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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Abstract

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

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

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

Abbreviations: BC-VFA, branched-chain volatile fatty acid; dr_N, nitrogen intestinal true digestibility; ED_6_N, nitrogen ruminal effective degradability assuming a particle outflow from the rumen of 0.06 h^{-1}; MFC, milk fat content; MPC, milk protein content; NANM N, non-ammoniacal non-microbial nitrogen; $PDIA_6$, dietary protein truly digestible in the intestine assuming a particle outflow from the rumen of 0.06 h^{-1}; PIA_6, dietary protein entering the intestine assuming a particle outflow from the rumen of 0.06 h^{-1}; PS, proteaginous seeds; SBM, soybean meal

1. Introduction

Replacing soybean meal (SBM) by proteaginous seeds (PS) in dairy cow diets has been studied for many years in different parts of the world (e.g. Ingalls and McKirdy, 1974; Yu et al., 1999; Puhakka et al., 2016). Compared to soybean meal, PS contain less crude protein (CP) (between 213 and 380 g/kg dry matter (DM) for PS versus 526 g/kg DM for SBM, INRA, 2018). In addition, proteins of raw PS are more degradable in the rumen (nitrogen ruminal degradability calculated with a particle outflow from the rumen of 0.06 h^{-1} (ED_6_N) between 0.70 and 0.81 g/g versus 0.63 g/g for SBM, INRA 2018), decreasing their nutritive value for ruminants. To counteract this, heat-treatments of PS have been developed and have become a well-known solution to reduce the rumen degradability of PS proteins (Cros et al., 1991; Benchaar et al., 1992) and thus to improve their nutritive value.

Our first hypothesis was that raw seeds, compared to SBM, would lead to higher ruminal degradation of proteins and lower dairy performance. Our second hypothesis was that treated PS compared to raw seeds would lead to lower ruminal degradation of proteins and higher dairy performance. To validate these hypotheses, the literature was searched for published data on the digestion of raw or treated PS in dairy cow diets and a meta-analysis performed to highlight the trends and quantify their effects on nitrogen ruminal degradability, nitrogen intestinal true digestibility, ruminal parameters, nitrogen partitioning and milk production and composition.

2. Materials and methods

2.1 Selection of articles

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

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

2.2 Creation of databases

Two file templates were created with Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA, version 2016), for the *in vivo* and *in situ* experiments, respectively, as methodologies and results differed between the two types of publications. For each publication, an individual sub-file, which gathered all the information contained in the publication, was completed using the corresponding template (*in vivo* and/or *in situ*) to be as exhaustive as possible. If one publication contained both *in vivo* and *in situ* results, two individual sub-files (one per template) were completed for this publication. Once all individual sub-files were completed, both databases (*in vivo* and *in situ*) were created by compiling their corresponding individual sub-files. For each database, a homogenization of qualitative terms used for some data was necessary before coding, and some units were converted when required. Moreover, additional calculations useful for interpretation were performed when not directly reported in the publication. In the *in vivo* database, this concerned mainly the proportion of dietary CP provided by the tested experimental feed (i.e. the proportion of this feed in the diet × CP content of this feed/CP content of the diet). As the calculation of N in milk differed between publications, this variable was systematically recalculated as (milk yield × milk protein content / 6.38 / 0.95), assuming 6.38 g of N in 100 g of milk proteins and 95 g of protein N in 100 g of total milk N (DePeters and Cant, 1992; Spanghero and Kowalski, 1997). Nitrogen balance was systematically recalculated as N intake – (N in milk + N in feces + N in urine), using N in milk calculated with the above-described formula. In the *in situ* database, the effective degradability of N (ED_6_N) was calculated using an outflow rate of particles from the rumen fixed at 0.06 h^{-1}, to make all values from the different publications comparable. The ED_6_N (g/g) was calculated according to Ørskov and McDonald (1979) as: $ED_6\_N = a + (b \times c) / (c + 0.06)$, with a the soluble fraction (g/g), b the degradable fraction (g/g) and c the rate of degradation of b (h^{-1}); or by the "step by step method" from the different points of N degradation kinetics (Kristensen et al., 1982). The content of dietary proteins entering the intestine (PIA_6) and of dietary proteins truly digestible in the intestine ($PDIA_6$) was calculated, respectively, as PIA_6 = CP content of the seed × (1 - ED_6_N) and $PDIA_6 = PIA_6 \times$ dr_N, with dr_N the intestinal true digestibility of N (in g/g) assimilated to the proportion of N apparently disappearing from mobile bags in the intestines (Vérité et al., 1987; INRA, 2018).

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

2.3 Treatment comparisons

For both *in vivo* and *in situ* data analyses, the PS studied were faba bean (*Vicia faba*), lupin (mainly *Lupinus albus*, and *Lupinus angustifolius*, considered without distinction because the two experiments with *Lupinus angustifolius* gave similar results to those with *Lupinus albus*) and pea (*Pisum sativum*). Only the following comparisons were considered in the present work (tested versus control): raw PS versus SBM; treated PS versus SBM; and treated versus raw for the same PS. This led to a selection from the *in vivo* and *in situ* databases, respectively, of 32 (representing 41 experiments) and 36 articles plus a non-published study, corresponding to the *in situ* measurements on the 11 experimental concentrates used in Mendowski et al. (2019 and 2020) by Chapoutot et al. (personal communication): 1 soybean meal, 7 faba bean-linseed blends (90:10) (raw, extruded at low or high temperature, with or without reducing sugars or an enzymatic cocktail), and 3 lupin-linseed blends (90:10) (raw or extruded at low or high temperature. The ED_6_N and the dr_N of these feeds were determined according to Michalet-Doreau et al. (1987) and Theodoridou et al. (2010), respectively, with N content in the residues measured using Dumas method for ED_6_N and Kjeldahl method for dr_N. Main results of this study were that extrusion of faba bean and lupin blends reduced ED_6_N by 16 to 23 points, and increased dr_N by between 20 and 30 points (depending on extrusion modalities) compared to raw blends. Extruded faba bean had similar ED_6_N to SBM, and dr_N of SBM was intermediate between raw and extruded PS.

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

2.4 Calculations and statistical analyses

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

3. Results

3.1 In situ *degradation and protein values*

The mean characteristics of all variables of interest from *in situ* database are summarized in Table 1. Lupin was the main PS represented, followed by faba bean, and pea. Direct comparisons between PS and SBM were scarce. Only six experiments from five publications simultaneously reported results for CP, ED_6_N and dr_N required for $PDIA_6$ calculation.

3.1.1 Raw or treated proteaginous seeds versus soybean meal

Results of *in situ* degradation of raw or treated PS compared to SBM are summarized in Table 4, and results on ED_6_N are represented in Figure 2. The PS contained less CP than SBM ($P < 0.001$), with differences averaging -203, -147 and -248 g/kg DM for faba bean, lupin and pea, respectively. All the raw PS had higher ED_6_N than SBM (+0.158, +0.181 and +0.151 g/g for faba bean, lupin and pea, respectively, $P < 0.004$, Figure 2). This led to lower PIA_6 values (-121, -123 and -130 g/kg DM for faba bean, lupin and pea, respectively, $P < 0.001$). Compared to SBM, dr_N tended to decrease with raw faba bean (-0.273 g/g, $P = 0.096$), but not with lupin. Only one comparison was found for raw pea (Solanas et al., 2005), which concluded that PS were associated with significantly lower dr_N than SBM (-0.102 g/g, $P < 0.050$).

In situ data reporting direct comparisons of treated PS vs. SBM were scarce and variable between studies (Solanas et al., 2005; Aguilera et al., 1992; Chapoutot et al., unpublished data). Briefly, extrusion of faba bean led to significantly lower ED_6_N than SBM (-0.009 g/g, $P < 0.001$), and extrusion of PS led to lower PIA_6, similar dr_N and lower $PDIA_6$ than SBM (on average, -70 g/kg DM, -0.03 g/g and -62 g/kg DM for all PS) mainly due to the lower CP content of PS. Autoclaving faba bean led to similar ED_6_N as SBM, but autoclaving lupin tended to lower ED_6_N (-0.093 g/g, $P = 0.066$). The only comparison found for autoclaving pea led to a lower ED_6_N (-0.153 g/g, Aguilera et al., 1992). Finally, roasting PS led to similar ED_6_N and PIA_6 as SBM.

3.1.2 Treated versus raw proteaginous seeds

The *in situ* degradation results for treated faba bean, lupin and pea compared to the same raw seeds are summarized in Table 5, and results for ED_6_N are represented in Figure 3. Treatments did not affect the CP content of PS, except for extrusion of faba bean and lupin (+15 and +10 g/kg DM, $P = 0.009$ and $P = 0.020$, n = 6 from 3 experiments and n = 3 from 2 experiment, respectively), which was an unexpected finding.

Extrusion of faba bean decreased ED_6_N (-0.111 g/g, $P = 0.001$) and consequently increased PIA_6 (+34 g/kg DM, $P = 0.002$), increased dr_N (+0.179 g/g, $P = 0.002$) and $PDIA_6$ (+62 g/kg DM, $P < 0.001$). When faba bean was toasted or roasted, the ED_6_N decreased by 0.093 g/g compared with raw faba bean ($P = 0.005$). No effect was observed on PIA_6 with toasted/roasted faba bean, whereas dr_N was increased by 0.051 g/g ($P < 0.001$), and $PDIA_6$ tended to be increased by 98.7 g/kg DM ($P = 0.083$, Table 5). Only one study comparing autoclaved to raw faba bean was found, which showed that ED_6_N decreased by 0.224 g/g, and PIA_6 consequently increased by 76.2 g/kg DM after autoclaving (Aguilera et al., 1992).

The ED_6_N was lower in extruded than raw lupin (-0.155 g/g, $P < 0.001$), whereas PIA_6, dr_N and $PDIA_6$ were all higher (+55 g/kg DM, $P < 0.001$, +0.324 g/g, $P = 0.001$ and +92 g/kg DM, $P = 0.020$, respectively). Toasting or roasting lupin reduced ED_6_N (-0.161 g/g, $P < 0.001$), and increased PIA_6 (+52.0 g/kg DM, $P < 0.001$) and dr_N (+0.018 g/g, $P < 0.001$), leading to an increase in $PDIA_6$ (+93.1 g/kg DM, $P = 0.040$). Publications about the effects of other treatments on lupin were scarce. Aguilera et al. (1992) showed that autoclaving lupin reduced ED_6_N by 0.313 g/g, thus increasing PIA_6 by 128 g/kg DM. Rodehutscord et al. (1999) observed that cooking or tanning lupin reduced ED_6_N by 0.035 and 0.071 g/g, while PIA_6 was increased by 10.8 and 18.9 g/kg DM, respectively.

When pea was treated by extrusion, the decrease in ED_6_N was not significant compared to raw pea, but PIA_6 was higher (+47 g/kg DM, $P = 0.010$). Solanas et al. (2005) observed an increase of 0.121 g/g in dr_N ($P < 0.050$) with extruded pea. Toasting pea reduced ED_6_N (-0.190 g/g, $P < 0.001$) and increased PIA_6 (+60 g/kg DM, $P = 0.017$) and dr_N (+0.033 g/g, P

< 0.001, Table 5). The only comparison between autoclaved and raw pea concluded that autoclaving reduced ED_6_N by 0.323 g/g and consequently increased PIA_6 by 86 g/kg DM (Aguilera et al., 1992).

3.2. In vivo *responses*

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

3.2.1 Raw and treated proteaginous seeds versus soybean meal

Results of the *in vivo* effects of feeding raw or treated PS (faba bean, lupin or pea) instead of SBM are summarized in Tables 6 to 8. Replacement of SBM by raw or treated PS was made in iso-nitrogenous conditions ($P > 0.100$ for all seeds). Only one exception has been observed for comparison between SBM and extruded lupin: the CP content of the diet was 5 g/kg DM higher with extruded lupin than with SBM ($P = 0.030$). Moreover, in most cases, the proportion of dietary CP provided by the experimental concentrate (PS or SBM) did not differ between tested and control treatments, except for extruded faba bean ($P = 0.039$, Table 6) and extruded lupin ($P = 0.060$, Table 7) diets, which provided more dietary CP than SBM. Replacement of SBM by PS in the diet did not affect DMI (measured on *ad libitum* or *sub ad* *libitum* diets), except for extruded lupin, which tended to decrease DMI by 1.4 kg/d compared to SBM (P = 0.093, Table 7).

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

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

Replacing SBM by raw pea led to an increase in NH_3 in ruminal fluid (+25.7 mg/L, P = 0.009; Figure 4), and tended to increase BC-VFA in the rumen (+0.2 mol/100 mol VFA) and to decrease N in milk (-6.1 g/d, P = 0.092, Table 8). Only one comparison was found about duodenal flow, and it showed that non-NH_3 N and microbial N were numerically increased (*P* > 0.050, Khorasani et al., 2000), whereas non-NH_3 non-microbial N (dietary + endogenous) was numerically decreased (*P* > 0.050, Khorasani et al., 2000). When pea was extruded, milk fat content (MFC) tended to decrease (-1.4 g/kg, *P* = 0.070), but N in milk tended to increase (+5.8 g/d, *P* = 0.090, Table 8 and Figure 6) compared to SBM. Petit et al. (1997) found that extrusion increased N excreted in urine and N balance (*P* < 0.050). When pea was flaked, milk urea was increased (+21.9 mg/L, *P* = 0.016, Table 8) compared to SBM. When pea was expanded and compared to SBM, no difference was observed on ruminal NH_3, milk yield, MFC and MPC (Masoero et al., 2006).

3.2.2 Treated versus raw proteaginous seeds

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

For faba bean, BC-VFA were lower (-0.5 mol/100 mol total VFA, *P* = 0.001), as was 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 and Figure 7) with extruded than with raw seeds. The only experiment reporting N duodenal flow documented an increase in both microbial and non-NH_3 non-microbial flow (*P* < 0.050, Benchaar et al., 1994b). When faba bean was toasted or roasted, N intake tended to decrease (-7.9 g N/d, *P* = 0.088) as did MFC (-1.6 g/kg, *P* = 0.023, Table 9) compared to raw faba bean. Only one publication was found about the effects of tanning faba bean, which concluded that tanning increased MPC and N intake (*P* < 0.050, Pelletier and Bouchard, 1978).

Replacing raw lupin by extruded seeds decreased NH_3 in ruminal fluid (-11.8 mg/L, *P* = 0.040, Figure 5), BC-VFA (-0.5 mol/100 mol VFA, *P* = 0.022) and MFC (-4.7 g/kg, *P* = 0.006), but increased N in milk (+4.0 g/j, *P* = 0.033, Table 10 and Figure 7); the only experiment with duodenal flow reported that there was an increase in both microbial and non-NH_3 non-microbial N duodenal flow (*P* < 0.050, Benchaar et al., 1994a). No difference in *in vivo* results was observed when lupin was toasted or roasted compared to raw lupin. Publications about the effects of expanding, tanning, autoclaving and cooking lupin were scarce. Pieper et al. (2006) concluded that expanding lupin led to better protection of CP, resulting in a better milk yield (*P* < 0.050), but no change in MFC and MPC. Emile et al. (1991) observed a higher milk yield but a lower MFC when lupin was tanned, with no difference in MPC.

For pea, few *in vivo* data were available about effects of extrusion, flaking, expanding and tanning (Table 11). Focant et al. (1990) concluded that extrusion led to a significant increase in microbial (*P* < 0.010) and a numerical increase in non-NH_3 non-microbial N flow in the duodenum, a decrease in NH_3 content from ruminal fluid (*P* < 0.001), but reported that steam-flaking had no observed effects. No beneficial effect on milk production was observed with extrusion, and Petit et al. (1997) concluded that extrusion decreased N excreted in feces (*P* < 0.050), but increased N balance (*P* < 0.010). Masoero et al. (2006) concluded that expanding had no effects on intake and milk production. Finally, Pelletier and Bouchard (1978) concluded that tanning pea had no effect on milk production, but increased MPC (*P* < 0.050) and had no effect on N partitioning despite an increase in N intake (*P* < 0.050).

4. Discussion

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

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

4.1. In situ *degradation*

With all PS, the ED_6_N was higher for raw seeds (on average 0.778 g/g) than for SBM (on average 0.613 g/g). In this analysis, the ED_6_N values for PS were slightly lower than those averaged from larger data sets, and presented in feed tables by INRA (2018) and INRA-CIRAD-AFZ (2017), i.e. 0.82, 0.86 and 0.86 g/g for raw faba bean, lupin and pea, respectively. Most of the treatments significantly decreased the ED_6_N: on average -0.100, -0.158 and -0.130 g/g with all treatments for faba bean, lupin and pea, respectively, with the strongest effect with autoclaving, followed by extrusion and finally toasting (Figure 3). The observed reductions in ED_6_N confirm that heat and/or pressure treatments can protect N from ruminal degradation, which led on average to an increase of +43 g PIA_6/kg DM for all PS and treatments. In this study, the ED_6_N reductions observed with extrusion were slightly lower than those reported in INRA Feed Tables (2018). This could partly be due to differences in treatment modalities (duration, temperatures reached, intensity of pressure treatments, etc.), which are often poorly described in the literature. For similar treatments (extrusion or other heat treatments except toasting/roasting), the greatest reductions in ED_6_N were observed for lupin, which appeared to be the seed the most reactive to treatment. This implies a higher increase in PIA_6 (in g/kg DM) for lupin (+53, n = 56) than for faba bean (+18, n = 25); pea also appeared reactive (+58, n = 6), but there were less available data. This finding was also supported by intra-experiment comparisons: reductions in ED_6_N were greater for lupin than for faba bean with autoclaving (Aguilera et al., 1992), extrusion (Chapoutot et al., 2016) and toasting (Goelema, 1999), and greater for lupin than for pea with extrusion (Aufrère et al., 2001) and toasting (Goelema, 1999; Goelema et al., 1998). Fewer data were available for dr_N than ED_6_N, but when PS were heat-treated, dr_N increased significantly (on average for all treatments: +0.10, +0.12 and +0.04 g/g for faba bean, lupin and pea, respectively). This suggests that the protection of proteins against ruminal degradation is reversible in the abomasum, which allows the absorption of amino acids in the intestine. In addition, the flow of amino acids potentially absorbed is greater. Some treated PS have similar or lower ED_6_N than SBM: autoclaved faba bean, lupin, or pea, and roasted lupin. However, due to their lower CP content, no treated PS reached a level of PIA_6 comparable to SBM despite the effects of heat treatments: PIA_6 values were on average 60 g/kg DM lower with treated PS than with SBM, and no direct comparison between treated PS and SBM was available for $PDIA_6$.

4.2. In vivo *responses*

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

According to the observed *in situ* results, a lower valorization of proteins by dairy cows might be expected when SBM is replaced by PS in their diet, and reciprocally a better valuation of proteins with treated PS compared to raw PS. Nevertheless, the expected effects of raw or treated PS were not systematically observed *in vivo*. This could be explained by the fact that differences between treatments on *in vivo* measured N flows were lower than those observed on N balance, which was mainly due to balance default (as a result of methodological limits) in dairy cows (Spanghero and Kowalski, 1997). It should also be noted that only Pereira et al. (2017) and Mendowski et al. (2019, 2020) explicitly reported on supplementation of metabolizable methionine. Thus, in all other studies, the diets containing faba bean, lupin and pea (particularly when extruded) could have provided a lower supply of digestible methionine than current recommendations (2.3% of PDI, INRA, 2018) for MPC and proteins secreted in milk. However, this may have been partly offset by the fact that comparisons between PS and SBM were performed with iso-CP diets (PS or SBM providing on average 39 g/100 g of dietary CP). Another explanation of differences between expected and observed effects when SBM is substituted by raw or treated PS *in vivo* could be related to the nature of the substitution within- experiments. Indeed, while in some publications, diets have been formulated to be iso-energetic, in others, PS diets provide more energy than SBM diets (the NE_L / CP ratio being higher for PS than for SBM). Thus, responses of dairy cows may also be partly related to a higher energy intake with PS diets than with SBM diets. Due to scarce available data, it has not been possible to split data between iso-energetic and non-iso-energetic supplementations for the analysis. Given the average values of 20.1 kg for DM intake, 165 g/kg DM for dietary CP content, and 39% of dietary CP for substitution, the increase in energy intake may reach 4.2 Mcal $NE_{L,}$ that may contribute to 2.6 g N in milk/d according to INRA (2018).

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

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

An evaluation of general trends provides a more global picture of the effects of dietary substitutions. When dairy cows were fed raw PS compared to SBM, ruminal NH_3 content increased (on average with all PS: +20 mg/L, n = 15), and the higher the proportion of CP provided by raw PS, the higher the NH_3 content in the rumen (as illustrated by the average within-experiment response law, Figure 8). The BC-VFA molar proportion tended to increase with faba bean compared to SBM (on average with all PS: +0.16 mol/100 mol total VFA, n = 11). The N in milk/N intake ratio decreased significantly with faba bean and numerically with lupin and pea (on average with all PS: -0.6 g/g, n = 29). The MPC decreased significantly with faba bean and lupin (on average -1.0 g/kg), but not with pea. Milk yield and N in milk did not change with lupin or pea (only N in milk tended to decrease with pea) but decreased with raw faba bean by 0.5 kg/d and 5 g/d, respectively. Taken together, these results are consistent with *in situ* observations: the increase in ruminal NH_3, and the lower MPC and N in milk reflecting higher ruminal N degradation and the induced lower $PDIA_6$ value. Our first hypothesis, which was that raw seeds compared to soybean meal would lead to lower dairy performance was verified with faba bean, and numerically the same trend was observed with lupin and pea, although it did not reach statistical significance. The N partitioning between urine and feces was not modified when SBM was replaced by raw PS, but very few data were available.

The attenuation observed between *in vivo* and *in situ* responses could partly be explained by the risk of over-estimating the ED_6_N of PS due to particle loss during incubation when using the nylon bag technique, which has been reported to be high with PS (Michalet-Doreau and Cerneau, 1992). Consequently, PDI values of PS may have been underestimated, which would imply a higher gap in dairy performance between SBM and PS.

The low content of digestible methionine of PS (1.48, 1.44 and 1.72 g/100 PDI for faba bean, lupin and pea, respectively, INRA 2018) should be noted. As very few publications, except for example Mendowski et al. (2019, 2020), reported supplementation with metabolizable methionine, diets containing PS could have had lower than recommended levels of digestible methionine, which could impair dairy performance, in particular MPC. This is consistent with what was obtained by Pereira et al. (2017), who observed that adding rumen-protected Lys and Met to a diet containing field pea increased MPC compared to the same diet with no AA supplement. In addition, Joch and Kudrna (2020) compared diets containing SBM in which either 0%, 30% or 50% of SBM was replaced by raw lupin (all diets containing protected Met) and observed no difference on MPC between diets: supplementing in protected Met may have prevented a decrease of MPC with lupin compared to SBM.

4.2.2. Effects of treatments on proteaginous seeds

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

Both raw and treated PS provided on average 29 g/100 g (from 14 to 53g/100 g) of CP in dairy cow diets. With extrusion there was a global decrease in ruminal NH_3, which was significant for lupin (-12 mg/L) and numerical for faba bean and pea (on average -17 mg/L, n = 7 with faba bean and pea), suggesting extrusion protected the proteins against ruminal degradation. This is consistent with observations on ED_6_N with extrusion. With other treatments than extrusion, the effects depended on seed and treatment. For toasted/roasted faba bean, there was a decrease in NH_3 in ruminal fluid (the change was not statistically significant, but only two comparisons were found), whereas for toasting/roasting lupin and flaking pea the opposite was observed with an increase in NH_3 in ruminal fluid (only few data available). The main significant zootechnical result on nitrogen utilization concerned extrusion of faba bean and lupin, which led to an increase in N in milk (as also illustrated by the within-experiment response law, Figure 9). However, some heat treatments of faba bean (extrusion and toasting/roasting) and lupin (extrusion) appeared to decrease MFC, and most of the other treatments followed the same trend. This could be explained by a greater availability of fat after extrusion of lupin compared to raw lupin (Chilliard et al., 2009), leading to an inhibition of *de novo* synthesis of fatty acids in the mammary gland by dietary polyunsaturated fatty acids or rumen biohydrogenation intermediates; Shingfield et al, 2010). For extruded faba bean, the five available comparisons concerned 10:90 linseed:faba bean blends (Mendowski et al., 2019 and 2020), in which fat could have been released from the linseed during extrusion. Moreover, faba bean has a high content of starch (44 % on a DM basis), and starch-rich diets have been shown to potentially induce milk fat depression in dairy cows (Bauman and Griinari, 2003). Consequently, the decrease in MFC observed with faba bean could result both from the release of polyunsaturated fatty acids from linseed and the faba bean seed itself, and its high starch content.

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

At similar dietary CP content, diets containing treated PS rather than SBM had non-significantly higher NH_3 rumen content and milk yield. The MPC was significantly lower with steam cooked faba bean and extruded lupin, and most other treated PS followed the same trend, so that N in milk tended to be lower than with SBM, but differences were small (-2 g N/day). Urinary or fecal N excretion and N balance values obtained with treated PS were comparable to those measured with SBM. The MFC tended to be lower with treated PS than with SBM, but differences were small (on average -1.2 g/kg, n = 16) and non-significant, except for a trend with extruded pea. When treated PS were compared to SBM either no change in values, or lower MFC and MPC values were observed, as reported with steam-cooked faba bean (-0.7 g/kg protein content, P = 0.090), extruded lupin (-2.2 g/kg protein content, P < 0.001) and extruded pea (-1.4 g/kg fat content, P = 0.070 and -5.8 g/d N in milk, P = 0.090). Heat treatments improved the nutritional value of PS, but still did not allow the seeds to reach the value of SBM. At iso-CP comparisons, this can be explained by the lower PDI content of PS compared to SBM, and also by the lack of digestible methionine contained in PS compared to SBM.

5. Conclusions

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

NH_3 measurements (which is in accordance to our first hypothesis). This ruminal N degradability was lowered by heat treatments (confirming our second hypothesis), but even so the amount of PIA_6 did not reach levels obtained with SBM because of the differences in CP content. Moreover, very few results on dr_N were available. Evaluations of *in vivo* dairy performance indicated that MPC and milk N secretion were decreased with raw PS compared to SBM (thereby confirming our first hypothesis). In contrast, no major effect was observed when PS were treated compared to raw PS (not supporting our second hypothesis), except for a decrease in MFC, especially for extruded faba bean and lupin. Finally, at similar CP content, N transfer as protein in milk remained lower with treated PS than with SBM, but differences were small. When SBM was replaced by raw PS, or when raw PS were replaced by treated PS, the observed variability of responses in milk production and composition could partly be explained by the variations specific to each study, and particularly the dietary level of digestible methionine. Moreover, when PS were heat-treated, technological processes were most often not fully described, which led to uncertainty about the intensity of the process the seeds may have undergone. A better description of these heat or pressure treatments would permit a better understanding of their effects on PS, and consequently the effect on dairy performance when these seeds are fed to dairy cows. This work revealed the very limited number of publications testing raw and treated PS in dairy cow diets, and how important it would be to perform more analytical feeding trials to improve both understanding of processes involved in N protection, and prevision of their nutritional interest as alternatives to SBM in dairy cow diets.

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Table 1: Descriptive statistics of the main variables of interest in the *in situ* database 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
ED_6_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
PIA_6 (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
$PDIA_6$ (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	-	-	-

[1] standard deviation

Table 2: Mean characteristics of variables of interest analyzed in the *in vivo* database, concerning diets including proteaginous seeds (PS) and soybean meal (SBM) whatever the 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 NH_3 (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

(1) standard deviation

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

(3) non-NH_3 and non-microbial N duodenal flow

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		*Ad libitum*		
Concentrate (g/100 g dietary DM)	94	44.0	13.5	16.1	75.7

(1) standard deviation

Table 4: *In situ* parameters and protein value of raw or treated proteaginous seeds (PS) 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	-	-

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

(2) standard error of the difference

Table 5: *In situ* parameters and protein value of raw proteaginous seeds (PS) compared to 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_6N (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

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

(2) standard error of the difference

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

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

(2) standard error of the difference

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

Table 7: Effects of replacing soybean meal (SBM) by raw or treated lupin seed on *in vivo* 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	-	-

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

[2] standard error of the difference

[3] branched-chain VFA (isobutyrate and isovalerate)

Table 8: Effects of replacing soybean meal (SBM) by raw or treated pea seed on *in vivo* 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	-	-

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

(2) standard error of the difference

(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	-	-

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

(2) standard error of the difference

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

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	-	-

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

(2) standard error of the difference

(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 milk (g/d)	1	94.8	97.7	2.8	-	-
	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	-	-

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

(2) standard error of the difference

Fig. 1. Prisma flow diagram

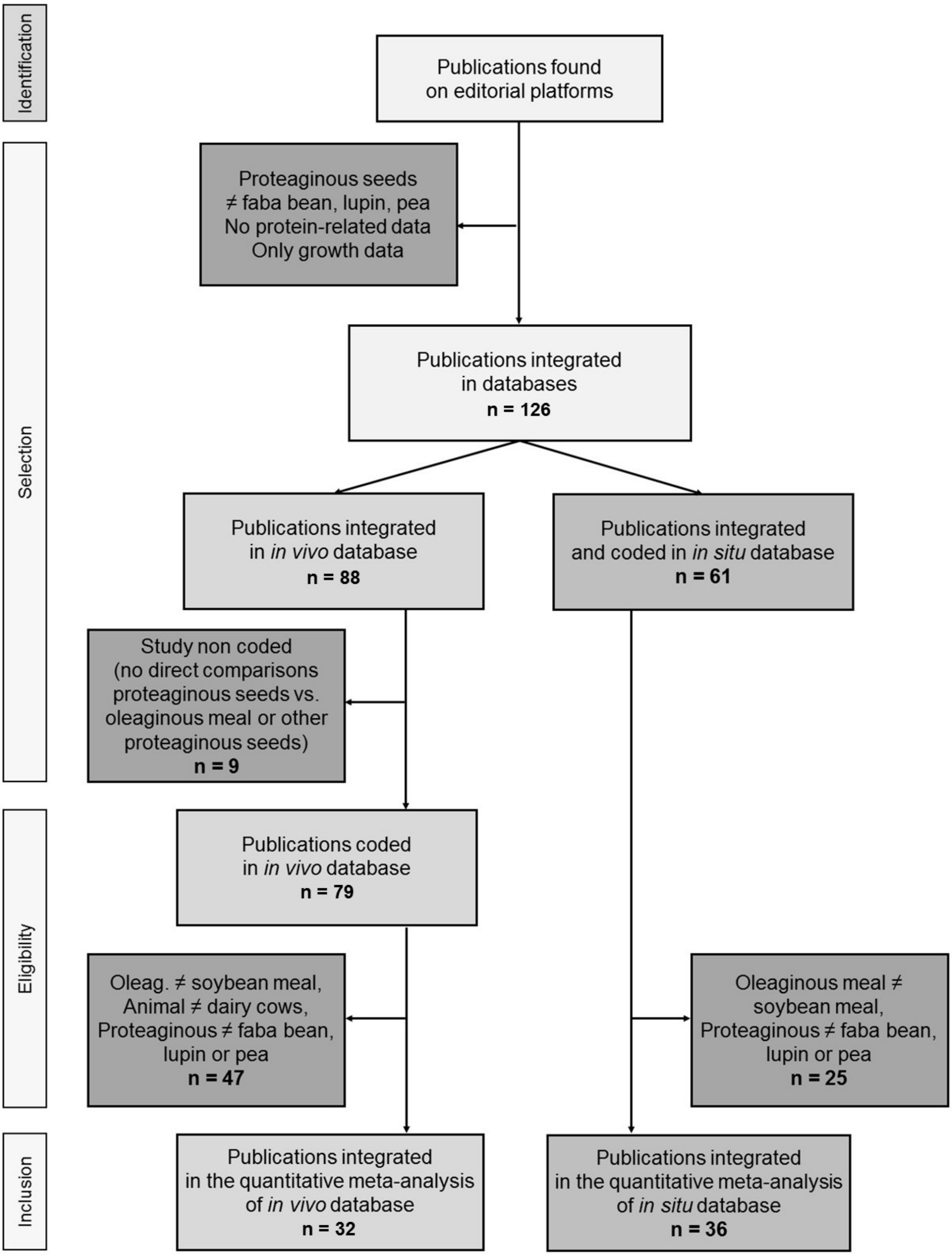


Fig 2. The variations in *in situ* effective degradability of N (ED_6_N) with raw or treated proteaginous seeds (PS) compared to soybean meal (SBM).

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

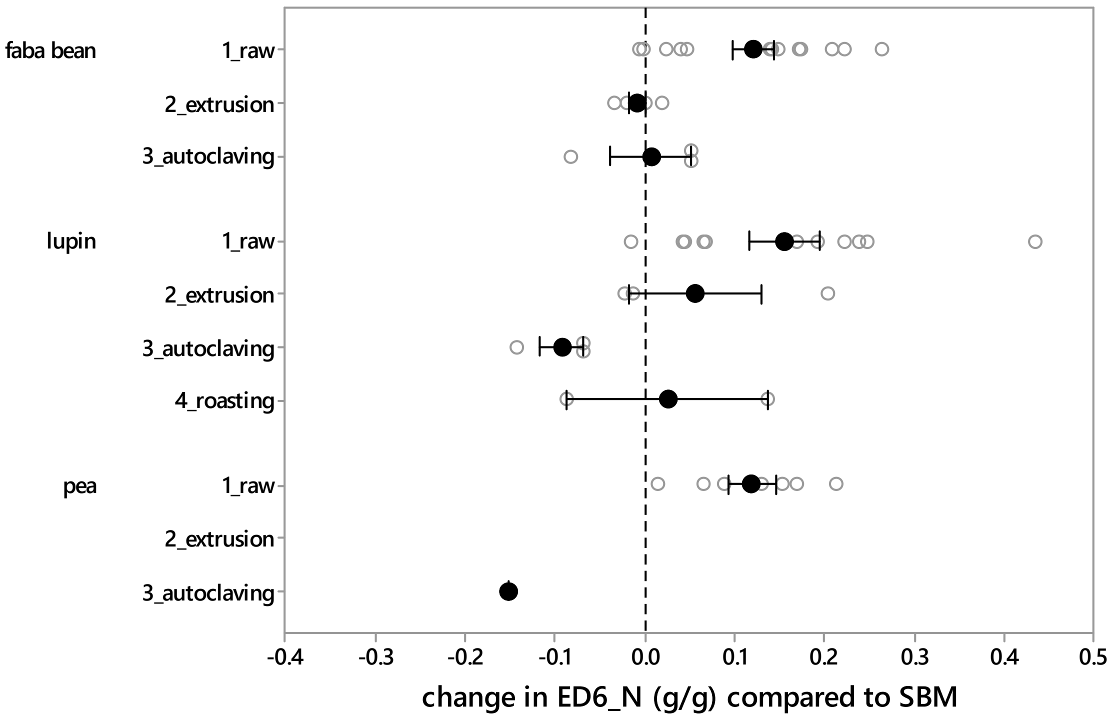


Fig 3. The variations in *in situ* effective degradability of N (ED_6_N) with treated proteaginous seeds (PS) compared to raw PS

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

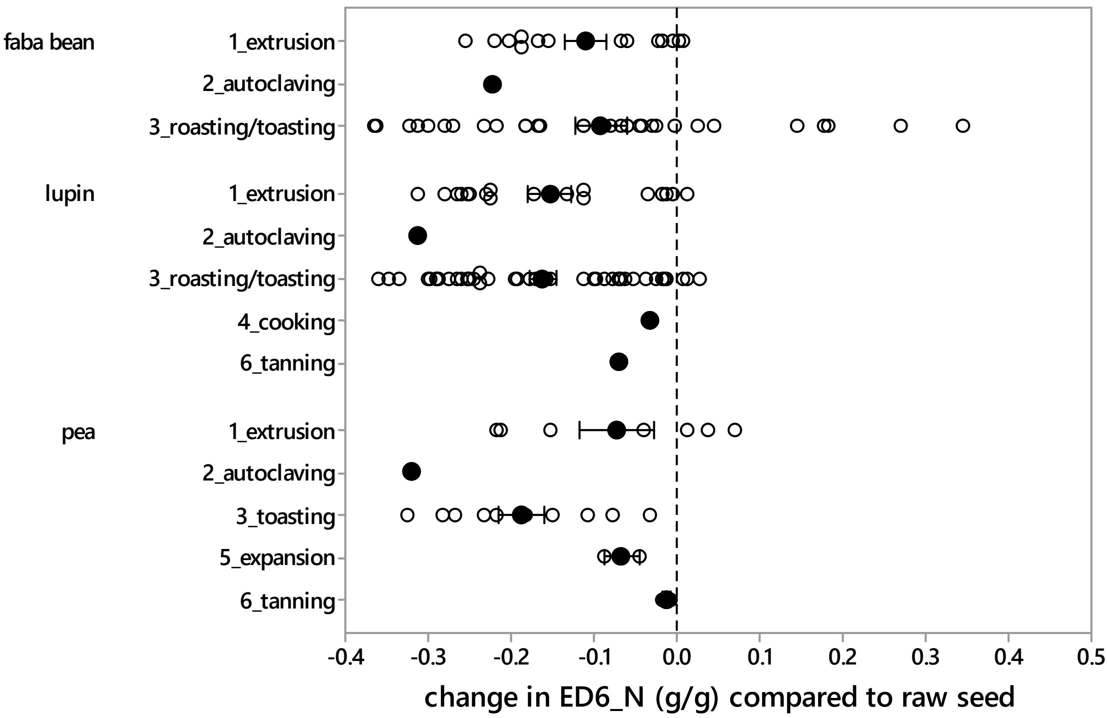


Fig 4. The variations of ammonia (NH_3) in ruminal fluid with raw or treated proteaginous seeds (PS) compared to soybean meal (SBM)

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

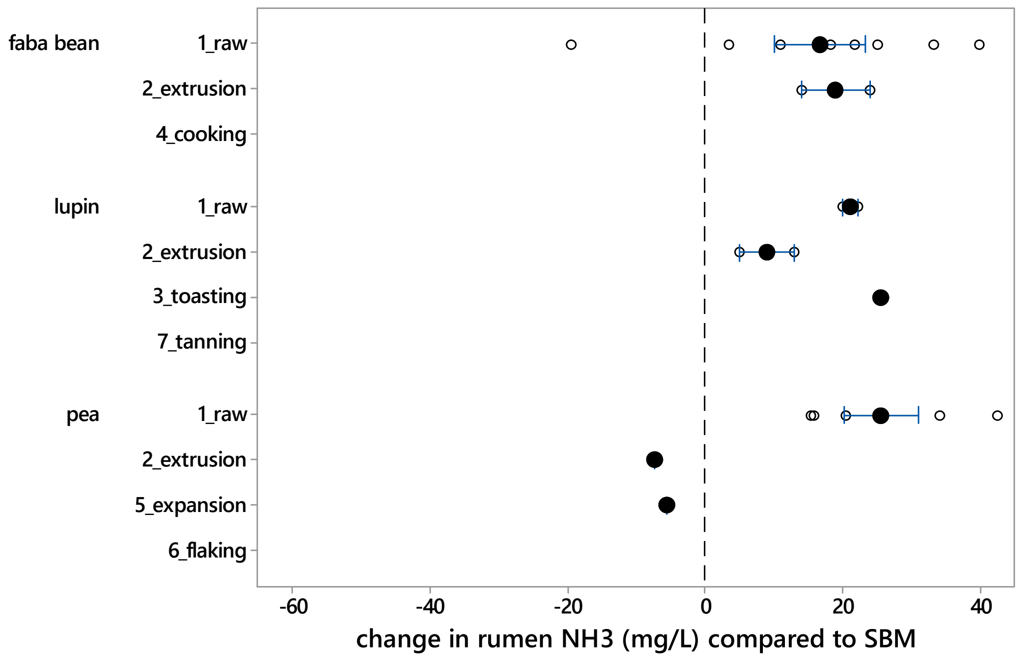


Fig 5. The variations of ammonia (NH_3) in ruminal fluid with treated proteaginous seeds (PS) compared to raw PS

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

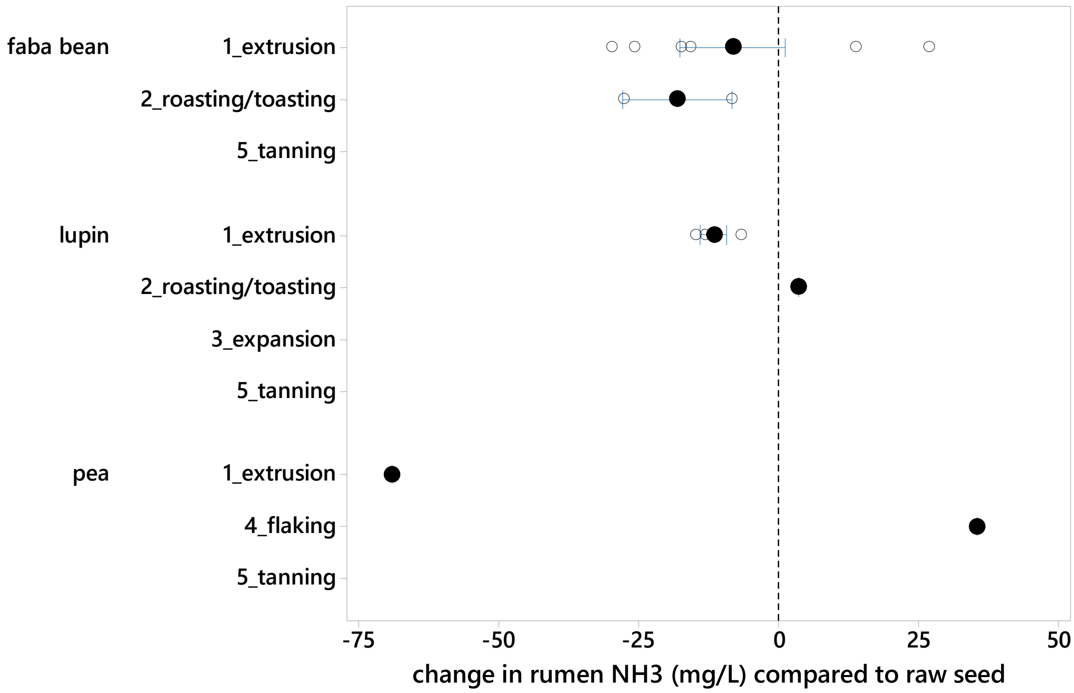


Fig 6. The variations in milk nitrogen secretion in dairy cows fed with raw or treated proteaginous seeds (PS) compared to soybean meal (SBM)

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

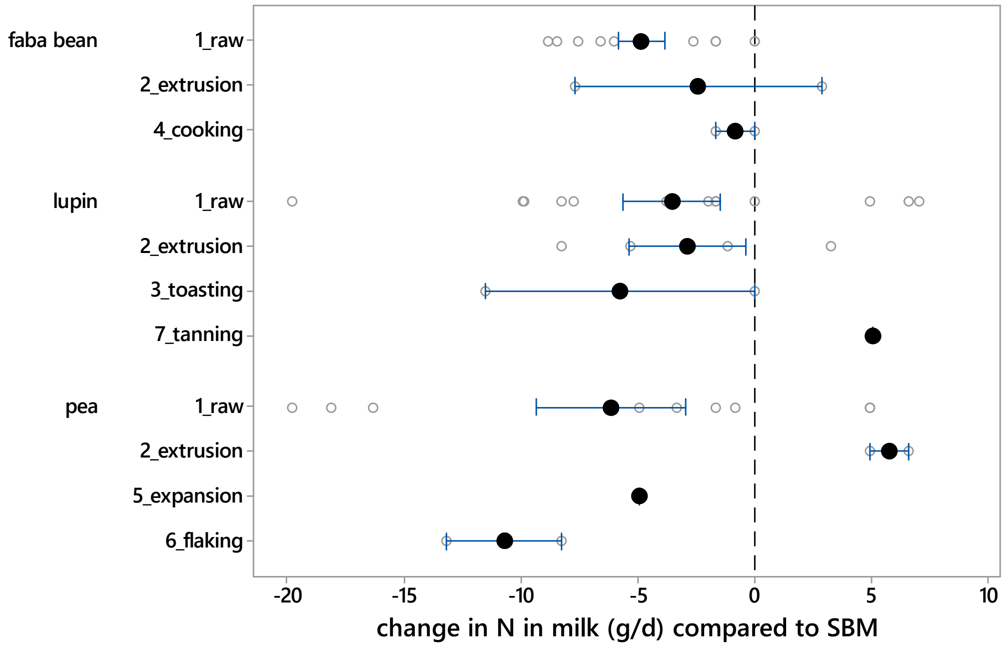


Fig 7. The variations in milk nitrogen secretion in dairy cows fed with treated proteaginous seeds (PS) compared to raw PS

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

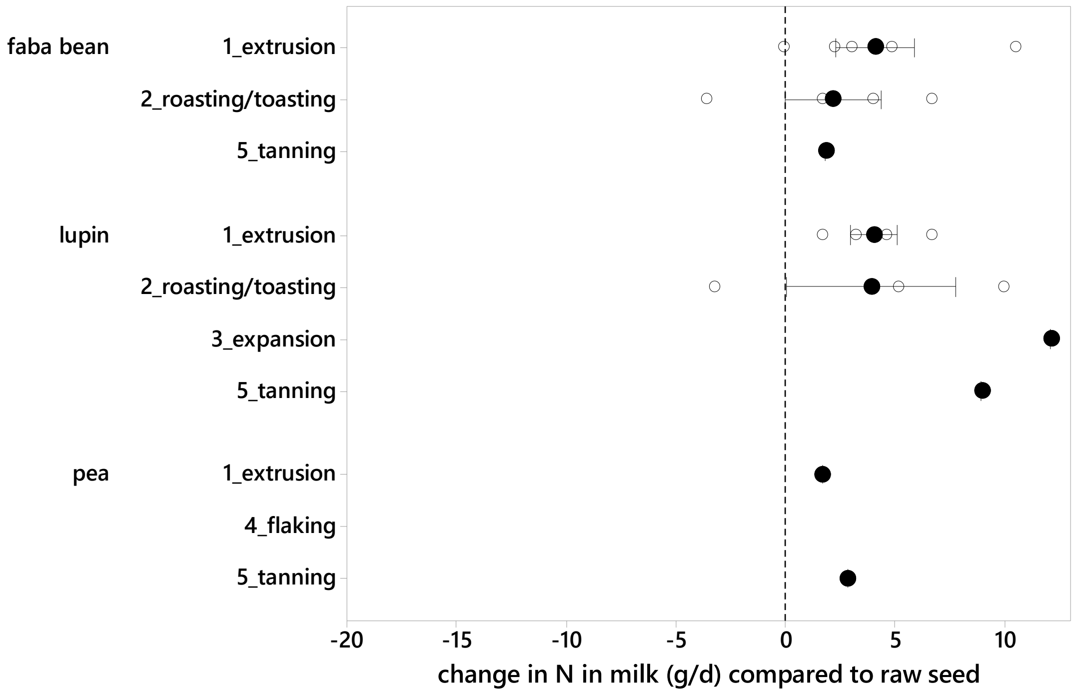


Fig 8. Within-experiment response law of NH_3 in ruminal fluid to iso-crude protein substitution of soybean meal (SBM) by raw proteaginous seeds (PS). Y = 126 + 0.75 X; n = 12, n_{exp} = 6, rmse = 6.3; *P* = 0.001. Data corrected for experiment effect, with experiment considered as fixed effect.

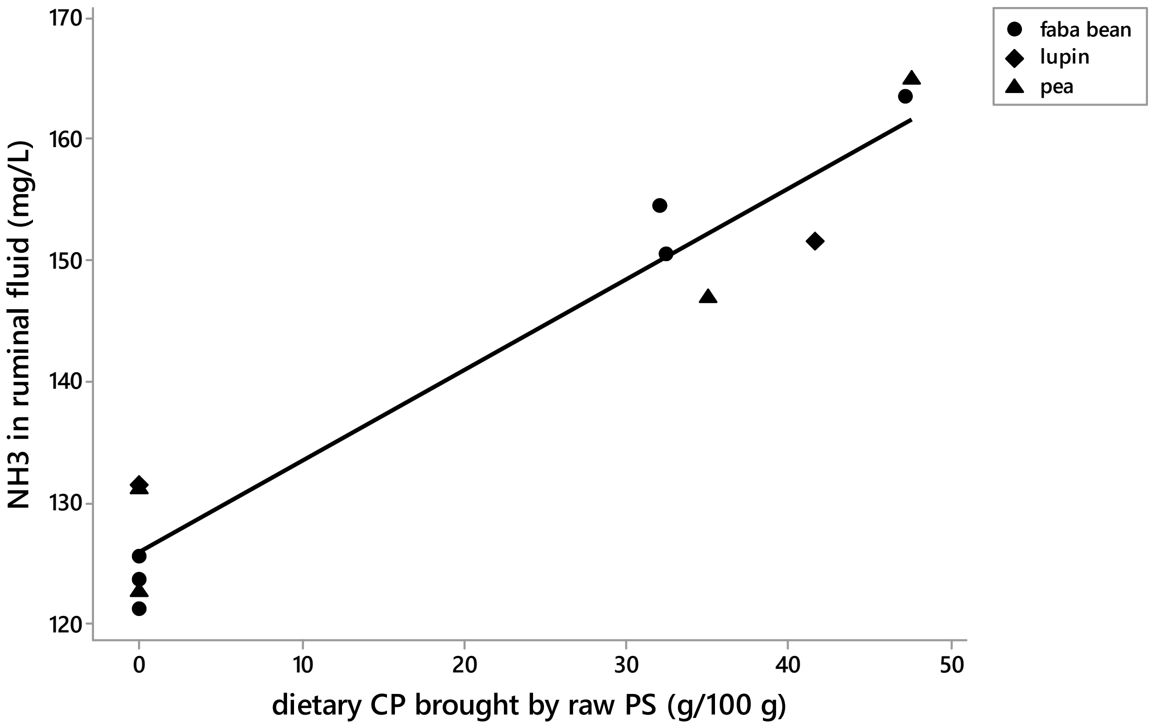
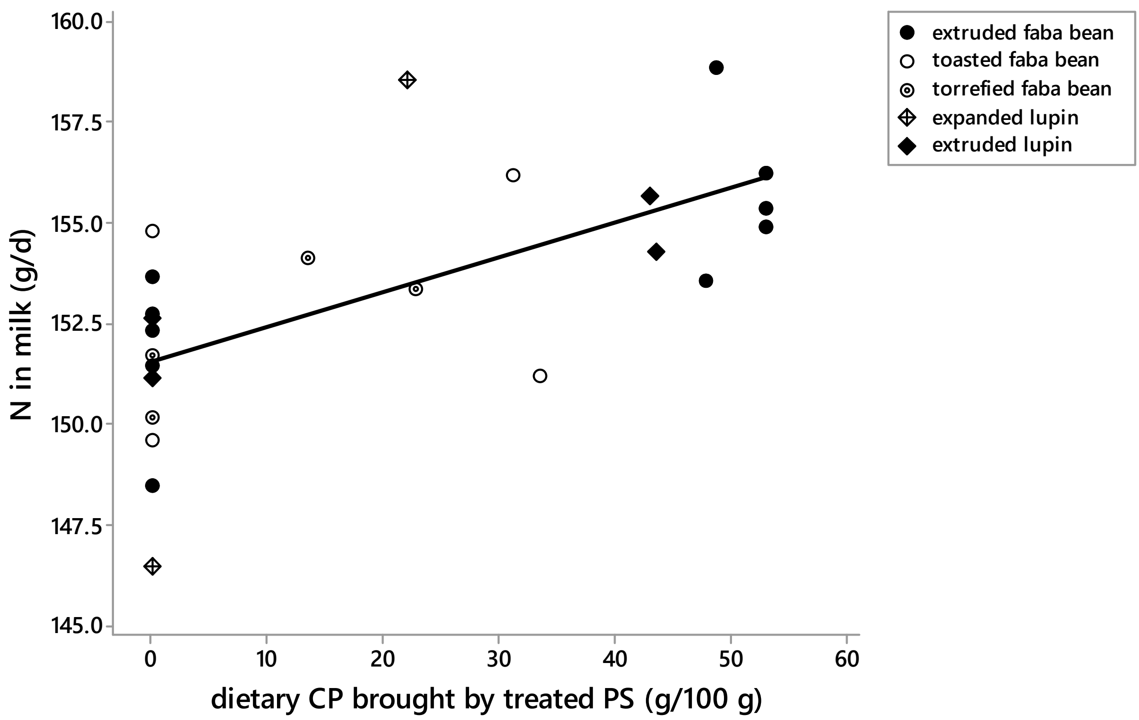


Fig 9. Within-experiment response law of N in milk to iso-crude protein substitution of raw by treated proteaginous seeds (PS). Y = 152 + 0.086 X; n = 24, n_{exp} = 12, rmse = 3.3, *P* = 0.024. Data corrected for experiment effect, with experiment considered as fixed effect.



Supplementary material

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

In situ *database*

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