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Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants

M. Eugène[a], H. Archimède[a,*], D. Sauvant[b]

[a] *INRA, Unité de Recherches Zootechniques Prise d'eau, Petit-Bourg, 97170 Guadeloupe, French West Indies*
[b] *INRA-INA PG, Unité de Physiologie de la Nutrition et Alimentation 16 rue Claude Bernard, 75005 Paris, France*

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Abstract

A quantitative meta-analysis was applied on 90 publications and 169 comparisons dealing with defaunation of the rumen (removal of protozoa from the rumen) in order to point out the major quantitative effects of defaunation and identify interfering factors. Generally speaking defaunation significantly ($P<0.01$) increased average daily gain (11% on average, 64 trials) but did not affect dry matter intake. As a consequence, defaunation improved feed conversion efficiency ($P<0.05$). These different effects were especially obvious as diets were both high in forage and low in nitrogen and as animals had a low growth potential. Defaunation significantly ($P<0.001$) decreased organic matter digestibility (-1.7 units, 52 trials) and cell wall carbohydrate digestibility (-5.7 units, 15 trials). The same trend was observed for rumen digestibility. On the other hand, duodenal nitrogen flow, expressed as a ratio of nitrogen intake, was enhanced by defaunation ($P<0.001$). Duodenal microbial nitrogen flow, expressed as the percentage of live-weight, increased by 21% after defaunation ($P<0.05$). The latter two results may account for the lower ammonia concentration in the rumen (-50.3 mgNH_3/l, 75 trials) and the higher microbial synthesis efficiency (11.8 g microbial N/kg OMDr 19 trials) observed for the ciliate-free animals ($P<0.001$). The level of concentrate in the diet strongly interfered with the effect of defaunation on the pH in the rumen. pH in the rumen was increased by defaunation with diets containing a high level of concentrate (PCO $\geq$ 50%), whereas it decreased with diets containing a low level of concentrate. The molar proportion of propionic acid was enhanced by defaunation, whereas the molar proportion of butyric acid was lowered ($P<0.01$) by defaunation, in the ruminal pool of volatile fatty acids (VFA). Finally, defaunation increased the ruminal volume and the liquid phase outflow rate ($P<0.05$), but to a lesser extent. We consider that these results strongly suggest a more efficient use of nutrients in ciliate-free animals, especially when they are given poor diets limiting animal production.

Keywords: Meta-analysis; Defaunation; Ruminants; Animal production; Digestion; Intake

1. Introduction

Domestic ruminants can valorise fibrous vegetal resources unused by men and other livestock animals. As primary consumers and transformers of this plant biomass, they represent an important source of animal protein for men. The microflora and microfauna play an important role in this process. The main role of this complex microbial ecosystem is to hydrolyse and ferment cell wall carbohydrates into available nutrients for the hosts they inhabit (Hungate, 1966). Protozoa can use most of the soluble carbohydrates,

*Corresponding author. Tel.: +33-590-255-943; fax: +33-590-255-936.
E-mail address: archi@antilles.inra.fr (H. Archimède).

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starch and cell wall carbohydrates as carbon and energy sources, after hydrolysis and fermentation in the rumen (Fonty et al., 1995). Dietary protein and bacteria are the main protein sources of protozoa which are degraded partly into AA and ammonia which is secreted into urea via urine, to be incorporated into their own proteins.

Many studies have tried, for many decades, to specify the interactions between the microbial population of the reticulo rumen (Hungate, 1955). Studies on defaunation of the rumen are hard to handle especially because protozoa are difficult to cultivate in vitro and defaunated animals are not easy to obtain and maintain without protozoa. As far as protozoa are concerned, in vivo studies have privileged the analysis of the differences of digestion and performances between conventional (faunated) and ciliate-free animals. Despite numerous studies on the effects and the zootechnical interest of defaunation (Nolan et al., 1981; Bird and Leng, 1985; Bird, 1989; Jouany and Ushida, 1998) some conclusions remain unclear. The main reviews on the topic used a qualitative approach to analyse published data (Demeyer and Van Nevel, 1979; Veira, 1986; Bird, 1989; Jouany, 1991; Williams and Coleman, 1992). The aim of the present work is to achieve a quantitative review of the published results of defaunation effects, with more data and especially using the meta-analysis which is a statistical method (St-Pierre, 2001).

2. Material and methods

Ninety publications, comprising 169 comparisons, were compiled into a database. In order to be integrated into this database, data on production (animal growth, milk or wool production) and/or data on digestion were obtained on defaunated and conventional animals in similar diets and environments. Most of the trials (60%) dealt exclusively with digestion and 40% dealt with production data (animal growth) and sometimes digestive information. All three animal species, cattle, sheep and goat, were represented in studied animals. Most of the animals were ovine males (80%), with few goats used. Growing animals represented 64% of the overall total. Generally, for digestion trials adult animals were chosen (36%) with stable ruminal flora and fauna.

Diets studied were diversified. Most of them (81%) were mixed diets, that is to say composed of forage supplemented with concentrates. Seven percent of the diets were only composed of concentrate whereas 12% were only composed of forage. Forages used were distributed as follows: hay (37%), straw (26%), roughage by-products (19%, corn cobs, oaten chaffs ...), pasture (13%), silage (6%). Concentrates were composed of common cereals (33%, corn, barley, wheat), proteaginous and oleaginous seed (21%, soya, groundnut, peas), cereal by-products (6%, bran, soja meal) and of less usual supplements (40%, molasses, milk, urea, amino-acid infusion).

Detergents (64%, Manoxol OT, alkanates, teric GN9, calcium peroxide, copper sulfate), ruminal manipulation (11%, emptying the rumen), natural dietary compounds (9%, plant saponin, concentrate given ad libitum, milk, vegetable oil) and isolation of new born animals from their mother (16%) were used in order to achieve defaunation of the animals. Trials where the effects of additives, other than defaunating, were significant, were discarded.

The average daily gain (ADG, g/d) and the wool production (g/d) were selected as the two major production parameters (Table 1). For the study of intake, the dry matter intake (DMI, g DM/d), the nitrogen intake (NI, gN/d) were selected. To be compared, the results were expressed as a percentage of live-weight (%LW). Feed conversion efficiency (FCE) was calculated (DMI/ADG, g/g), for trials where DMI differences were observed between defaunated and faunated animals. Usual analyses of the diet determined were: dry matter (DM), organic matter (OM), total crude protein ($CP = N \times 6.25$) contents of the diets and cell wall constituents, NDF, ADF and ADL.

Digestion parameters selected were OM total tract digestibility (DtOM) or OM ruminal digestibility (DrOM), cell wall total tract digestibility (DtNDF) or ruminal digestibility (DrNDF) and nitrogen total tract digestibility (DtN). Fermentative parameters of the rumen were volatile fatty acids concentrations (VFAs, mM), ammonia concentrations (NH_3, mgN/l), and pH. Duodenal nitrogen daily flows considered were non ammonia nitrogen (Duo.NAN, gN/d), microbial nitrogen (Duo.MN, gN/d). Urinary and faecal nitrogen daily flows were also considered (Table 2).

Table 1
Effects of defaunation of the rumen on growth, intake and digestion in ruminants. Mean values of parameters analysed in the bibliographic database (Part I)

Parameters	Nb data	Mean		S.D.	Refs.[c]
		Defaunated	Faunated		
Diet characteristics on DM[a]					
Organic matter (OM, %)	222	90.6	90.62	3.24	1–8;11–27;31;33–35;38–40;42–44; 48;49;51–57;60;62;66;68–74; 77–81;83–88
Crude protein (CP = N × 6.25, %)	328	14.17	14.18	4.01	1–90
Neutral detergent fiber (NDF, %)	306	45.69	45.69	16.5	1–90
Percentage of concentrate (PCO, %)	328	46.14	46.16	32.74	1–44;46–49;51–57;60–90
Production, on LW[b]					
Average daily gain (ADG, %)	128	0.444	0.401	0.319	1–4;6–9;11–34;42–44;85
Daily wool growth (Wool, %)	56	6.23	5.48	2.66	7–9;10–12;23;38;39;42–44
Intake, on LW					
Dry matter intake (DMI, %)	294	2.53	2.51	1.02	1–4;6–9;11–44;46;48–52; 54–56;60–74;76–90
Organic matter intake (OMI, %)	232	2.29	2.28	0.89	1–4;6–9;11–35;38–44; 51–55;57–74;76–90
Nitrogen intake (NI, %)	264	6.02	6.02	4.01	1–4;6–57;60–90
Digestibilities					
OM total tract digestibility (DtMO, %)	104	65.89	67.54	11.83	11;12;16;17;27;31–38;48; 52–61;66–78;81;83;87;90
NDF total tract digestibility (DtNDF, %)	30	55.37	61.09	6.70	16;27;31;48;54;66;69; 71;74;78;81;83;87
Nitrogen total tract digestibility (DtN, %)	76	68.68	69.92	9.71	16;27;31;35;48;53;54;56;58; 60–62;66–78;87;89;90
OM ruminal digestibility (DrMO, %)	38	41.58	48.21	8.39	12;48;49;60;62;68; 70–72;77;78;80–82;90
NDF ruminal digestibility (DrNDF, %)	22	46.77	51.90	9.39	48;51;54;60;66;71;77;79;81

[a] DM: dry matter.
[b] LW: Live weight (kg).
[c] Refs.: see References used in the database.

Table 2
Effects of defaunation of the rumen on growth, intake and digestion in ruminants. Mean values of parameters analysed in the bibliographic database (Part II)

Parameters	Nb data	Mean		S.D.	Refs.[a]
		Defaunated	Faunated		
Nitrogen flows					
Duodenal non-ammonia nitrogen (Duo.NAN, gN/d)	44	31.16	26.89	14.66	27;48;49;53;60;62;66;68; 70–72;77;78;80–90
Duodenal microbial nitrogen flow (Duo.MN, gMN/d)	38	17.58	15.75	8.71	48;49;60;62;66;68;70–72; 77;78;80;89;90
Urinary nitrogen flow (Nu, gN/d)	16	27.59	31.77	22.87	31;66;73;78;90
Fecal nitrogen flow (Nfec, gN/d)	64	13.00	12.67	14.99	16;17;27;31;35;48;53–56; 60–62;66–71;73–78;87–90
Digestion in the rumen					
Ruminal pH (pH unit)	88	6.28	6.33	0.32	2;14;17;19;26;27;32;36;40; 48–55;61–69;72–84;86–90
Volatile fatty acids concentration (VFA, mM)	136	85.90	89.15	18.19	1;12;18–36;40–57;60–90
Acetate molar proportion (C2, %)	118	62.78	64.22	8.62	1;6;19–27;32;33;37;40;44–57;61–90
Propionate molar proportion (C3, %)	120	23.05	20.20	5.99	1;6;19–27;32;33;37;40;44–57;61–90
Butyrate molar proportion (C4, %)	116	10.94	12.57	5.49	1;6;19–27;32;33;37;40;44–57;61–90
Ammonia concentrations (NH_3, mgN/l)	150	117.36	167.65	69.33	1;13–22;27–39;40–48; 51–57;60–72;74–90
Microbial synthesis efficiency (MSE, g MN/kg OMDr)	38	39.96	28.14	13.29	27;48;49;60;62;66;68;70; 71;77;78;80;90
OM apparently degraded in the rumen (OMDr, g)	38	442	506	451	12;48;49;60;62;68;70–72; 77;78;80–82;90
Ruminal volume and turn-over					
Ruminal fluid volume (Vr, %LW[b])	34	11.12	10.46	1.49	41;51;54;55;57;60;62;
Fractional turn-over rate					70;78–81;83;88;90
Liquid phase (Kl, %/h)	36	8.19	8.26	2.65	41;54;55;57;62;70;78–80;83;87;88;90
Solid phase (Kp, %/h)	30	5.36	5.17	2.07	41;54;55;60;62;66;70;79;83;87;88

[a] Refs.: see References used in the database.
[b] LW: live weight (kg).

As the experimental procedure was unbalanced due to the gathering of various data in the database, the interpretation of data has favoured the comparison two by two of parameters studied for defaunated and faunated animals given the same diet. In a first step, systematic graphical analysis has allowed to put in evidence the global relationships observed between all the literature data. A primary comparison of results between defaunated and faunated animals on the same trial, considered as blocks, was conducted, by analysis of variance using GLM (Minitab).

$$Y_{ij} = \mu + E_i + D_j + e_{ij} [\text{model 1}]$$

Y_{ij} is the observed character in trial i, μ the global mean, E_i the effect of the trial i, D_j the defaunation effect within a trial i (1 DF), and e_{ij} represents the unexplained residual error.

When another measured character (X) appeared to interfere with the defaunation effect, it was introduced as a covariate using GLM, following model 2:

$$Y_{ij} = \mu + E_i + D_j + aX_{ij} + e_{ij} [\text{model 2}]$$

a is the regression coefficient of Y on the covariable X; all other symbols are as defined in model 1.

In order to be more specific and to better interpret interactions between some factors, analyses were also conducted on the outcome for measured variable Y according to the following model 3:

$$Y_{ij} = \mu + E_i + a_j X + e_j [\text{model 3}]$$

Y_{ij} is the difference between parameter Y for defaunated and faunated groups observed in trials i, respectively. Thus, it is considered as the response to defaunation (Res.); μ is the global mean, E_i the effect among studies i, X is the covariable, a_j is the regression coefficient of Y on X and e_j represents the unexplained residual error.

3. Results

3.1. Effect of defaunation on animal growth

Mean average daily gain measured in the database, expressed as a percentage of live-weight (ADG ± s.d. %LW), was 0.4223 ± 0.3187 %LW. The first analysis conducted, considering available data from 64 trials, revealed a significant increase in average daily gain ($P < 0.01$) after defaunation (0.444 vs. 0.401, s.e.m. = 0.095 %LW). A similar effect ($P < 0.01$) was observed when average daily gain was expressed as g/kg of metabolic weight (ADG/MW = 10.67 vs. 9.68, s.e.m. = 2.15 g/kg MW).

A second analysis was conducted, relating the growth with the level of dry matter intake (DMI %LW = 3.22 ± 0.98 %LW). Fig. 1 indicates intra and global relationships between ADG %LW and DMI %LW. A significant effect of the covariate DMI %LW, with a regression coefficient of 0.198 ± 0.023 %LW was observed. In that case the effect of defaunation still remained significant and of similar amplitude (0.440 vs. 0.404, s.e.m. = 0.082 %LW, $P < 0.02$).

A third analysis of residual variations of the previous model was conducted in order to point out other possible interfering factors. Two characteristics of the diet interfered significantly, the mean percentage of concentrate in the trials (PCO = 38.43 ± 28.61%) and the mean percentage of nitrogen content/neutral detergent fiber content in the diet (N %NDF = 6.40 ± 4.18%). Hence, an analysis was conducted with trial, defaunation and defaunation interaction with PCO as qualitative factors, and integrating DMI %LW and PCO as covariates into the model. All the effects (trial, defaunation, defaunation × PCO, DMI %LW), except PCO effect ($P = 0.09$), were highly significant ($P < 0.001$), s.e.m. was 0.073 %LW. In that case, the regression coefficient of covariate DMI %LW was 0.211 ± 0.034 %LW and that of PCO was 0.090 ± 0.053% versus 0.092 ± 0.053% for defaunated and faunated groups respectively. This result indicates that the positive effect of defaunation on average daily gain was more pronounced with diets containing high forage levels. Another analysis was conducted with trial, defaunation and defaunation interaction with N %NDF ratio as qualitative factors, and integrating DMI %LW and N %NDF ratio as covariates into the model. All the effects (trial, defaunation, defaunation × N %NDF, DMI %LW), except N %NDF ratio effect ($P = 0.42$), were highly significant ($P < 0.001$), s.e.m. was 0.073 %LW. In that case, the regression coefficient of covariate DMI %LW was 0.226 ± 0.036 %LW and that of N %NDF ratio was − 0.167 ± 0.194% versus − 0.160 ± 0.194% for defaunated and faunated

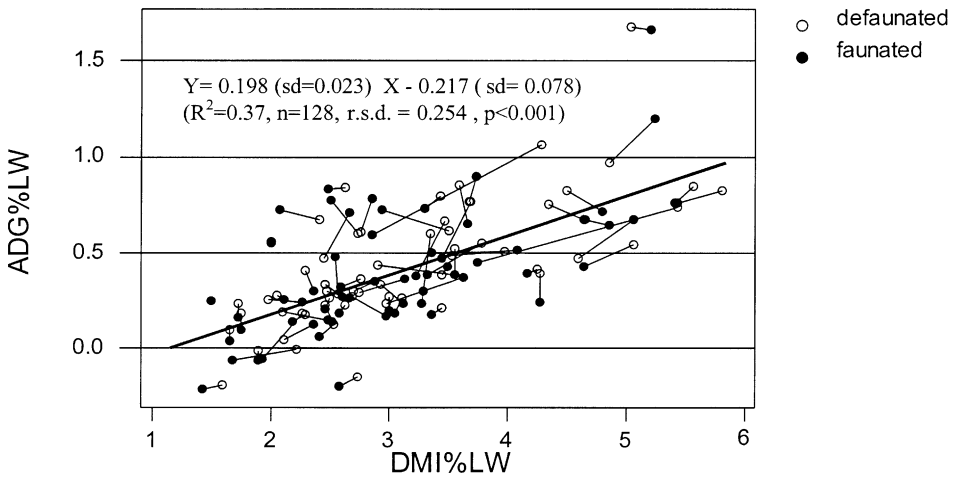

Fig. 1. Effect of defaunation on average daily gain (ADG %LW) of ruminants in relation to dry matter intake (DMI %LW). Synthesis of the literature data. LW: large weight.

groups respectively. The effect of defaunation still remained comparable to previous approaches and was 0.035 (0.440 vs. 0.405 %LW). As there is a significant interaction between defaunation and the N %NDF ratio effect, this result indicates that the effect of defaunation is more obvious for diets low in nitrogen compared to cell wall contents (NDF). To sum up these interactions, a multiple regression of the response to defaunation ('defaunated–faunated') of average daily gain %LW on either the response of DMI %LW, PCO and N %NDF ratio was calculated:

$$\text{Response ADG\%LW} = 0.128 - 0.0074\text{N\%NDF} - 0.0013\text{PCO} + 0.213$$

$$\text{Response DMI\%LW} \quad (n = 62, R = 0.68, \text{r.s.d.} = 0.099)$$

All the coefficients were significant, except for N %NDF ratio ($P = 0.08$). Fig. 2 indicates that the prediction of the response of ADG was well correlated to the observed response, however the slope is significantly different from 1.

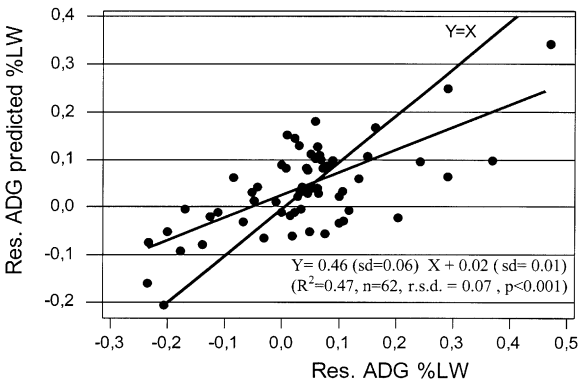

Fig. 2. Prediction of average daily gain response to defaunation (Res. ADG %LW predicted) in relation to dry matter intake (DMI %LW), percentage of concentrate (PCO) and nitrogen over energy ratio (N %NDF) in the dietary regimen. Synthesis of the literature data. Res. ADG %LW: observed response.

3.2. Effect of defaunation on voluntary intake

An analysis of variance of DMI %LW was conducted, considering only trials where intake was not limited and where there was a difference between faunated and defaunated groups (83 among 146 trials). Even with these trials no effect of defaunation on voluntary dry matter intake appeared ($P = 0.37$).

3.3. Effect of defaunation on feed conversion efficiency

Feed conversion efficiency (FCE = 8.44 ± 4.02 g/g, $n = 115$) was reduced by 1.41 g/g (7.78 vs. 9.19 g/g) after defaunation. This effect of defaunation was

more pronounced with trials where ADG %LW was low, especially when it was less than 0.5 %LW (Fig. 3). There was no difference between ovine and bovine responses to defaunation.

3.4. Effect of defaunation on wool production

An analysis of variance of wool production was conducted with 28 trials. A highly significant increase ($P<0.001$) of this production appeared after defaunation (6.23 vs. 5.48, s.e.m. = 0.56 g/j). Furthermore, response of wool production (Res. Wool) is higher with high level of DM intake and low level of N %NDF ratio ($P=0.003$):

$$\text{Res.Wool} = 0.36 + 0.46\text{DMI\%LW} - 0.130\text{N\%NDF}$$
$$(n = 25, R^2 = 0.77, \text{r.s.d.} = 0.41).$$

3.5. Effect of defaunation on feed digestibility and digestive flows

3.5.1. Total tract digestibilities

An analysis of variance integrating 52 trials was applied to total tract OM digestibility (DtOM = 66.7 ± 11.8%), total tract cell wall digestibility (DtNDF = 58.2 ± 6.7%) and total tract nitrogen digestibility (DtN = 66.9 ± 9.7%). Analysis of the 52 trials led to the conclusion that there was a significant depression ($P=0.001$) of DtOM by 1.7 units after defaunation (65.9 vs. 67.5, s.e.m. = 2.3%). The trials indicating DtNDF values were less numerous (15), nevertheless, depression of DtNDF due to defaunation appeared to be more important (5.7 units) than that of DtOM and significant ($P<0.001$, 55.4 vs. 61.09, s.e.m. = 2.6%).

When DtOM and DtNDF were measured simultaneously ($n=15$), DtOM appeared to decrease by 0.26 ± 0.13 unit, while DtNDF dropped by 1 unit. Only a tendency ($P=0.07$) towards the decrease in DtN appeared after defaunation (38 trials, 68.68 vs. 69.92, s.e.m. = 2.9%). Examination of residual variations of these different models revealed no major interfering factors with feed digestibility.

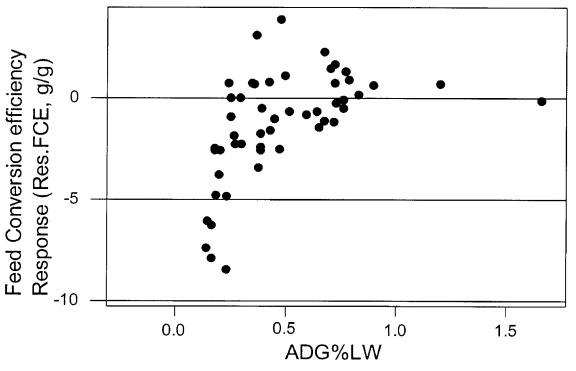

Fig. 3. Defaunation and average daily gain (ADG %LW) effects on the response of feed conversion efficiency (Res. FCE) of ruminants. Synthesis of the literature data.

3.5.2. Feed digestibility in the reticulo-rumen

An analysis of variance was applied to apparent OM digestibility in the rumen (DrOM = 66.7 ± 11.8%), and apparent cell wall digestibility in the rumen (DrNDF = 49.3 ± 9.4%). The analysis of DrOM through 19 trials led to the conclusion that there was a significant decrease ($P=0.001$) of DrOM by 7.3 units after defaunation (41.6 vs. 48.9, s.e.m. = 3.1%). Trials indicating DrNDF values were less numerous (12). Depression of DrNDF due to defaunation appeared nearly significant ($P<0.07$) and lower (4.6 units) than that of DrOM (46.8 vs. 51.4, s.e.m. = 5.1). For these trials the percentage of OM digestible in total tract which is degraded in the rumen was significantly lower ($P=0.002$) for defaunated animals (62.3 vs. 70.5, s.e.m. = 5.1%).

3.5.3. Duodenal, urinary and faecal nitrogen outflows

For all the statistical analyses on duodenal nitrogen outflows, nitrogen intake, expressed as %LW, was taken into account as a covariate in statistical models, except for duodenal outflows of nitrogen expressed as %NI (Duo.N %NI).

An analysis of variance of 21 trials with duodenal non ammonia nitrogen outflow values (expressed as %LW) was performed. Defaunation induced a significant ($P<0.001$) increase in the latter (0.047 vs. 0.040 ± 0.003 %LW). Duo.N %NI ($n=44$, Duo.N %NI = 103.39 ± 24.67%) significantly increased ($P<0.001$, 22 trials) after defaunation (111.3 vs. 91.8, s.e.m. = 9.8%). Twenty-two trials have allowed us to calculate duodenal crude protein/kg DMI out-

flows ($n=44$, Duo.CP = 143.2 ± 38.2 g/kg MSI). Defaunation significantly ($P<0.001$) increased this flow (152.4 vs. 129.8, s.e.m. = 8.3 g/kg DMI). Examination of residual variations of these different models revealed no major interfering factors with digestibilities.

Among the 22 trials mentioned above, 18 indicated both duodenal non ammonia and microbial nitrogen outflows. The analysis of the latter trials indicated that defaunation induced a significant ($P=0.007$) increase in duodenal microbial nitrogen outflow (0.028 vs. 0.023, s.e.m. = 0.004 %LW). The ratio of duodenal microbial nitrogen outflow/duodenal non ammonia nitrogen outflow was not affected by defaunation (59.58 ± 2.4%, $P=0.910$).

The analysis of the few trials (8) indicating urinary nitrogen excretion enabled to point out a meaningless decrease ($P=0.079$) of the latter after defaunation (0.016 vs. 0.019, s.e.m. = 0.001 %LW). On the contrary, defaunation induced a highly significant ($P=0.003$, $n=30$) increase in faecal nitrogen excretion (0.015 vs. 0.014, s.e.m. = 0.007 %LW).

3.6. Fermentations in the rumen

3.6.1. Acidity in the rumen

The 68 trials concerning VFA allowed to point out a significant decrease ($P=0.024$) of VFA concentration in rumen juice (85.9 vs. 89.1, s.e.m. = 8.2 mM) for defaunated animals. Furthermore, an analysis of the residuals of the previous statistical model indicated that the DMI level interfered with the effects of defaunation on the decrease in VFA concentrations. The decrease in VFA concentration is more marked with trials in which the level of intake was high (about superior to 3 %LW; Fig. 4).

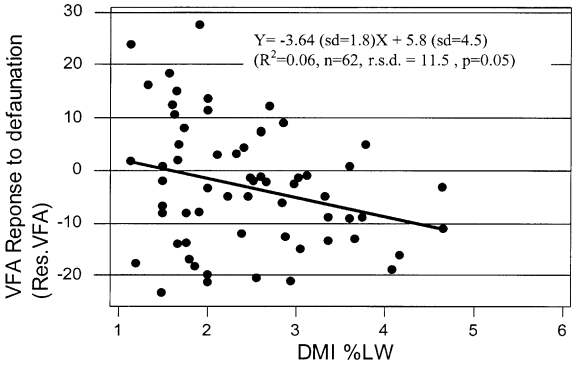

Fig. 4. Defaunation and dry matter intake influences upon volatile fatty acids concentration (VFA) in the ruminal fluid. Synthesis of the literature data.

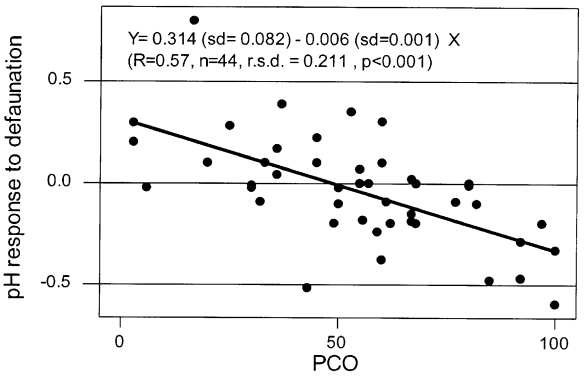

Fig. 5. Effect of the percentage of concentrate (PCO) on the response of pH to defaunation (Res.pH). Synthesis of the literature data.

No significant effect of defaunation on pH in the rumen was observed (6.28 vs. 6.33, s.e.m. = 0.16) after analysis of 44 concerned trials whereas defaunation significantly ($P<0.009$) decreased pH, when VFA accounted as a covariate in the model (5.6 vs. 6.2, s.e.m. = 0.15). Moreover, a highly significant interaction appeared between the response of pH to defaunation (Res.pH, $P=0.001$) and the percentage of concentrate in the diet. There is a significant negative regression link between the latter two parameters (Fig. 5).

$$\text{Res.pH} = 0.31 - 0.006\ \text{PCO}$$
$$(n = 44, R^2 = 0.34, \text{r.s.d.} = 0.21)$$

With low levels of concentrate, the acidity in the rumen decreased after defaunation, whereas the inverse effect appeared with high levels of concentrate. This response of pH is also negatively and logically linked to the response of VFA to defaunation. As a consequence the above equation can be significantly enhanced:

$$\text{Res.pH} = 0.17 - 0.005\ \text{PCO} - 0.005\ \text{Res.AGV}$$
$$(n = 34, R^2 = 0.41, \text{r.s.d.} = 0.18).$$

3.6.2. Molar proportions of the VFAs

Defaunation induced a decrease in the molar proportion of acetate in the rumen (%C2, $P = 0.08$) after analysis of 59 concerned trials (62.8 vs. 64.2, s.e.m. = 4.4%). A significant increase in the molar proportion of propionate in the rumen by 2.85 units ($P = 0.006$) was observed after defaunation (%C3 = 23.05 vs. 20.20, s.e.m. = 5.43%). Defaunation induced a significant decrease by 1.63 units ($P = 0.009$) of the molar proportion of butyrate in the rumen after defaunation (%C4 = 10.94 vs. 12.57, s.e.m. = 3.23%) as was shown by analysis of the 57 concerned trials.

Average VFA concentration ratios of the fermentation pathway C2/C3, C2 + C4/C3 and C3/C4 were calculated. The analysis of the 58 concerned trials, integrating the significant covariate DMI %LW ($P = 0.005$), concluded that there was a significant decrease ($P = 0.001$) of C2/C3 ratio (2.96 vs. 3.49, s.e.m. = 0.72) after defaunation. A similar decrease ($P = 0.040$) was observed for 53 trials where C2 + C4/C3 ratio was available (3.40 vs. 4.20, s.e.m. = 0.95). As concerning C3/C4 ratio, a highly significant ($P = 0.001$) increase appeared after defaunation (2.65 vs. 1.82, s.e.m. = 1.1).

Total concentration and molar proportion of VFA allowed us to calculate the different concentrations of each VFA. Acetate and propionate concentrations in the rumen juice were not affected by defaunation. Butyrate concentration was significantly reduced ($P = 0.001$) after defaunation (52 trials, 8.3 vs. 9.9, s.e.m. = 2.5 mmol/l).

3.6.3. Ammonia concentration

For faunated and defaunated groups, respectively, a global positive and inter-study relationship appeared between NH_3 concentration in the rumen and percentage of total crude protein in the diet (CP%DM).

$$\text{Faunated: } NH_3 = 30.9 + 9.7\ \text{CP \%DM}$$
$$(n = 75, R = 0.49, \text{r.s.d.} = 62.8)$$

$$\text{Defaunated: } NH_3 = 8.4\ \text{CP \%DM}$$
$$(n = 75, R = 0.93, \text{r.s.d.} = 47.9)$$

A highly significant ($P < 0.001$) decrease in NH_3 in the rumen juice by 50.3 mgN/l appeared after defaunation (117.4 vs. 167.7, s.e.m. = 30.0 mgN/l), as indicated by analysis of the 75 concerned trials. Taking the previous relationship into account, defaunation would approximately account for a 5 units increase in the diet crude protein content. No covariate had a significant effect on ammonia in the rumen. However, the effect of defaunation on NH_3 decreased with diets low in CP %DM contents. Thus for 9 trials, with CP %DM contents inferior to 10 %DM, NH_3 adjusted values were 58.3 and 94.3 mgN/l for defaunated and faunated groups respectively, a difference of 36.0 mgN/l. Fig. 6 indicates that the NH_3 concentration of the rumen juice of defaunated animals is equal to 2/3 of that of faunated animals.

3.7. Microbial growth

Microbial synthesis efficiency (M.S.E., expressed in g microbial N (MN)/kg OM apparently digested in the rumen (OMDr)) significantly ($P = 0.001$) increased after defaunation (19 trials, 39.96 vs. 28.14, s.e.m. = 6.26 g MN/kg OMDr). Amongst the 19 trials, 15 indicated both duodenal microbial nitrogen flow and M.S.E. For these 15 trials defaunation significantly ($P < 0.001$) increased duodenal microbial nitrogen flow (15.5 vs. 11.8, s.e.m. 1.9 g MN/d). In parallel with that, analysis of variance of OMDr (474 ± 451 g) conducted with the same 15 trials, led to the conclusion that there was a significant decrease in OMDr after defaunation (343 vs. 400, s.e.m. = 27 g). The latter two results account for ($P < 0.001$) M.S.E. increase after defaunation (44.7 vs. 30.0, s.e.m. = 5.1 g MN/kg OMDr).

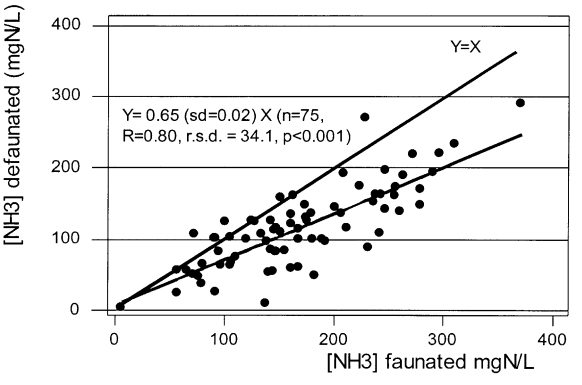

Fig. 6. Effect of defaunation on ammonia concentration (NH_3) in ruminal fluid. Synthesis of the literature data.

3.8. Ruminal volume, solid and liquid phase turn over rates in the rumen

DMI %LW was taken into account as a covariate in all analyses of variance related to ruminal fluid volume and turn-over rates. To make the results comparable, ruminal fluid volume was expressed as %LW (Vr = 8.7 ± 1.2 %LW). An analysis of variance of 17 trials indicated an increase ($P = 0.12$) in the ruminal liquid volume after defaunation (11.1 vs. 10.5, s.e.m. = 1.1 %LW). Fractional turn-over rate in the rumen of the liquid phase (Kl = 8.2 ± 2.6 %/h) and solid phase (Kp = 5.3 ± 2.1 %/h) were measured in 18 and 15 trials respectively. Those two parameters were positively correlated when simultaneously measured ($R^2 = 0.43$, $n = 22$). Kl slowly but significantly ($P = 0.021$) increased after defaunation (8.4 vs. 8.3, s.e.m. = 0.7 %/h) whereas Kp's increase was not significant ($P = 0.527$) after defaunation (5.4 vs. 5.1, s.e.m. = 1.0 %/h). On the other hand, response of Kp to defaunation was significantly and negatively correlated to the response of ruminal volume ($Y = 0.58 - 0.63\ X$; $n = 12$, $R^2 = 0.50$, r.s.d. = 1.1 %/h). This relationship indicates that particle flow tends to remain constant within a similar trial and that variations of these two parameters correspond to compensatory phenomena between ruminal volume and turn-over rate.

4. Discussion

Meta-analysis confirmed most of the reported results of defaunation and brought to light original conclusions on subjects which were still conflicting. Furthermore, this work contributed to reveal interfering factors and statistical relationships, which could be useful to elaborate or validate mechanistic ruminal digestion models. Such a meta-analysis appears to be more powerful than a single study to reveal some new effects of defaunation of the rumen and mainly new interfering factors.

Average daily gain and dry matter intake results explain the better feed coefficient efficiency for the ciliate-free animals. Furthermore, the positive effects of defaunation on average daily gain appeared obvious, especially as diets were poor and average daily gain was low. Bird (1989) thought that the level of concentrate in the diet might influence the outcome of defaunation on live-weight gain. Our results confirmed this hypothesis, and furthermore developed it. Indeed defaunation effects appeared more pronounced when the ratio N %NDF was roughly inferior to 6%, for diets with high level of straw or roughage. In the same way, the effects of defaunation were higher when the percentage of concentrate in the diet was roughly inferior to 35–40%. Nevertheless, results obtained for diets with high levels of concentrate must be carefully analysed. Indeed, providing high levels of concentrate to conventional animals decreased the number of protozoa in the rumen and even removed them all (Eadie et al., 1970), thus no significant differences between animals with reduced fauna and ciliatre-free animals have been observed.

As defaunation has no effect on intake and as ciliate-free animals have lower cell-wall carbohydrate and OM digestibility, absorbed energy is lower than it is in conventional animals. Thus, the better feed conversion efficiency after defaunation may be mainly attributed to the higher yield of metabolic use of absorbed nutrients, for maintenance and growth. It confirms Bird's hypothesis (Bird, 1989). This higher yield may partly be explained by the higher nitrogen and essential AA absorption in the duodenum, as is suggested by the high increase in duodenal nitrogen flow (18%) reported by our results. Increase in duodenal nitrogen flow is explained, above all, by increase in duodenal microbial nitrogen flow, as reported by authors (Demeyer et al., 1982; Ushida et al., 1990). On average, the latter increased by 22% versus 13% for duodenal non microbial nitrogen flow. The economy of dietary protein, suggested by some authors (Rowe et al., 1985; Ushida et al., 1986) was partially confirmed by the analysis of our database, maybe because few data on nitrogen balance were available.

The high efficiency of nutrients metabolic use could also be due to the profile of energetic nutrients in the rumen. Indeed, defaunation undoubtedly decreased VFAs, while the ratio (C2 + C4)/C3 decreased. Jouany et al. (1988) had already hypothesised that defaunation promoted propionate production whatever the diet responsible for the profile of VFAs in the rumen. Increase in the molar proportion of propionate is in favour of a higher metabolic use of the nutrients (Kreuzer et al., 1986), although this hypothesis was not shared by other authors (Ørskov et al., 1979; Demeyer and Van Nevel, 1986). Finally, as fatty acid concen-

tration is high in bacteria, especially for attached bacteria which are more numerous, the increased absorption of long chain fatty acids may partially account for higher metabolic use of absorbed nutrients in ciliate-free animals. Thus Sauvant and Bas (2002) indicate that mean fatty acid concentration of adherent bacteria, free bacteria and protozoa are on average equal to 15–20, 6–10 and 2–4 %DM of the microorganism, respectively.

Our results confirmed that protozoa may prevent the abrupt drop of the pH in the rumen, in some instances, and are in agreement with other results (Chamberlain et al., 1983; Coleman, 1988; Ushida et al., 1991). Furthermore, Williams and Coleman (1992) have reported that lactic acid utilisation by protozoa contributed to prevent the decrease of the pH in the rumen. Ruminal lactate is cleared less effectively from defaunated animals (Chamberlain et al., 1983; Newbold et al., 1986). Besides, we pointed out an interaction between the effect of defaunation on pH and the level of concentrates in the diet. Thus, in diets containing less than 50% of concentrate, ruminal liquid is less acid in ciliate-free animals than in conventional animals. Higher buffering capacities of conventional animals, given diets very rich in concentrate, may be explained as protozoa can store particles, starch grains and other carbohydrates inside their cells and delay the fermentation process.

As reported by numerous authors, ammonia concentrations in the rumen were by far much lower in ciliate-free animals compared to the control ones (Ushida et al., 1986; Ivan et al., 1992; Jouany and Ushida, 1998; Santra and Karim, 2000). Part of these results should, on the one hand, be attributed to higher microbial synthesis, and on the other hand, to less bacterial recycling (Firkins et al., 1998; Koenig et al., 2000) and bacteria proteolysis when protozoa are missing (Coleman, 1975; Ueda et al., 1975; Onodera et al., 1977; Demeyer and Van Nevel, 1979). To a lesser extent, absence of protozoal lysis, which occurs during nycthemeral phases could be responsible for those results (Abou Akkada and Howard, 1962). Furthermore, ruminal dietary degradation is lowered, especially the proteic fraction. In conventional animals, nitrogen availability is higher than the requirements of the present microorganisms. Hence, there is an excess of nitrogen in the rumen of faunated animals. Higher microbial protein production observed in ciliate-free animals shows that part of free nitrogen compounds in excess in ruminal fluid, reported above, is directed towards bacterial cell growth. As observed by Jouany (1996) and Eugène et al. (in press) our analysis shows a lower nitrogen urinary excretion in ciliate-free animals, despite the fact that the differences were not significant. Higher nitrogen efficiency of ruminal ecosystem of ciliate free animals may explain these results. Thus nitrogen microbial nitrogen bioavailability of the diet is higher in ciliate-free animals (Koenig et al., 2000).

Our results demonstrated that the higher microbial synthesis efficiency is due to both a better microbial proteosynthesis (31%) and a lower OM digestion in ciliate-free animals. The increase in net synthesis of N may account for higher microbial synthesis efficiency observed in the rumen of ciliate-free animals (Demeyer and Van Nevel, 1979). Moreover, a decrease in energetic requirements for maintenance of rumen microorganisms may occur in ciliate-free animals, in which the microbial biomass is reduced (Jouany, 1978). Furthermore, ecological characteristics and dynamics of the fauna and flora in the rumen are different. Protozoa residence time in the rumen is more than 4 times higher than that of bacteria, according to studies on ^{15}N binding of bacteria and protozoa (Tarakanow et al., 1984). Considering the rumen as a chemostat, energetic requirements for maintenance may presumably be higher for protozoa, expressed as the ratio of protein leaving the rumen. Thus, protozoa may negatively affect the biological efficiency of the rumen ecosystem.

Finally, defaunation presumably affects molecular hydrogen metabolism. Moss et al. (2000) showed that a good relationship exists between (C2 + C4)/C3 ratio and methane production. Defaunation decreases methane production as indicated by many authors (Jouany et al., 1981; Whitelaw et al., 1984; Kreuzer et al., 1986; Vermorel and Jouany, 1989; Williams and Coleman, 1992). These results are logical as the protozoa are also an important site for methanogens (Newbold et al., 1995; Ushida and Jouany, 1996). Indeed, the C3/C4 ratio greatly increased as a consequence of defaunation, which may reveal an excess of hydrogen (Sauvant and Van Milgen, 1995). When methanogenesis decreases, hydrogen production may be oriented in propionic acid production, at the expense of methane production (Demeyer and Van

Nevel, 1986). This suggests that higher OM digestibility, and therefore higher energy digestibility, observed in the rumen and in the total tract in faunated animals is partly lost in methane emission.

The increase in weight of ruminal contents after defaunation is presumably due to the fill effect of the lower OM digestibility in the rumen. On the contrary, no obvious global rule can be drawn from the effects of defaunation on the liquid phase and the solid phase fractional turnover rate of rumen contents. These results confirmed, with more data, those reported by Demeyer (1988).

The significant increase in wool production after defaunation is well admitted by all authors in the literature. As wool growth is very sensitive to the amounts of sulphur containing AA, defaunation presumably increased the amount of these sulphur containing AA, of microbial origin, in the N duodenal flow at the duodenum (Bird and Leng, 1984; Cottle, 1988a,b). Only one exception has occurred: inverse results on wool production reported by Cottle (1988a,b) may be explained by the very special characteristics of the diet (percentage of concentrate >90%). These results are coherent with the higher nitrogen nutritive value of the diet for ciliate-free animals. As few data on milk production were available (Yang and Varga, 1993), we could not use quantitative analysis to study the effects of defaunation. Therefore, further investigations have to be carried out in order to test the effects of defaunation on milk production, even if apparently the response to defaunation for meat and milk production efficiency should not be different.

5. Conclusion

The results indicate a more efficient use of diets in ciliate-free animals, especially when poor diets, limiting growth, are given. Thanks to this meta-analysis it becomes possible to identify types of diets and production systems, in which defaunation potentially better valorises feed and enhances animal nutrition. That effect of defaunation is pointed out, despite lower parietal constituents and OM digestibility of the diet. Hence, better zootechnical efficiency of ciliate-free animals results mainly from more efficient metabolic use of absorbed nutrients. The factors put forward and which may account for higher efficiency of nutrients use are the higher amino acids supply to the host animal, a more balanced VFAs and an enhanced microbial fatty acids synthesis.

6. Notation

ADG, Average daily gain
DMI, dry matter intake
Dt, total tract digestibility
Dr, rumen digestibility
NH_3, ammonia concentrations in the rumen
VFA, volatile fatty acids concentrations in the rumen
NI, nitrogen intake
Duo.NAN, duodenal non ammonia nitrogen flow
Duo.MN, duodenal microbial nitrogen flow

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