0.001$), as was
319 MFC (-2.7 g/kg, $P = 0.020$), and N in milk tended to be higher (+4.1 g/j, $P = 0.085$, Table 9
320 and Figure 7) with extruded than with raw seeds. The only experiment reporting N duodenal
321 flow documented an increase in both microbial and non-NH₃ non-microbial flow ($P < 0.050$,
322 Benchaar et al., 1994b). When faba bean was toasted or roasted, N intake tended to decrease
323 (-7.9 g N/d, $P = 0.088$) as did MFC (-1.6 g/kg, $P = 0.023$, Table 9) compared to raw faba
324 bean. Only one publication was found about the effects of tanning faba bean, which
325 concluded that tanning increased MPC and N intake ($P < 0.050$, Pelletier and Bouchard,
326 1978).

327 Replacing raw lupin by extruded seeds decreased NH₃ in ruminal fluid (-11.8 mg/L, $P =$
328 0.040, Figure 5), BC-VFA (-0.5 mol/100 mol VFA, $P = 0.022$) and MFC (-4.7 g/kg, $P =$
329 0.006), but increased N in milk (+4.0 g/j, $P = 0.033$, Table 10 and Figure 7); the only
330 experiment with duodenal flow reported that there was an increase in both microbial and non-
331 NH₃ non-microbial N duodenal flow ($P < 0.050$, Benchaar et al., 1994a). No difference in *in*
332 *vivo* results was observed when lupin was toasted or roasted compared to raw lupin.
333 Publications about the effects of expanding, tanning, autoclaving and cooking lupin were
334 scarce. Pieper et al. (2006) concluded that expanding lupin led to better protection of CP,
335 resulting in a better milk yield ($P < 0.050$), but no change in MFC and MPC. Emile et al.
336 (1991) observed a higher milk yield but a lower MFC when lupin was tanned, with no
337 difference in MPC.

338 For pea, few *in vivo* data were available about effects of extrusion, flaking, expanding and
339 tanning (Table 11). Focant et al. (1990) concluded that extrusion led to a significant increase
340 in microbial ($P < 0.010$) and a numerical increase in non-NH₃ non-microbial N flow in the
341 duodenum, a decrease in NH₃ content from ruminal fluid ($P < 0.001$), but reported that steam-
342 flaking had no observed effects. No beneficial effect on milk production was observed with
343 extrusion, and Petit et al. (1997) concluded that extrusion decreased N excreted in feces ($P <$
344 0.050), but increased N balance ($P < 0.010$). Masoero et al. (2006) concluded that expanding
345 had no effects on intake and milk production. Finally, Pelletier and Bouchard (1978)
346 concluded that tanning pea had no effect on milk production, but increased MPC ($P < 0.050$)
347 and had no effect on N partitioning despite an increase in N intake ($P < 0.050$).

348

349 **4. Discussion**

350 The aims of this study were twofold: first, to make an inventory of the available data
351 about the *in situ* degradation and the *in vivo* responses of dairy cows fed raw or treated faba
352 bean, lupin and pea; and second, to quantify the main trends related to the substitution of

353 SBM by PS, or the replacement of raw PS by treated PS, on N ruminal degradability and
354 intestinal true digestibility, ruminal parameters, milk production and composition, and N
355 partitioning.

356 4.1. In situ degradation

357 With all PS, the ED₆_N was higher for raw seeds (on average 0.778 g/g) than for SBM (on
358 average 0.613 g/g). In this analysis, the ED₆_N values for PS were slightly lower than those
359 averaged from larger data sets, and presented in feed tables by INRA (2018) and INRA-
360 CIRAD-AFZ (2017), i.e. 0.82, 0.86 and 0.86 g/g for raw faba bean, lupin and pea,
361 respectively. Most of the treatments significantly decreased the ED₆_N: on average -0.100, -
362 0.158 and -0.130 g/g with all treatments for faba bean, lupin and pea, respectively, with the
363 strongest effect with autoclaving, followed by extrusion and finally toasting (Figure 3). The
364 observed reductions in ED₆_N confirm that heat and/or pressure treatments can protect N
365 from ruminal degradation, which led on average to an increase of +43 g PIA₆/kg DM for all
366 PS and treatments. In this study, the ED₆_N reductions observed with extrusion were slightly
367 lower than those reported in INRA Feed Tables (2018). This could partly be due to
368 differences in treatment modalities (duration, temperatures reached, intensity of pressure
369 treatments, etc.), which are often poorly described in the literature. For similar treatments
370 (extrusion or other heat treatments except toasting/roasting), the greatest reductions in ED₆_N
371 were observed for lupin, which appeared to be the seed the most reactive to treatment. This
372 implies a higher increase in PIA₆ (in g/kg DM) for lupin (+53, n = 56) than for faba bean
373 (+18, n = 25); pea also appeared reactive (+58, n = 6), but there were less available data. This
374 finding was also supported by intra-experiment comparisons: reductions in ED₆_N were
375 greater for lupin than for faba bean with autoclaving (Aguilera et al., 1992), extrusion
376 (Chapoutot et al., 2016) and toasting (Goelema, 1999), and greater for lupin than for pea with
377 extrusion (Aufrère et al., 2001) and toasting (Goelema, 1999; Goelema et al., 1998). Fewer
378 data were available for dr_N than ED₆_N, but when PS were heat-treated, dr_N increased

379 significantly (on average for all treatments: +0.10, +0.12 and +0.04 g/g for faba bean, lupin
380 and pea, respectively). This suggests that the protection of proteins against ruminal
381 degradation is reversible in the abomasum, which allows the absorption of amino acids in the
382 intestine. In addition, the flow of amino acids potentially absorbed is greater. Some treated PS
383 have similar or lower ED₆_N than SBM: autoclaved faba bean, lupin, or pea, and roasted
384 lupin. However, due to their lower CP content, no treated PS reached a level of PIA₆
385 comparable to SBM despite the effects of heat treatments: PIA₆ values were on average 60
386 g/kg DM lower with treated PS than with SBM, and no direct comparison between treated PS
387 and SBM was available for PDIA₆.

388 4.2. *In vivo responses*

389 The PS seem to have a satisfying palatability for dairy cows as, in most cases, DMI was
390 not reduced compared to diets containing SBM (the majority of experiments were conducted
391 with diets distributed *ad libitum*).

392 According to the observed *in situ* results, a lower valorization of proteins by dairy cows
393 might be expected when SBM is replaced by PS in their diet, and reciprocally a better
394 valuation of proteins with treated PS compared to raw PS. Nevertheless, the expected effects
395 of raw or treated PS were not systematically observed *in vivo*. This could be explained by the
396 fact that differences between treatments on *in vivo* measured N flows were lower than those
397 observed on N balance, which was mainly due to balance default (as a result of
398 methodological limits) in dairy cows (Spanghero and Kowalski, 1997). It should also be noted
399 that only **Pereira et al. (2017) and** Mendowski et al. (2019, 2020) explicitly reported on
400 supplementation of metabolizable methionine. Thus, in all other studies, the diets containing
401 faba bean, lupin **and pea** (particularly when extruded) could have provided a lower supply of
402 digestible methionine than current recommendations (2.3% of PDI, INRA, 2018) for MPC
403 and proteins secreted in milk. However, this may have been partly offset by the fact that
404 comparisons between PS and SBM were performed with iso-CP diets (PS or SBM providing

405 on average 39 g/100 g of dietary CP). Another explanation of differences between expected
406 and observed effects when SBM is substituted by raw or treated PS *in vivo* could be related to
407 the nature of the substitution within- experiments. Indeed, while in some publications, diets
408 have been formulated to be iso-energetic, in others, PS diets provide more energy than SBM
409 diets (the NE_L / CP ratio being higher for PS than for SBM). Thus, responses of dairy cows
410 may also be partly related to a higher energy intake with PS diets than with SBM diets. Due to
411 scarce available data, it has not been possible to split data between iso-energetic and non-iso-
412 energetic supplementations for the analysis. Given the average values of 20.1 kg for DM
413 intake, 165 g/kg DM for dietary CP content, and 39% of dietary CP for substitution, the
414 increase in energy intake may reach 4.2 Mcal NE_L , that may contribute to 2.6 g N in milk/d
415 according to INRA (2018).

416 4.2.1. Effects of substitution of soybean meal by raw proteaginous seeds

417 Even if the effects on protein degradation in the rumen appeared to follow a general trend
418 when SBM was replaced by raw PS (Vander Pol et al., 2009; Cherif et al., 2018; Mendowski
419 et al., 2019), the effects of these substitutions on milk production and composition were
420 highly variable.

421 An evaluation of general trends provides a more global picture of the effects of dietary
422 substitutions. When dairy cows were fed raw PS compared to SBM, ruminal NH_3 content
423 increased (on average with all PS: +20 mg/L, $n = 15$), and the higher the proportion of CP
424 provided by raw PS, the higher the NH_3 content in the rumen (as illustrated by the average
425 within-experiment response law, Figure 8). The BC-VFA molar proportion tended to increase
426 with faba bean compared to SBM (on average with all PS: +0.16 mol/100 mol total VFA, $n =$
427 11). The N in milk/N intake ratio decreased significantly with faba bean and numerically with
428 lupin and pea (on average with all PS: -0.6 g/g, $n = 29$). The MPC decreased significantly
429 with faba bean and lupin (on average -1.0 g/kg), but not with pea. Milk yield and N in milk
430 did not change with lupin or pea (only N in milk tended to decrease with pea) but decreased

431 with raw faba bean by 0.5 kg/d and 5 g/d, respectively. Taken together, these results are
432 consistent with *in situ* observations: the increase in ruminal NH₃, and the lower MPC and N in
433 milk reflecting higher ruminal N degradation and the induced lower PDIA₆ value. Our first
434 hypothesis, which was that raw seeds compared to soybean meal would lead to lower dairy
435 performance was verified with faba bean, and numerically the same trend was observed with
436 lupin and pea, although it did not reach statistical significance. The N partitioning between
437 urine and feces was not modified when SBM was replaced by raw PS, but very few data were
438 available.

439 The attenuation observed between *in vivo* and *in situ* responses could partly be explained
440 by the risk of over-estimating the ED₆_N of PS due to particle loss during incubation when
441 using the nylon bag technique, which has been reported to be high with PS (Michalet-Doreau
442 and Cerneau, 1992). Consequently, PDI values of PS may have been underestimated, which
443 would imply a higher gap in dairy performance between SBM and PS.

444 The low content of digestible methionine of PS (1.48, 1.44 and 1.72 g/100 PDI for faba
445 bean, lupin and pea, respectively, INRA 2018) should be noted. As very few publications,
446 except for example Mendowski et al. (2019, 2020), reported supplementation with
447 metabolizable methionine, diets containing PS could have had lower than recommended
448 levels of digestible methionine, which could impair dairy performance, in particular MPC.

449 This is consistent with what was obtained by Pereira et al. (2017), who observed that adding
450 rumen-protected Lys and Met to a diet containing field pea increased MPC compared to the
451 same diet with no AA supplement. In addition, Joch and Kudrna (2020) compared diets
452 containing SBM in which either 0%, 30% or 50% of SBM was replaced by raw lupin (all
453 diets containing protected Met) and observed no difference on MPC between diets:
454 supplementing in protected Met may have prevented a decrease of MPC with lupin compared
455 to SBM.

456 4.2.2. *Effects of treatments on proteaginous seeds*

457 Only a few *in vivo* measures varied significantly following PS treatments. As observed
458 previously for the comparison between raw PS and SBM, the effects on protein degradation in
459 the rumen appeared to follow a general trend when raw PS was replaced by heat-treated PS
460 (Focant et al., 1990; Benchaar et al., 1992; Mendowski et al., 2019), but the effects of these
461 substitutions on milk production and composition were highly variable.

462 Both raw and treated PS provided on average 29 g/100 g (from 14 to 53g/100 g) of CP in
463 dairy cow diets. With extrusion there was a global decrease in ruminal NH₃, which was
464 significant for lupin (-12 mg/L) and numerical for faba bean and pea (on average -17 mg/L,
465 n = 7 with faba bean and pea), suggesting extrusion protected the proteins against ruminal
466 degradation. This is consistent with observations on ED₆_N with extrusion. With other
467 treatments than extrusion, the effects depended on seed and treatment. For toasted/roasted
468 faba bean, there was a decrease in NH₃ in ruminal fluid (the change was not statistically
469 significant, but only two comparisons were found), whereas for toasting/roasting lupin and
470 flaking pea the opposite was observed with an increase in NH₃ in ruminal fluid (only few data
471 available). The main significant zootechnical result on nitrogen utilization concerned
472 extrusion of faba bean and lupin, which led to an increase in N in milk (as also illustrated by
473 the within-experiment response law, Figure 9). However, some heat treatments of faba bean
474 (extrusion and toasting/roasting) and lupin (extrusion) appeared to decrease MFC, and most
475 of the other treatments followed the same trend. This could be explained by a greater
476 availability of fat after extrusion of lupin compared to raw lupin (Chilliard et al., 2009),
477 leading to an inhibition of *de novo* synthesis of fatty acids in the mammary gland by dietary
478 polyunsaturated fatty acids or rumen biohydrogenation intermediates; Shingfield et al, 2010).
479 For extruded faba bean, the five available comparisons concerned 10:90 linseed:faba bean
480 blends (Mendowski et al., 2019 and 2020), in which fat could have been released from the
481 linseed during extrusion. Moreover, faba bean has a high content of starch (44 % on a DM
482 basis), and starch-rich diets have been shown to potentially induce milk fat depression in

483 dairy cows (Bauman and Grinari, 2003). Consequently, the decrease in MFC observed with
484 faba bean could result both from the release of polyunsaturated fatty acids from linseed and
485 the faba bean seed itself, and its high starch content.

486 4.2.3. Effects of substitution of soybean meal by treated proteaginous seeds

487 At similar dietary CP content, diets containing treated PS rather than SBM had non-
488 significantly higher NH₃ rumen content and milk yield. The MPC was significantly lower
489 with steam cooked faba bean and extruded lupin, and most other treated PS followed the same
490 trend, so that N in milk tended to be lower than with SBM, but differences were small (-2 g
491 N/day). Urinary or fecal N excretion and N balance values obtained with treated PS were
492 comparable to those measured with SBM. The MFC tended to be lower with treated PS than
493 with SBM, but differences were small (on average -1.2 g/kg, n = 16) and non-significant,
494 except for a trend with extruded pea. When treated PS were compared to SBM either no
495 change in values, or lower MFC and MPC values were observed, as reported with steam-
496 cooked faba bean (-0.7 g/kg protein content, P = 0.090), extruded lupin (-2.2 g/kg protein
497 content, P < 0.001) and extruded pea (-1.4 g/kg fat content, P = 0.070 and -5.8 g/d N in milk,
498 P = 0.090). Heat treatments improved the nutritional value of PS, but still did not allow the
499 seeds to reach the value of SBM. At iso-CP comparisons, this can be explained by the lower
500 PDI content of PS compared to SBM, and also by the lack of digestible methionine contained
501 in PS compared to SBM.

502

503 5. Conclusions

504 For some treatment comparisons, very few (if any) data were available for dairy cows, and
505 results concerning N partitioning in particular were scarce. However, despite the limited
506 amounts of available data and the great diversity of feeding practices, some general trends
507 appear from this quantitative review. Proteins from raw PS were more degradable in the
508 rumen than proteins from SBM, as revealed both by *in situ* studies and by *in vivo* ruminal

509 NH₃ measurements (which is in accordance to our first hypothesis). This ruminal N
510 degradability was lowered by heat treatments (confirming our second hypothesis), but even so
511 the amount of PIA₆ did not reach levels obtained with SBM because of the differences in CP
512 content. Moreover, very few results on dr_N were available. Evaluations of *in vivo* dairy
513 performance indicated that MPC and milk N secretion were decreased with raw PS compared
514 to SBM (thereby confirming our first hypothesis). In contrast, no major effect was observed
515 when PS were treated compared to raw PS (not supporting our second hypothesis), except for
516 a decrease in MFC, especially for extruded faba bean and lupin. Finally, at similar CP
517 content, N transfer as protein in milk remained lower with treated PS than with SBM, but
518 differences were small. When SBM was replaced by raw PS, or when raw PS were replaced
519 by treated PS, the observed variability of responses in milk production and composition could
520 partly be explained by the variations specific to each study, and particularly the dietary level
521 of digestible methionine. Moreover, when PS were heat-treated, technological processes were
522 most often not fully described, which led to uncertainty about the intensity of the process the
523 seeds may have undergone. A better description of these heat or pressure treatments would
524 permit a better understanding of their effects on PS, and consequently the effect on dairy
525 performance when these seeds are fed to dairy cows. This work revealed the very limited
526 number of publications testing raw and treated PS in dairy cow diets, and how important it
527 would be to perform more analytical feeding trials to improve both understanding of
528 processes involved in N protection, and prevision of their nutritional interest as alternatives to
529 SBM in dairy cow diets.

530

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535

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- 658

659 Table 1: Descriptive statistics of the main variables of interest in the *in situ* database
 660 concerning proteaginous seeds and soybean meal whatever the treatment.

	n	Mean	sd ¹	Min	Max
CP content (g/kg DM)					
Faba bean	57	308	45	246	471
Lupin	111	361	42	283	513
Pea	21	248	17	215	268
Soybean meal	18	503	18	478	541
ED₆_N (g/g)					
Faba bean	63	0.720	0.134	0.434	0.937
Lupin	112	0.683	0.154	0.370	0.954
Pea	41	0.676	0.129	0.428	0.910
Soybean meal	14	0.600	0.087	0.400	0.769
dr_N (g/g)					
Faba bean	27	0.889	0.082	0.642	0.982
Lupin	24	0.899	0.130	0.500	0.969
Pea	16	0.959	0.029	0.870	0.992
Soybean meal	5	0.970	0.14	0.956	0.987
PIA₆ (g/kg DM)					
Faba bean	45	66	42	19	173
Lupin	94	113	60	17	353
Pea	19	80	36	29	153
Soybean meal	16	208	92	120	499
PDIA₆ (g/kg DM)					
Faba bean	11	58	51	14	170
Lupin	7	91	55	14	171
Pea	4	89	40	48	132
Soybean meal	1	122	-	-	-

661 ¹ standard deviation

662 Table 2: Mean characteristics of variables of interest analyzed in the *in vivo* database,
 663 concerning diets including proteaginous seeds (PS) and soybean meal (SBM) whatever the
 664 treatment.

	n	Mean	sd ⁽¹⁾	Min	Max
CP in diet (g/kg) DM					
Faba bean	23	166	13	140	186
Lupin	20	173	18	145	204
Pea	15	153	9	134	169
Soybean meal	20	161	15	138	203
CP provided by SBM or PS (g/100 g dietary CP)					
Faba bean	19	39.7	12.3	13.6	53.4
Lupin	10	38.9	10.9	22.0	50.0
Pea	4	38.3	11.9	22.9	47.7
Soybean meal	10	37.8	6.8	26.8	49.5
DMI (kg/d)					
Faba bean	27	20.7	3.0	16.8	26.0
Lupin	30	20.1	2.0	15.4	23.9
Pea	16	19.7	5.1	8.1	26.3
Soybean meal	28	20.0	4.2	7.8	25.9
Ruminal NH₃ (mg/L)					
Faba bean	20	172.4	42.4	110.5	268.4
Lupin	7	165.1	31.0	125.0	194.2
Pea	10	147.4	48.9	54.2	223.4
Soybean meal	12	131.4	35.9	38.4	168.6
Ruminal total VFA (mmol/L)					
Faba bean	20	105.7	15.6	74.0	129.5
Lupin	7	106.4	5.2	101.0	114.1
Pea	7	100.1	11.7	86.2	121.0
Soybean meal	11	104.1	10.6	83.4	123.0
Ruminal acetate/propionate (mol/mol)					
Faba bean	20	3.3	0.8	1.7	4.9
Lupin	7	2.9	0.1	2.7	3.1
Pea	7	3.6	0.5	2.3	4.5
Soybean meal	11	2.8	0.6	1.6	3.7
Ruminal BC-VFA⁽²⁾ (% mol of total VFA)					
Faba bean	15	2.4	0.8	0.9	3.6
Lupin	7	2.7	0.1	2.3	3.5
Pea	4	2.3	0.8	1.8	2.9
Soybean meal	10	2.2	0.6	1.0	3.4
Rumen protein balance (g/kg DMI)					
Faba bean	10	18.8	21.7	-22.6	51.0
Lupin	2	5.0	28.3	-15.0	25.1
Pea	1	48.8	-	-	-
Soybean meal	1	47.0	-	-	-

Non-NH ₃ N duodenal flow (g/d)					
Faba bean	6	194.6	149.8	94.5	408.7
Lupin	2	414.4	81.0	357.1	471.7
Pea	4	214.9	126.6	136.0	401.7
Soybean meal	1	383.3	-	-	-
Microbial N duodenal flow (g/d)					
Faba bean	2	205.2	15.6	194.2	216.2
Lupin	2	206.1	3.1	203.9	208.3
Pea	4	123.8	88.5	68.0	254.1
Soybean meal	1	221.4	-	-	-
NANM N duodenal flow ⁽³⁾ (g/d)					
Faba bean	2	182.4	14.5	172.1	192.6
Lupin	2	208.2	77.9	153.1	263.3
Pea	4	91.2	38.1	68.0	147.6
Soybean meal	1	161.9	-	-	-
Milk yield (kg/d)					
Faba bean	27	26.9	5.0	19.1	36.0
Lupin	28	29.5	4.2	23.9	37.7
Pea	16	29.2	7.0	19.6	42.7
Soybean meal	30	27.9	6.1	20.2	42.9
Milk fat content (g/kg)					
Faba bean	27	37.5	6.0	24.5	46.1
Lupin	28	36.7	4.2	27.0	43.0
Pea	16	36.0	3.2	28.5	40.8
Soybean meal	30	36.0	3.8	23.3	42.6
Milk protein content (g/kg)					
Faba bean	27	32.6	2.7	28.1	37.5
Lupin	28	30.0	1.6	27.0	33.7
Pea	16	31.5	1.9	28.6	35.1
Soybean meal	30	32.2	2.1	29.1	35.3
Milk urea (mg/L)					
Faba bean	17	230.8	53.9	123.0	329.3
Lupin	7	262.4	134.9	102.0	416.0
Pea	8	256.2	78.8	154.0	376.2
Soybean meal	12	273.6	87.3	148.0	403.0
N intake (g/d)					
Faba bean	30	485.4	181.7	110.7	714.2
Lupin	24	565.3	86.0	394.2	717.6
Pea	14	537.2	94.6	343.0	656.0
Soybean meal	23	546.1	85.2	383.0	673.4
N in milk (g/d)					
Faba bean	27	143.9	28.0	94.6	201.3
Lupin	28	145.2	19.6	112.4	185.4
Pea	16	150.9	34.0	94.8	204.6

Soybean meal	30	147.4	28.1	112.4	206.2
N in urine (g/d)					
Faba bean	12	165.3	47.7	86.0	232.0
Lupin	3	127.7	5.9	120.9	132.1
Pea	8	181.3	54.9	100.0	256.0
Soybean meal	8	180.5	47.4	122.0	238.0
N in feces (g/d)					
Faba bean	16	155.9	78.4	31.9	237.3
Lupin	3	154.9	8.5	147.9	164.4
Pea	8	171.9	26.2	136.0	201.0
Soybean meal	8	182.4	22.8	156.0	224.0
N balance (g/d)					
Faba bean	12	37.6	32.1	-8.4	93.7
Lupin	3	36.9	8.4	29.3	46.0
Pea	8	32.2	27.2	-3.8	67.7
Soybean meal	8	24.4	23.9	-10.4	58.8
N in milk / N intake ratio					
Faba bean	24	0.27	0.03	0.23	0.32
Lupin	22	0.26	0.04	0.20	0.34
Pea	14	0.28	0.04	0.22	0.34
Soybean meal	23	0.28	0.04	0.22	0.34

665 ⁽¹⁾ standard deviation

666 ⁽²⁾ branched-chain VFA (isobutyrate and isovalerate)

667 ⁽³⁾ non-NH₃ and non-microbial N duodenal flow

668 Table 3: Description of cow milk production and diets in the *in vivo* database.

	n	Mean	sd ⁽¹⁾	Min	Max
Cows					
Days in milk (d)	94	93.8	44.3	21	200
Initial milk yield (kg/d)	49	31.5	4.7	21.6	41
Main forage offered (g/100 g dietary DM)	112	41.5	15.5	17.9	84.0
Maize silage	61	41.1	14.8	17.9	74.6
Grass silage	23	44.2	18.8	25.0	84.0
Hay	23	44.1	2.4	25.0	70.0
Straw	3	24.4	0.2	24.3	24.6
Cut grass	2		<i>Ad libitum</i>		
Concentrate (g/100 g dietary DM)	94	44.0	13.5	16.1	75.7

669 ⁽¹⁾ standard deviation

670

671

672 Table 4: *In situ* parameters and protein value of raw or treated proteaginous seeds (PS)
 673 compared to soybean meal (SBM).

PS	Treatment	Variable (unit)	n	Mean SBM	Mean PS	Δ PS – SBM ⁽¹⁾	SE ⁽²⁾ Δ	P- value	
Faba bean	Raw	CP (g/kg DM)	10	500	297	-203	9.4	<0.001	
		ED ₆ _N (g/g)	10	0.629	0.787	0.153	0.023	<0.001	
		PIA ₆ (g/kg DM)	10	185	64	-121	7.4	<0.001	
		dr_N (g/g)	2	0.956	0.684	-0.273	0.042	0.096	
		PDIA ₆ (g/kg DM)	2	156	24	-131	4.8	0.023	
	Extrusion	CP (g/kg DM)	5	521	299	-222	7.0	<0.001	
		ED ₆ _N (g/g)	5	0.687	0.678	-0.009	0.090	<0.001	
		PIA ₆ (g/kg DM)	5	163	96	-67	3.4	<0.001	
		dr_N (g/g)	5	0.956	0.915	-0.041	0.013	0.038	
		PDIA ₆ (g/kg DM)	5	156	88	-68	4.0	<0.001	
	Autoclaving	CP (g/kg DM)	1	478	341	-138	-	-	
		ED ₆ _N (g/g)	3	0.543	0.549	0.006	0.045	0.905	
		PIA ₆ (g/kg DM)	1	201	171	-29	-	-	
	Lupin	Raw	CP (g/kg DM)	10	503	356	-147	17.7	<0.001
			ED ₆ _N (g/g)	9	0.593	0.773	0.181	0.044	0.003
PIA ₆ (g/kg DM)			9	206	83	-123	21.6	<0.001	
dr_N(g/g)			2	0.964	0.752	-0.213	0.070	0.201	
PDIA ₆ (g/kg DM)			1	156	16	-139	-	-	
Extrusion		CP (g/kg DM)	4	515	367	-147	27.6	0.013	
		ED ₆ _N (g/g)	3	0.644	0.700	0.557	0.074	0.529	
		PIA ₆ (g/kg DM)	3	185	111	-74	23.6	0.089	
		dr_N (g/g)	3	0.961	0.961	-0.001	0.008	0.940	
		PDIA ₆ (g/kg DM)	2	156	109	-47	2.9	0.039	
Autoclaving		CP (g/kg DM)	1	478	407	-71	-	-	
		ED ₆ _N (g/g)	3	0.543	0.450	-0.093	0.025	0.066	
		PIA ₆ (g/kg DM)	1	200	229	29	-	-	
Roasting		CP (g/kg DM)	2	525	362	-163	60.0	0.224	
		ED ₆ _N (g/g)	2	0.512	0.537	0.025	0.112	0.860	
	PIA ₆ (g/kg DM)	2	258	168	-90	86.9	0.487		
Pea	Raw	CP (g/kg DM)	6	492	244	-248	11.2	<0.001	
		ED ₆ _N (g/g)	5	0.617	0.769	0.151	0.021	0.002	
		PIA ₆ (g/kg DM)	5	187	57	-130	8.0	<0.001	
		dr_N (g/g)	1	0.972	0.870	-0.102	-	-	
	Extrusion	dr_N (g/g)	1	0.972	0.991	0.019	-	-	
	Autoclaving	CP (g/kg DM)	1	478	268	-211	-	-	
		ED ₆ _N (g/g)	1	0.561	0.428	-0.153	-	-	
		PIA ₆ (g/kg DM)	1	201	153	-48	-	-	

674 ⁽¹⁾ difference between proteaginous seed (PS) and soybean meal (SBM)

675 ⁽²⁾ standard error of the difference

676 Table 5: *In situ* parameters and protein value of raw proteaginous seeds (PS) compared to
677 treated PS

PS	Treatment	Variable (unit)	n	Mean raw	Mean treated	Δ Treated – raw ⁽¹⁾	SE ⁽²⁾ Δ	P- value	
Faba bean	Extrusion	CP (g/kg DM)	13	307	322	15	4.9	0.009	
		ED ₆ _N (g/g)	14	0.876	0.765	-0.111	0.025	0.001	
		PIA ₆ (g/kg DM)	12	33	67	34	8.7	0.002	
		dr_N (g/g)	7	0.735	0.913	0.179	0.034	0.002	
		PDIA ₆ (g/kg DM)	6	24	87	62	5.2	<0.001	
	Autoclaving	CP (g/kg DM)	1	341	341	0	-	-	
		ED ₆ _N (g/g)	1	0.721	0.497	-0.224	-	-	
		PIA ₆ (g/kg DM)	1	95	171	76	-	-	
	Toasting/ roasting	CP (g/kg DM)	13	310	311	1	1.5	0.703	
		ED ₆ _N (g/g)	33	0.737	0.644	-0.093	0.031	0.005	
		PIA ₆ (g/kg DM)	12	124	121	-3	20.7	0.879	
		dr_N (g/g)	12	0.868	0.919	0.051	0.007	<0.001	
		PDIA ₆ (g/kg DM)	2	59	157	99	12.9	0.083	
	Lupin	Extrusion	CP (g/kg DM)	20	372	382	10	3.9	0.020
			ED ₆ _N (g/g)	19	0.931	0.776	-0.155	0.026	<0.001
PIA ₆ (g/kg DM)			16	28	83	55	11.1	<0.001	
dr_N (g/g)			6	0.622	0.946	0.324	0.047	0.001	
PDIA ₆ (g/kg DM)			2	16	109	92	2.9	0.020	
Autoclaving		CP (g/kg DM)	1	407	407	0	-	-	
		ED ₆ _N (g/g)	1	0.750	0.437	-0.313	-	-	
		PIA ₆ (g/kg DM)	1	102	229	128	-	-	
Toasting/ roasting		CP (g/kg DM)	37	355	357	3	1.8	0.169	
		ED ₆ _N (g/g)	47	0.773	0.611	-0.161	0.016	<0.001	
		PIA ₆ (g/kg DM)	37	83	135	52	6.9	<0.001	
		dr_N (g/g)	12	0.938	0.956	0.018	0.001	<0.001	
		PDIA ₆ (g/kg DM)	2	72	165	93	5.9	0.040	
Cooking		CP (g/kg DM)	1	302	304	2	-	-	
		ED ₆ _N (g/g)	1	0.938	0.903	-0.035	-	-	
		PIA ₆ (g/kg DM)	1	19	30	11	-	-	
Tanning		CP (g/kg DM)	1	302	283	-19	-	-	
		ED ₆ _N (g/g)	1	0.938	0.867	-0.071	-	-	
	PIA ₆ (g/kg DM)	1	19	38	19	-	-		
Pea	Extrusion	CP (g/kg MS)	4	237	237	0	2.5	0.904	
		ED ₆ N (g/g)	7	0.782	0.708	-0.074	0.046	0.159	
		PIA ₆ (g/kg DM)	3	29	76	47	4.8	0.010	
		dr_N (g/g)	1	0.870	0.991	0.121	-	-	
	Autoclaving	CP (g/kg DM)	1	268	268	0	-	-	
		ED ₆ _N (g/g)	1	0.751	0.428	-0.323	-	-	
		PIA ₆ (g/kg DM)	1	67	153	86	-	-	
	Toasting	CP (g/kg DM)	2	261	261	0	0	-	
		ED ₆ _N (g/g)	11	0.783	0.593	-0.190	0.027	<0.001	
		PIA ₆ (g/kg DM)	2	66	126	60	10.3	0.017	
		dr_N (g/g)	11	0.932	0.964	0.033	0.004	<0.001	

	PDIA ₆ (g/kg DM)	2	63	123	60	9.7	0.103
Expansion	ED _{6_N} (g/g)	2	0.604	0.536	-0.068	0.021	0.191
Tanning	ED _{6_N} (g/g)	2	0.910	0.895	-0.014	0.004	0.195

678 ⁽¹⁾ difference between treated proteaginous seed (PS) raw PS

679 ⁽²⁾ standard error of the difference

680 Table 6: Effects of replacing soybean meal (SBM) by raw or treated faba bean seed on *in vivo*
 681 parameters in dairy cows

Treatment	Variable (unit)	n	Mean SBM	Mean PS	Δ PS – SBM ⁽¹⁾	SE ⁽²⁾ Δ	P- value
Raw	Diet CP (g/kg DM)	8	159	159	0	0.4	0.718
	CP provided by SBM or PS (g/100 g CP)	4	35	41	6	2.8	0.104
	DM intake (kg/d)	9	20.7	20.7	0.0	0.08	0.876
	NH ₃ (mg/L)	8	144.9	161.6	16.7	6.59	0.039
	Total VFA (mmol/L)	8	105.4	98.6	-6.8	2.61	0.036
	Acetate/propionate (mol/mol)	8	2.7	2.9	0.2	0.10	0.052
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	5	2.3	2.5	0.2	0.10	0.079
	Milk yield (kg/d)	10	26.8	26.3	-0.5	0.23	0.079
	Milk fat (g/kg)	10	33.3	33.9	0.6	0.67	0.424
	Milk protein (g/kg)	10	33.2	32.6	-0.7	0.23	0.018
	Milk urea (mg/L)	2	217.0	187.0	-30.0	4.00	0.084
	N intake (g/d)	9	522.8	519.8	-3.0	3.13	0.370
	N milk (g/d)	10	146.4	141.6	-4.8	1.00	0.001
	N urine (g/d)	4	182.6	179.5	-3.1	4.96	0.572
	N feces (g/d)	4	197.9	194.4	-3.5	2.79	0.296
N balance (g/d)	4	2.9	10.1	7.2	3.12	0.104	
N milk/N intake (g/100 g)	9	28.6	28.0	-0.7	0.24	0.026	
Extrusion	Diet CP (g/kg DM)	2	144	144	0	4.3	0.953
	CP provided by SBM or PS (g/100 g CP)	2	40	48	8	0.5	0.039
	DM intake (kg/d)	2	21.6	21.8	0.3	0.11	0.256
	NH ₃ (mg/L)	2	112.0	131.0	19.0	5.00	0.164
	Total VFA (mmol/L)	2	101.0	102.0	1.0	7.00	0.910
	Acetate/propionate (mol/mol)	2	3.1	3.1	0.08	0.17	0.713
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	2	2.6	2.2	-0.4	0.13	0.183
	Milk yield (kg/d)	2	29.7	30.2	0.5	1.30	0.778
	Milk fat (g/kg)	2	31.6	29.7	-1.8	1.16	0.359
	Milk protein (g/kg)	2	30.6	29.1	-1.5	0.26	0.107
	Milk urea (mg/L)	2	177.0	129.0	-48.0	6.00	0.079
	N intake (g/d)	2	499.9	506.5	6.6	19.70	0.794
	N milk (g/d)	2	146.5	144.1	-2.4	5.28	0.729
	N urine (g/d)	2	148.5	155.7	7.2	4.28	0.343
	N feces (g/d)	2	184.7	180.6	-4.1	0.80	0.123
N balance (g/d)	2	20.3	26.1	5.8	11.00	0.688	
N milk/N intake (g/100 g)	2	29.3	28.5	-0.9	0.06	0.047	
Steam cooking	Milk yield (kg/d)	2	21.2	21.3	0.1	0.08	0.425
	Milk fat (g/kg)	2	38.4	37.5	-0.9	1.20	0.590
	Milk protein (g/kg)	2	34.5	33.8	-0.7	0.10	0.090
	Milk urea (mg/L)	2	309.7	281.9	-27.8	11.40	0.246
	N milk (g/d)	2	120.4	119.6	-0.8	0.83	0.500

682 ⁽¹⁾ difference between proteaginous seed (PS) and soybean meal (SBM)

683 ⁽²⁾ standard error of the difference

684 ⁽³⁾ branched-chain VFA (isobutyrate and isovalerate)

685 Table 7: Effects of replacing soybean meal (SBM) by raw or treated lupin seed on *in vivo*
 686 parameters in dairy cows.

Treatment	Variable (unit)	n	Mean SBM	Mean PS	Δ PS – SBM ⁽¹⁾	SE ⁽²⁾ Δ	P-value
Raw	Diet CP (g/kg DM)	9	176	173	-3	3.1	0.389
	CP provided by SBM or PS (g/100 g CP)	4	41	40	-2	3.5	0.656
	DM intake (kg/d)	11	20.3	19.9	-0.4	0.33	0.296
	NH ₃ (mg/L)	2	144.3	165.4	21.1	1.07	0.032
	Total VFA (mmol/L)	2	111.0	106.1	-5.0	1.05	0.133
	Acetate/propionate (mol/mol)	2	2.9	2.9	0.03	0.07	0.706
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	2	2.4	2.5	0.0	0.13	0.852
	Milk yield (kg/d)	13	28.5	28.8	0.3	0.35	0.400
	Milk fat (g/kg)	13	36.5	37.1	0.6	0.53	0.266
	Milk protein (g/kg)	13	31.2	30.0	-1.2	0.20	<0.001
	Milk urea (mg/L)	3	325.0	303.7	-21.3	17.20	0.340
	N intake (g/d)	11	577.7	574.6	-3.1	11.90	0.799
	N milk (g/d)	13	146.2	142.6	-3.5	2.11	0.119
	N urine (g/d)	1	139.0	132.0	-7.2	-	-
	N feces (g/d)	1	171.0	148.0	-22.6	-	-
	N balance (g/d)	1	40.0	36.0	-4.1	-	-
N milk/N intake (g/100 g)	11	26.2	26.0	-0.2	0.38	0.683	
Extrusion	Diet CP (g/kg DM)	3	145	150	5	0.8	0.030
	CP provided by SBM or PS (g/100 g CP)	2	40	43	3	0.3	0.060
	DM intake (kg/d)	3	20.1	18.7	-1.4	0.45	0.093
	NH ₃ (mg/L)	2	120.0	129.0	9.0	4.00	0.266
	Total VFA (mmol/L)	2	108.0	101.0	-7.0	0	-
	Acetate/propionate (mol/mol)	2	2.7	2.9	0.2	0.12	0.337
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	2	2.5	2.3	-0.2	0.02	0.051
	Milk yield (kg/d)	4	29.2	30.7	1.5	0.72	0.135
	Milk fat (g/kg)	4	34.4	31.2	-3.2	1.47	0.119
	Milk protein (g/kg)	4	30.5	28.4	-2.2	0.07	<0.001
	Milk urea (mg/L)	2	178.0	111.0	-67.0	9.00	0.085
	N intake (g/d)	3	503.2	484.3	-18.9	16.80	0.378
	N milk (g/d)	4	146.1	143.2	-2.9	2.52	0.339
	N urine (g/d)	2	139.3	125.5	-13.8	4.55	0.202
	N feces (g/d)	2	170.5	158.4	-12.2	6.02	0.293
	N balance (g/d)	2	39.6	37.7	-2.0	8.35	0.854
N milk/N intake (g/100 g)	3	30.8	31.2	0.5	0.36	0.310	
Roasting	DM intake (kg/d)	2	23.7	22.5	-1.2	0.34	0.169
	NH ₃ (mg/L)	1	168.6	194.2	25.6	-	-
	Total VFA (mmol/L)	1	114.0	114.1	0.1	-	-
	Acetate/propionate (mol/mol)	1	3.1	2.9	-0.2	-	-

	BC-VFA ⁽³⁾ (mol/100 mol VFA)	1	2.4	2.2	-0.2	-	-
	Milk yield (kg/d)	2	31.8	31.9	0.1	0.90	0.926
	Milk fat (g/kg)	2	37.8	38.0	0.2	0.95	0.900
	Milk protein (g/kg)	2	32.8	31.6	-1.2	-	-
	N intake (g/d)	2	611.2	584.8	-26.4	-	-
	N milk (g/d)	2	168.3	162.5	-5.8	-	-
	N milk/N intake (g/100 g)	2	27.6	28.1	0.5	-	-
Tanning	DM intake (kg/d)	1	18.2	18.6	0.4	-	-
	Milk yield (kg/d)	1	25.2	26.9	1.7	-	-
	Milk fat (g/kg)	1	42.6	41.1	-1.5	-	-
	Milk protein (g/kg)	1	30.2	29.6	-0.6	-	-
	N milk (g/d)	1	125.6	130.7	5.1	-	-

687 ⁽¹⁾ difference between proteaginous seed (PS) and soybean meal (SBM)

688 ⁽²⁾ standard error of the difference

689 ⁽³⁾ branched-chain VFA (isobutyrate and isovalerate)

690 Table 8: Effects of replacing soybean meal (SBM) by raw or treated pea seed on *in vivo*
 691 parameters in dairy cows.

Treatment	Variable (unit)	n	Mean SBM	Mean PS	Δ PS – SBM ⁽¹⁾	SE ⁽²⁾ Δ	P-value
Raw	Diet CP (g/kg DM)	9	159	157	-2	4.7	0.759
	CP provided by SBM or PS (g/100 g CP)	4	36	38	2	8.03	0.799
	DM intake (kg/d)	9	22.4	22.2	-0.2	0.34	0.612
	NH ₃ (mg/L)	5	108.1	133.8	25.7	5.43	0.009
	Total VFA (mmol/L)	4	102.9	107.1	4.2	1.79	0.101
	Acetate/propionate (mol/mol)	4	3.0	3.2	0.2	0.08	0.113
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	4	2.1	2.2	0.2	0.06	0.085
	Rumen protein balance (g/kg DMI)	1	47.0	48.8	1.8	-	-
	Non-NH ₃ N duodenal flow (g/d)	1	383.3	401.7	18.4	-	-
	Microbial N duodenal flow (g/d)	1	221.4	254.1	32.7	-	-
	Non-NH ₃ non-microbial N duodenal flow (g/d)	1	161.9	147.6	-14.3	-	-
	Milk yield (kg/d)	9	32.0	30.9	-1.1	0.64	0.140
	Milk fat (g/kg)	9	36.8	37.1	0.3	0.50	0.563
	Milk protein (g/kg)	9	31.4	31.2	-0.2	0.12	0.169
	Milk urea (mg/L)	5	243.3	238.1	-5.2	27.70	0.861
	N intake (g/d)	9	569.0	563.3	-5.7	17.00	0.744
	N milk (g/d)	9	162.3	156.2	-6.1	3.2	0.092
	N urine (g/d)	5	207.4	211.8	4.4	12.60	0.745
	N feces (g/d)	5	175.4	181.0	5.6	8.82	0.560
	N balance (g/d)	5	38.4	36.2	-2.3	8.86	0.809
N milk/N intake (g/100 g)	9	28.6	27.7	-0.9	0.51	0.120	
Extrusion	Diet CP (g/kg DM)	2	161	154	-7	7.9	0.530
	DM intake (kg/d)	2	20.7	21.4	0.7	0.50	0.395
	NH ₃ (mg/L)	1	143.8	136.3	-7.4	-	-
	Milk yield (kg/d)	2	34.1	34.5	0.5	0.65	0.614
	Milk fat (g/kg)	2	37.1	35.7	-1.4	0.15	0.070
	Milk protein (g/kg)	2	31.8	32.2	0.4	0.60	0.626
	Milk urea (mg/L)	1	186.2	234.2	48.1	-	-
	N intake (g/d)	2	540.9	585.7	44.8	37.20	0.441
	N milk (g/d)	2	176.5	182.3	5.8	0.83	0.090
	N urine (g/d)	1	148.0	180.0	32.0	-	-
	N feces (g/d)	1	196.0	181.0	-15.0	-	-
	N balance (g/d)	1	9.3	67.7	58.4	-	-
	N milk/N intake (g/100 g)	2	32.6	31.2	-1.4	1.81	0.582
Flaking	Milk yield (kg/d)	2	23.0	22.6	-0.4	0.24	0.364
	Milk fat (g/kg)	2	36.6	35.6	-1.0	0.55	0.307
	Milk protein (g/kg)	2	34.3	33.0	-1.3	0.30	0.144
	Milk urea (mg/L)	2	290.4	312.3	21.9	0.55	0.016
	N milk (g/d)	2	143.5	132.8	-10.7	2.47	0.144
Expansion	Diet CP (g/kg DM)	1	159	159	1	-	-

DM intake (kg/d)	1	22.3	22.7	0.4	-	-
NH ₃ (mg/L)	1	143.8	138.2	-5.6	-	-
Milk yield (kg/d)	1	34.4	34.4	-0.01	-	-
Milk fat (g/kg)	1	36.7	36.0	-0.7	-	-
Milk protein (g/kg)	1	34.0	33.2	-0.8	-	-
N intake (g/d)	1	566.9	578.1	11.2	-	-
N milk (g/d)	1	191.4	186.4	-5.0	-	-
N milk/N intake (g/100 g)	1	33.8	32.2	-1.5	-	-

692 ⁽¹⁾ difference between proteaginous seed (PS) and soybean meal (SBM)

693 ⁽²⁾ standard error of the difference

694 ⁽³⁾ branched-chain VFA (isobutyrate and isovalerate)

695 Table 9: Effects of replacing raw by treated faba bean seed on *in vivo* parameters in dairy
 696 cows.

Treatment	Variable (unit)	n	Mean raw	Mean treated	Δ Treated – raw ⁽¹⁾	SE ⁽²⁾ Δ	P-value
Extrusion	Diet CP (g/kg DM)	6	160	161	1	1.6	0.430
	CP provided by faba bean (g/100 g CP)	6	24	24	0	0.3	0.223
	DM intake (kg/d)	6	21.3	21.5	0.2	0.17	0.248
	NH ₃ (mg/L)	6	168.3	160.0	-8.3	9.37	0.418
	Total VFA (mmol/L)	6	109.5	112.9	3.4	2.25	0.191
	Acetate/propionate (mol/mol)	6	3.1	3.2	0.1	0.09	0.449
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	6	2.6	2.1	-0.5	0.06	0.001
	Rumen protein balance (g/kg DMI)	1	-3.5	-22.6	-19.2	-	-
	Non-NH ₃ N duodenal flow (g/d)	1	365.5	408.7	43.2	-	-
	Microbial N duodenal flow (g/d)	1	194.2	216.2	22.0	-	-
	Non-NH ₃ non-microbial N duodenal flow (g/d)	1	172.1	192.6	20.5	-	-
	Milk yield (kg/d)	5	30.2	31.2	1.0	0.48	0.106
	Milk fat (g/kg)	5	38.0	35.2	-2.7	0.73	0.020
	Milk protein (g/kg)	5	30.2	30.1	-0.2	0.11	0.211
	Milk urea (mg/L)	5	213.2	213.6	0.4	7.81	0.962
	N intake (g/d)	6	541.9	549.0	7.0	7.43	0.388
	N milk (g/d)	5	150.4	154.4	4.1	1.78	0.085
	N urine (g/d)	5	175.6	175.5	-0.1	3.08	0.977
	N feces (g/d)	5	213.8	209.9	-3.9	3.66	0.344
	N balance (g/d)	5	43.1	60.6	17.5	11.50	0.204
N milk/N intake (g/100 g)	5	26.1	26.4	0.3	0.29	0.360	
Toasting/ roasting	Diet CP (g/kg DM)	4	181	179	-2	1.5	0.267
	CP provided by faba bean (g/100 g CP)	4	28	25	-3	2.1	0.310
	DM intake (kg/d)	4	21.4	21.3	-0.1	0.04	0.092
	NH ₃ (mg/L)	2	227.7	209.5	-18.2	9.71	0.312
	Total VFA (mmol/L)	2	117.0	98.0	-19.0	9.00	0.282
	Acetate/propionate (mol/mol)	2	4.1	4.9	0.8	0.33	0.262
	Rumen protein balance (g/kg DMI)	4	29.8	23.7	-6.1	2.87	0.126
	Non-NH ₃ N duodenal flow (g/d)	2	99.2	97.5	-1.8	0.59	0.205
	Milk yield (kg/d)	4	27.0	27.4	0.4	0.30	0.275
	Milk fat (g/kg)	4	46.1	44.5	-1.6	0.37	0.023
	Milk protein (g/kg)	4	35.6	35.4	-0.2	0.37	0.630
	Milk urea (mg/L)	4	234.2	238.6	4.4	10.50	0.707
	N intake (g/d)	6	455.0	447.0	-7.9	3.74	0.088
	N milk (g/d)	4	155.5	157.6	2.1	2.17	0.397
	N feces (g/d)	2	34.6	35.2	0.6	1.60	0.772
N milk/N intake (g/100 g)	4	25.3	26.1	0.8	0.41	0.160	
Tanning	Milk yield (kg/d)	1	20.4	19.8	-0.6	-	-

Milk fat (g/kg)	1	32.5	32.6	0.1	-	-
Milk protein (g/kg)	1	28.1	29.5	1.4	-	-
N intake (g/d)	1	357.0	379.0	22.0	-	-
N milk (g/d)	1	94.6	96.4	1.8	-	-
N urine (g/d)	1	86.0	109.0	23.0	-	-
N feces (g/d)	1	143.0	148.0	5.0	-	-
N balance (g/d)	1	33.4	25.6	-7.8	-	-
N milk/N intake (g/100 g)	1	26.5	25.4	-1.1	-	-

697 ⁽¹⁾ difference between treated proteaginous seed (PS) and raw PS

698 ⁽²⁾ standard error of the difference

699 ⁽³⁾ branched-chain VFA (isobutyrate and isovalerate)

700 Table 10: Effects of replacing raw by treated lupin seed on *in vivo* parameters in dairy cows.

Treatment	Variable (unit)	n	Mean raw	Mean treated	Δ Treated – raw ⁽¹⁾	SE ⁽²⁾ Δ	P- value
Extrusion	Diet CP (g/kg DM)	3	149	151	2	0.3	0.014
	CP provided by lupin (g/100 g CP)	3	44	46	1	0.6	0.195
	DM intake (kg/d)	3	18.6	18.7	0.1	0.27	0.727
	NH ₃ (mg/L)	3	157.7	145.9	-11.8	2.44	0.040
	Total VFA (mmol/L)	3	103.5	103.9	0.4	1.43	0.791
	Acetate/propionate (mol/mol)	3	2.8	2.9	0.1	0.08	0.215
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	3	2.9	2.4	-0.5	0.07	0.022
	Rumen protein balance (g/kg DMI)	1	25.1	-15.0	-40.1	-	-
	Non-NH ₃ N duodenal flow (g/d)	1	357.1	471.7	114.6	-	-
	Microbial N duodenal flow (g/d)	1	203.9	208.3	4.4	-	-
	Non-NH ₃ non-microbial N duodenal flow (g/d)	1	153.1	263.3	110.2	-	-
	Milk yield (kg/d)	4	27.9	28.8	1.0	0.58	0.199
	Milk fat (g/kg)	4	37.0	32.3	-4.7	0.69	0.006
	Milk protein (g/kg)	4	28.9	28.9	-0.0	0.23	0.975
	Milk urea (mg/L)	3	209.0	193.0	-16.0	14.00	0.371
	N intake (g/d)	3	447.0	453.9	6.9	5.06	0.306
	N milk (g/d)	4	133.6	137.6	4.0	1.06	0.033
	N urine (g/d)	2	132.1	125.5	-6.6	4.55	0.383
	N feces (g/d)	2	147.9	158.4	10.5	6.02	0.333
	N balance (g/d)	3	-6.3	36.1	42.4	40.60	0.405
N milk/N intake (g/100 g)	2	30.9	31.0	0.1	0.23	0.774	
Expansion	Diet CP (g/kg DM)	1	177	177	0	-	-
	CP provided by lupin (g/100 g CP)	1	22	22	0	-	-
	DM intake (kg/d)	1	21.1	21.3	0.2	-	-
	Milk yield (kg/d)	1	32.6	35.2	2.6	-	-
	Milk fat (g/kg)	1	36.5	34.9	-1.6	-	-
	Milk protein (g/kg)	1	29.5	29.4	-0.1	-	-
	N intake (g/d)	1	597.6	603.2	5.6	-	-
	N milk (g/d)	1	158.7	170.7	12.1	-	-
N milk/N intake (g/100 g)	1	26.6	28.3	1.8	-	-	
Toasting/ roasting	Diet CP (g/kg DM)	1	204	204	0	-	-
	DM intake (kg/d)	3	22.1	21.7	-0.4	0.398	0.405
	NH ₃ (mg/L)	1	190.8	194.2	3.4	-	-
	Total VFA (mmol/L)	1	110.1	114.1	4.0	-	-
	Acetate/Propionate (mol/mol)	1	3.1	2.9	-0.2	-	-
	BC-VFA ⁽³⁾ (mol/100 mol VFA)	1	2.3	2.2	-0.1	-	-
	Milk yield (kg/d)	3	28.8	29.8	1.0	0.79	0.322

	Milk fat (g/kg)	3	39.1	38.9	-0.2	0.32	0.597
	Milk protein (g/kg)	3	32.0	31.8	-0.3	0.15	0.208
	N intake (g/d)	3	618.1	608.6	-9.6	11.3	0.484
	N milk (g/d)	3	148.8	152.7	3.9	3.86	0.418
	N milk/N intake (g/100 g)	3	24.6	25.5	0.9	0.94	0.438
Tanning	DM intake (kg/d)	1	18.2	18.6	0.4	-	-
	Milk yield (kg/d)	1	25.4	26.9	1.5	-	-
	Milk fat (g/kg)	1	43.0	41.1	-1.9	-	-
	Milk protein (g/kg)	1	29.1	29.6	0.5	-	-
	N milk (g/d)	1	121.8	130.7	8.9	-	-

701 ⁽¹⁾ difference between treated proteaginous seed (PS) and raw PS

702 ⁽²⁾ standard error of the difference

703 ⁽³⁾ branched-chain VFA (isobutyrate and isovalerate)

704 Table 11: Effects of replacing raw by treated pea seed on *in vivo* parameters in dairy cows

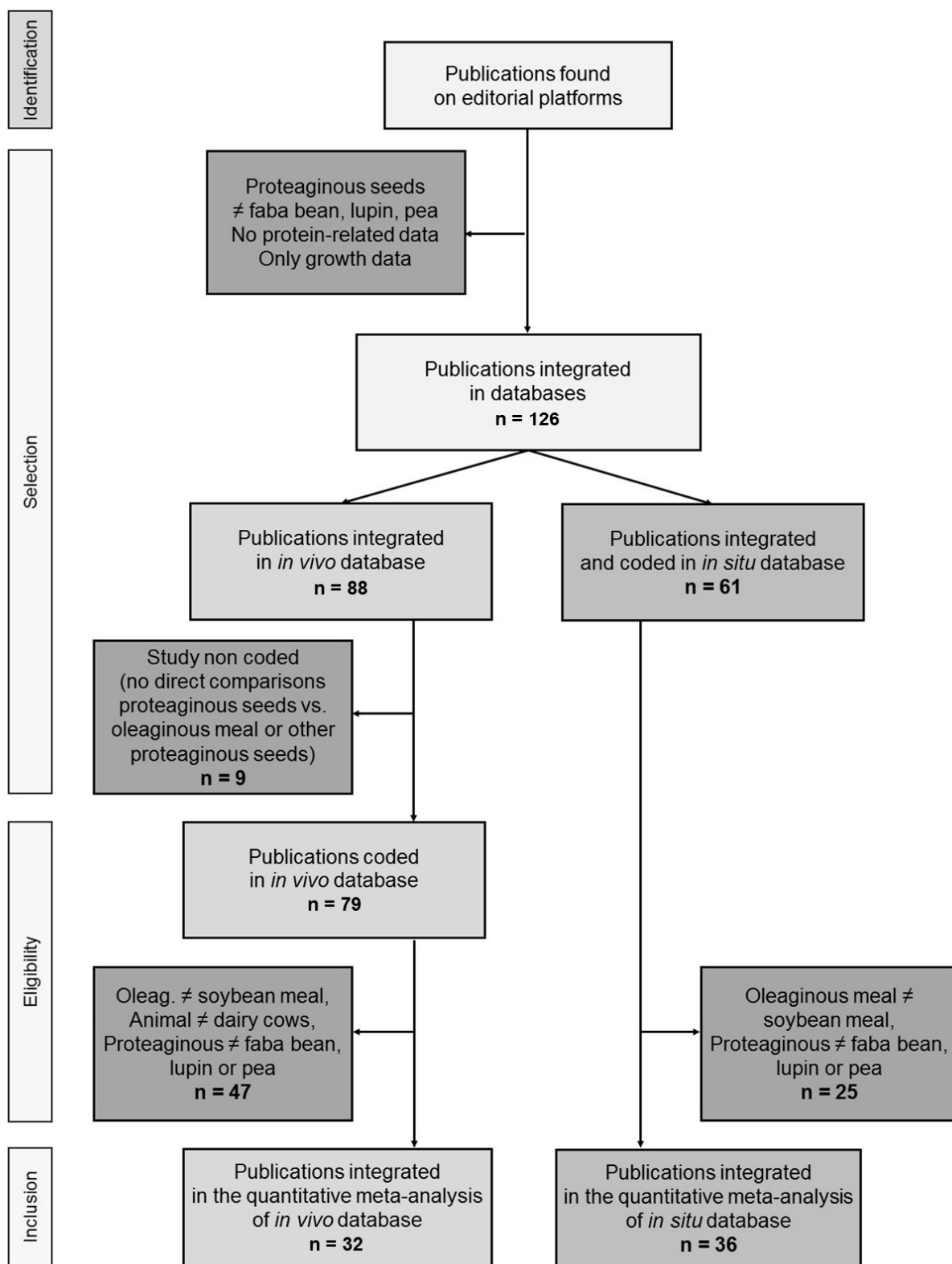
Treatment	Variable (unit)	n	Mean raw	Mean treated	Δ Treated – raw ⁽¹⁾	SE ⁽²⁾ Δ	P-value
Extrusion	Diet CP (g/kg DM)	3	154	151	-3	9.0	0.807
	DM intake (kg/d)	2	21.6	21.4	-0.2	0.13	0.395
	NH ₃ (mg/L)	2	183.1	127.7	-55.4	13.8	0.155
	Total VFA (mmol/L)	1	87.3	98.5	11.2	-	-
	Acetate/propionate (mol/mol)	1	4.5	4.0	-0.5	-	-
	Non-NH ₃ N duodenal flow (g/d)	1	136.0	185.0	49.0	-	-
	Microbial N duodenal flow (g/d)	1	68.0	104.0	36.0	-	-
	Non-NH ₃ non-microbial N duodenal flow (g/d)	1	68.0	81.0	13.0	-	-
	Milk yield (kg/d)	2	34.3	34.6	-0.3	0.99	0.821
	Milk fat (g/kg)	2	36.2	35.7	-0.5	0.75	0.656
	Milk protein (g/kg)	2	31.7	32.2	0.6	0.35	0.361
	Milk urea (mg/L)	1	222.2	234.2	12.0	-	-
	N intake (g/d)	2	567.0	585.7	18.7	19.30	0.509
	N milk (g/d)	2	177.4	182.3	5.0	3.30	0.374
	N urine (g/d)	1	194.0	180.0	-14.0	-	-
	N feces (g/d)	1	201.0	181.0	-20.0	-	-
	N balance (g/d)	1	-2.6	67.7	70.4	-	-
	N milk/N intake (g/100 g)	2	31.3	31.2	-0.1	1.54	0.968
	Steam-flaking	Diet CP (g/kg DM)	1	134	140	6	-
NH ₃ (mg/L)		1	188.2	223.4	35.2	-	-
Total VFA (mmol/L)		1	87.3	86.2	-1.1	-	-
Acetate/propionate (mol/mol)		1	4.5	4.4	-0.1	-	-
Expanding	Diet CP (g/kg DM)	1	159	159	0	-	-
	DM intake (kg/d)	1	22.6	22.7	0.1	-	-
	NH ₃ (mg/L)	1	178.0	138.2	-39.8	-	-
	Milk yield (kg/d)	1	34.2	34.4	0.2	-	-
	Milk fat (g/kg)	1	36.4	36.0	-0.4	-	-
	Milk protein (g/kg)	1	33.6	33.2	-0.4	-	-
	N intake (g/d)	1	575.1	578.1	3.0	-	-
	N milk (g/d)	1	188.1	186.4	-1.6	-	-
N milk/N intake (g/100 g)	1	32.7	32.2	-0.5	-	-	
Tanning	Milk yield (kg/d)	1	20.1	19.6	-0.5	-	-
	Milk fat (g/kg)	1	32.9	31.1	-1.8	-	-
	Milk protein (g/kg)	1	28.6	30.2	1.6	-	-
	N intake (g/d)	1	343.0	359.0	16.0	-	-
	N urine (g/d)	1	111.0	100.0	-11.0	-	-

N feces (g/d)	1	141.0	148.0	7.0	-	-
N balance (g/d)	1	-3.8	13.3	17.2	-	-
N milk/N intake (g/100 g)	1	27.7	27.2	-0.4	-	-

705 ⁽¹⁾ difference between treated proteaginous seed (PS) and raw PS

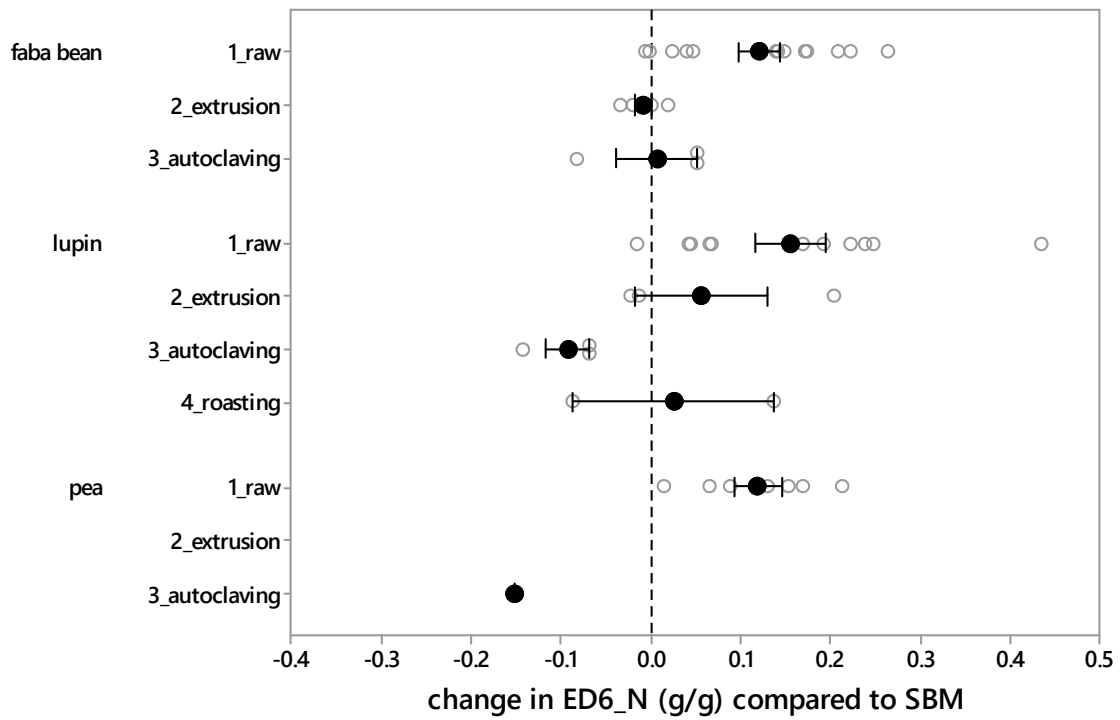
706 ⁽²⁾ standard error of the difference

707 Fig. 1. Prisma flow diagram



709 Fig 2. The variations in *in situ* effective degradability of N (ED₆_N) with raw or treated
710 proteaginous seeds (PS) compared to soybean meal (SBM).

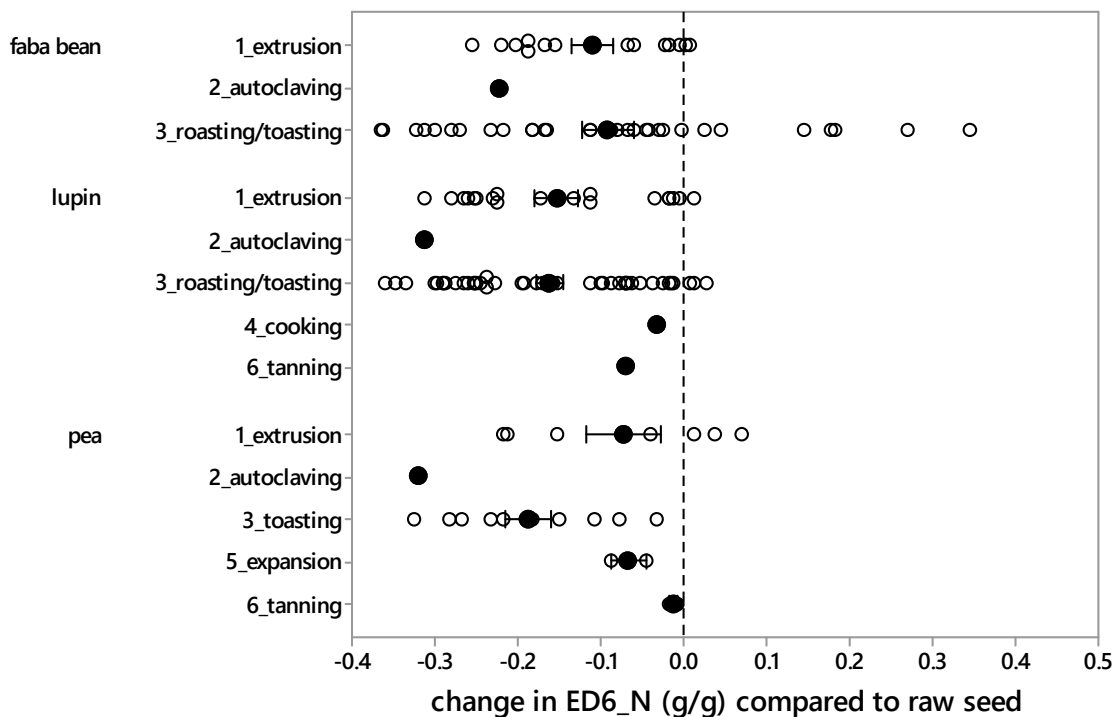
711 White dots represent each comparison within each publication, and black dots represent the
712 average. Horizontal bars are standard error of the mean



713

714 Fig 3. The variations in *in situ* effective degradability of N (ED₆_N) with treated proteaginous
 715 seeds (PS) compared to raw PS

716 White dots represent each comparison within each publication, and black dots represent the
 717 average. Horizontal bars are standard error of the mean

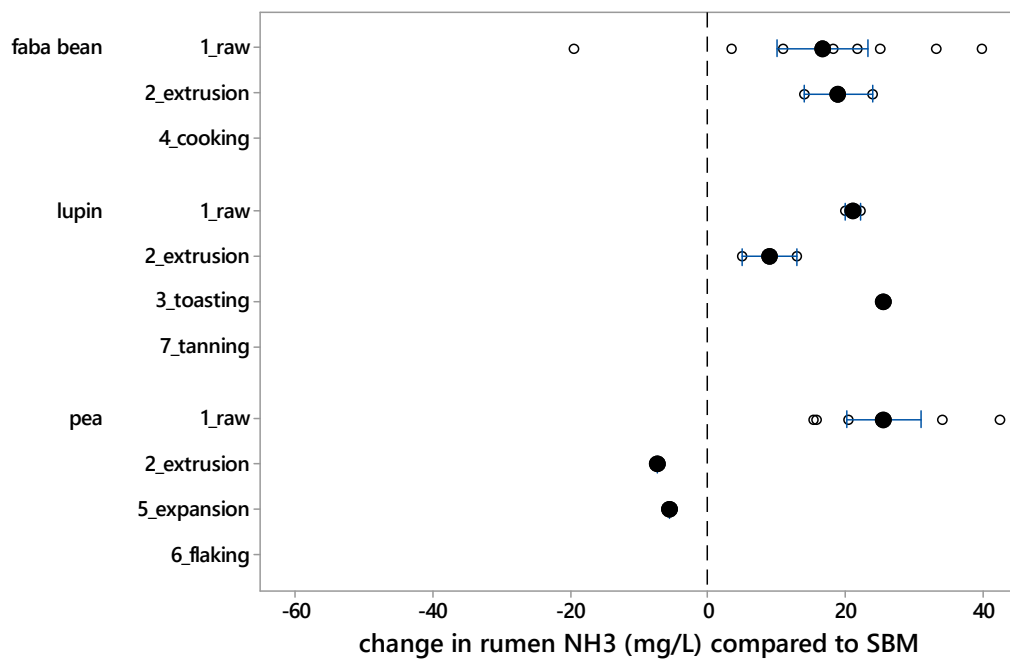


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719

720 Fig 4. The variations of ammonia (NH_3) in ruminal fluid with raw or treated proteaginous
 721 seeds (PS) compared to soybean meal (SBM)

722 White dots represent each comparison within each publication, and black dots represent the
 723 average. Horizontal bars are standard error of the mean

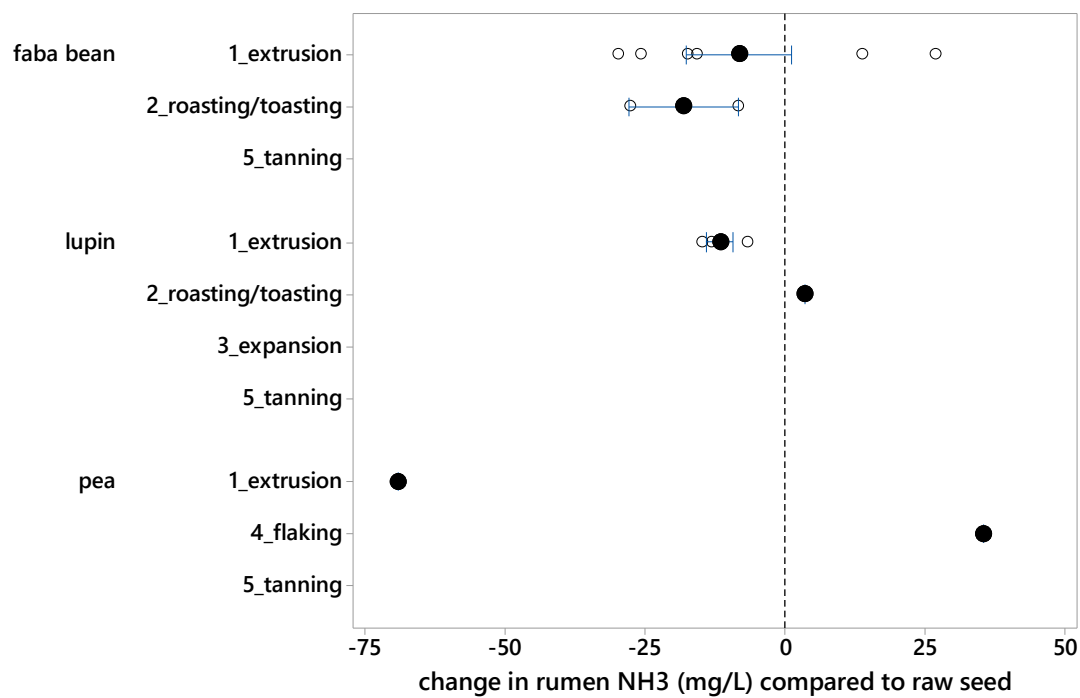


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725

726 Fig 5. The variations of ammonia (NH_3) in ruminal fluid with treated proteaginous seeds (PS)
 727 compared to raw PS

728 White dots represent each comparison within each publication, and black dots represent the
 729 average. Horizontal bars are standard error of the mean

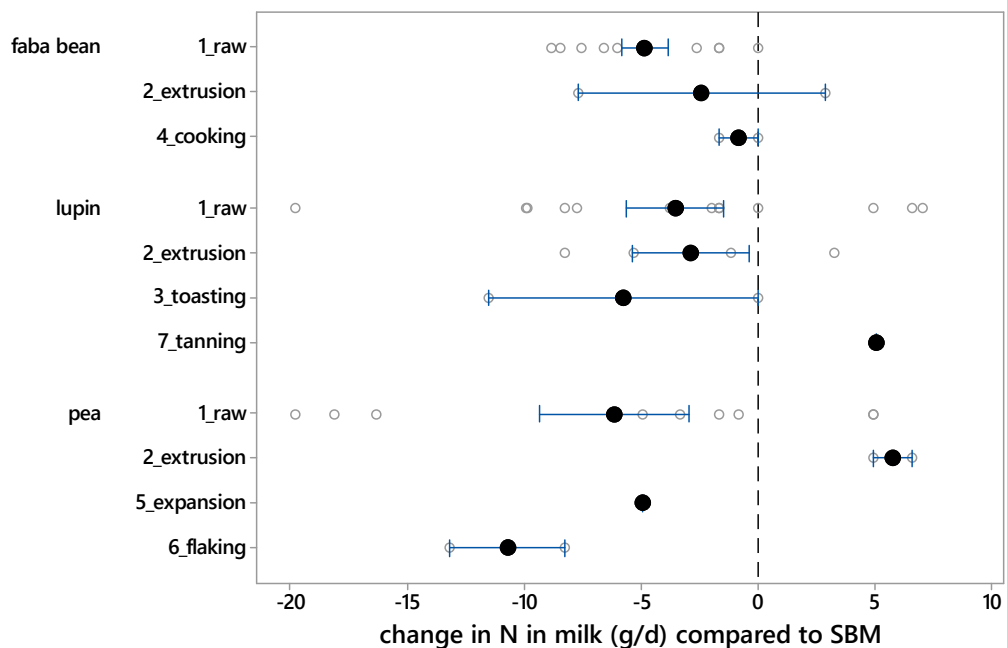


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731

732 Fig 6. The variations in milk nitrogen secretion in dairy cows fed with raw or treated
 733 proteaginuous seeds (PS) compared to soybean meal (SBM)

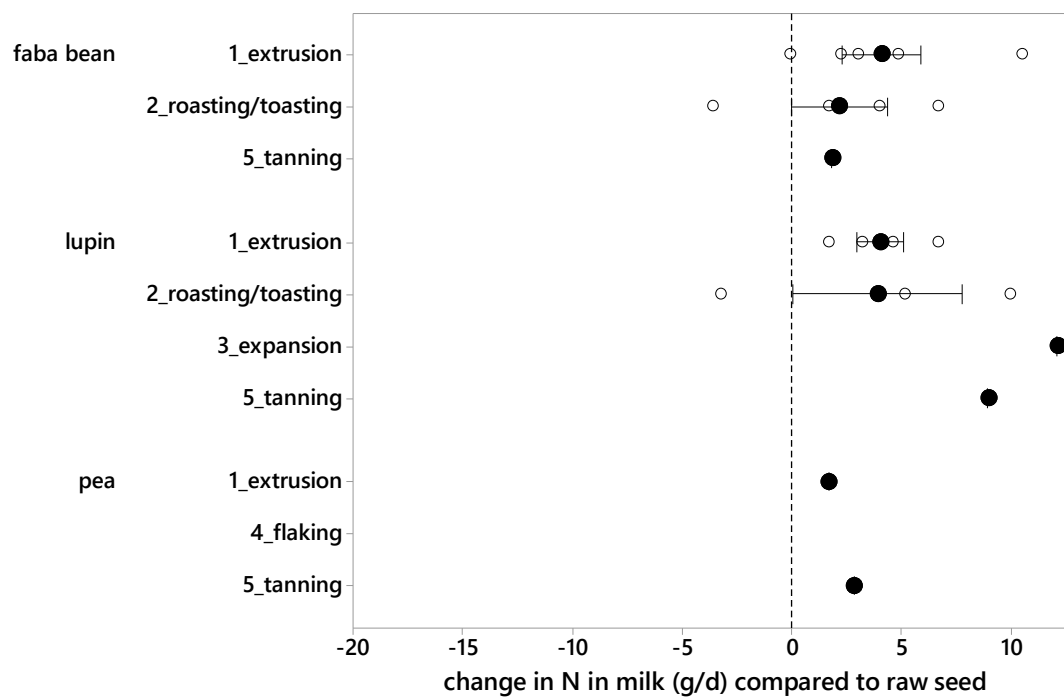
734 White dots represent each comparison within each publication, and black dots represent the
 735 average. Horizontal bars are standard error of the mean



736

737 Fig 7. The variations in milk nitrogen secretion in dairy cows fed with treated proteaginous
 738 seeds (PS) compared to raw PS

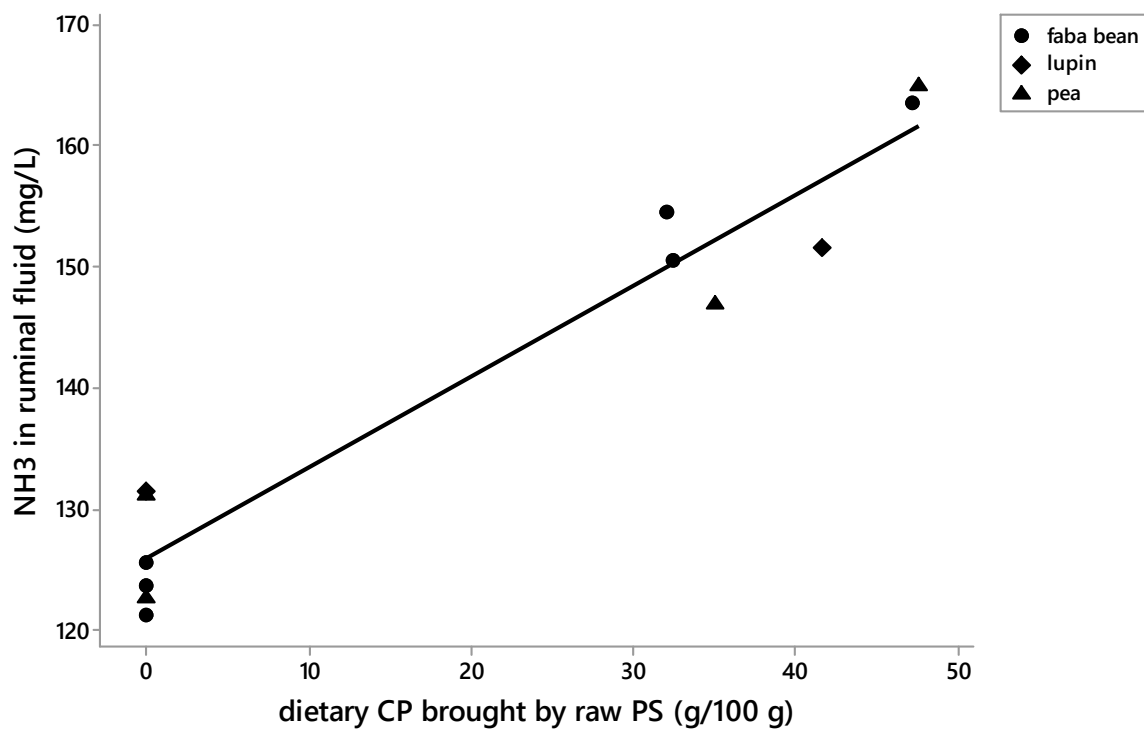
739 White dots represent each comparison within each publication, and black dots represent the
 740 average. Horizontal bars are standard error of the mean



741

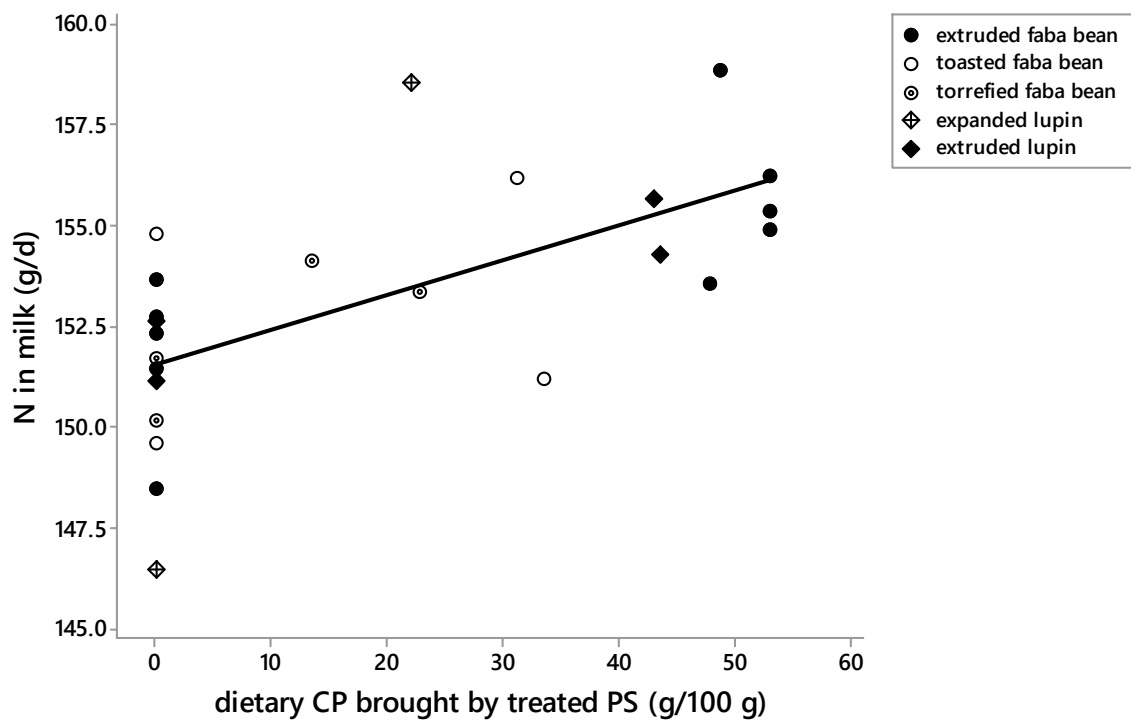
742

743 Fig 8. Within-experiment response law of NH_3 in ruminal fluid to iso-crude protein
744 substitution of soybean meal (SBM) by raw proteaginous seeds (PS). $Y = 126 + 0.75 X$; $n =$
745 12 , $n_{\text{exp}} = 6$, $\text{rmse} = 6.3$; $P = 0.001$. Data corrected for experiment effect, with experiment
746 considered as fixed effect.



747

748 Fig 9. Within-experiment response law of N in milk to iso-crude protein substitution of raw
749 by treated proteaginous seeds (PS). $Y = 152 + 0.086 X$; $n = 24$, $n_{\text{exp}} = 12$, $\text{rmse} = 3.3$, $P =$
750 0.024 . Data corrected for experiment effect, with experiment considered as fixed effect.



751

752 **Supplementary material**

753 List of publications whose data are included in the databases analyzed in this work

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