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Protein degradability and intestinal digestibility of blood meals: comparison of two processing methods

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Abstract

Crude protein degradability (nylon bag technique) and intestinal digestibility (mobile bag procedure) of blood dried by two processes: batch (BBM) (temperature: 100–160°C; drying time: 5–7 h) or batch modified (BMBM) (vacuum applied: 0.2 atm; temperature: 70°C; drying time: 1–2 h) were estimated. Blood meals obtained from industrial plants (four samples per process) averaging 92 (BBM) and 86% (BMBM) CP on DM, were evaluated using three Holstein dairy cows fed alfalfa hay (10 kg per day). Two degradability (in sacco technique) and one intestinal (mobile bag technique) assays were performed. Particle size was characterized by dry sieving (arithmetic mean, mean particle size and percent sample retained in 50 μ mesh), and particle washout dynamics was studied. Physical characterization of particles suggest BBM had higher number of particles escaping initially from bags while, as incubation time increased, BBM and BMBM washouts from bags were similar. In both assays, no time effect ($P > 0.3$) on nitrogen disappearing from rumen was observed suggesting N losses occurred at, or before, first incubation time. N degradability of BMBM was higher ($P < 0.05$) than BBM (25 versus 14% and 28 versus 22% assays 1 and 2, respectively). Differences in degradability between assays could result from increased particle losses from bags in assay 2 when meals were ground before incubation, since BBM and BMBM zero — time incubation values in assay 1 and 2 were similar ($\gamma = 0.95$). N soluble fraction was estimated incubating meals samples in buffer solution, as N losses from bags not incubated in rumen (T_0), and as T_0 values corrected by proportion of particles lost through sieve 50 μ (Top). Estimates of N rapidly degraded in rumen were similar for buffer procedure (3 and 4%, BBM and BMBM) and Top (4 and 6%, BBM and BMBM) and lower than T_0 (17 and 21%, BBM and BMBM). Potentially rumen degradable fractions (N degradability — Top) were 19 and 24% for BBM and BMBM, representing 85% of rumen degradable protein. There were not detected differences in intestinal digestibility of undegraded N (BBM: 20%; BMBM: 27%) between processes ($P > 0.7$). Variations in meals N intestinal digestibility were registered within each process (BBM: 14–33%; BMBM: 15–60%). Variation in N ruminal dynamics and intestinal protein

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digestibility within and between processes may be attributed to lack of uniformity in the industrial procedures, particularly protein coagulation before drying. Results indicate blood meals quality may be variable and vacuum application reducing exposure of blood to heat may increase protein availability. Estimates of rapidly degraded N by sample incubation in buffer and zero time correction by $<50\mu$ particles were similar, indicating convenience to sieve blood meals before rumen incubation. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Blood meal; Degradability; Intestinal digestibility

1. Introduction

Blood is a tissue high in water (80%), containing 14% nitrogen on its DM. The globular proteins — hemoglobin, albumin and globulins — represent, respectively, 59, 16 and 13% of total nitrogen. High lysine content and water solubility, and moderately high arginine content made these proteins susceptible to rapid enzymatic degradation by rumen microbial “trypsin-like” proteases which would suggest blood meals would be a source of N rapidly degraded in rumen (Clark et al., 1987; Cheftel, 1989; Wadhwa et al., 1993). However, these meals are considered a valuable feedstuff for high producing ruminants for its low protein degradability.

Heat applied to dry blood for animal feeding purposes, may reduce protein digestive availability mainly through physicochemical modifications (denaturation) and linkage with other substances (i.e. Maillard reaction); degree of protein damage will depend on amount of heat applied and length of heating. For this, differences in industrial processes may greatly influence nutritional quality of blood meals and may explain contradictions in animal performances when this by-product is included in ruminant diets (Waltz et al., 1989; NRC, 1996; Valentine and Bartsh, 1996; Grant and Haddad, 1998).

This study was performed to evaluate nutritional impact on blood meal protein quality of a technical modification of batch drying process.

2. Materials and methods

Evaluated blood meals were obtained from two rendering and meat plants differing in drying processes, samples — from different batches — were taken in four consecutive weeks (one sample per process per week). In the batch (BBM) method, blood was first steam coagulated, separating liquid from solids by filtration, then blood solids were dried in a batch dryer, where the surrounding shell provided the water steam heat source (water steam: 1.5 kg/cm^2). Blood solids remained in dryer 5–7 h, temperatures achieved inside the batch ranged from 100 to 160°C . In the batch modified procedure (BMBM), vacuum (0.2 atm) was applied in the chimney which extracted air from system, what made blood water to evaporate at 70°C , and reduce drying time to 1.5–2 h. In the latter, cattle bones and animal fat were added during drying to keep solids from sticking on drier sides. Temperatures and times quoted were given by industrial managers and were not specially measured for the present study.

2.1. Particle characterization

Particle size was characterized by dry sieving (2 min) three samples (3 g) of each blood meal in a sieve shaker (2000 rev/min) with five sieves (1, 0.5, 0.25, 0.125, 0.05 mm mesh apertures sizes). Particle characteristics were expressed as percent sample retained in 0.05 mm mesh, and real particle size was estimated as arithmetic mean and mean particle size. Arithmetic mean (Israelsen, 1968) was calculated from the median of each particle size class indexed to its percentage by weight. To estimate mean particle size, cumulative percent of particles passing the screen was plotted against sieve size, and a log-normal distribution fitting was used (Waldo et al., 1971). Mean particle size was calculated as the screen size of the sieve on which 50% of the particles would be collected. Particle washout dynamics was studied by measuring dry matter losses from bags placed in cold water for different length of time. Three bags (same bags and sample size as in degradability studies) per blood meal were placed in cold water (one bag/flask/250 ml of water) under continuous agitation for 30 min, 1, 2, 4, 8, 12, and 24 h. Upon removal from water, bags were dried (60°C, 48 h) and weighed. Residual water was filtered using paper (Whatman 540) cones, filters were dried (60°C, 48 h) and weighed. Particle losses from bags were estimated as the proportion of dry particles retained in cone filter referred to total washouts (soluble + particles) estimated as difference between initial and final DM weights of sample contained in bags.

2.2. Protein degradability

Rumen protein degradability was estimated in sacco (Orskov et al., 1980) in two assays. Six bags, per meal and incubation time, containing approximately 3 g of air-dry matter (0.0119 g sample per cm²) were placed (weighed with a 700 g weight) in the ventral part of the rumen of three dry Holstein cows (450 kg, average) with permanent rumen and duodenum fistulas. In each assay, on two consecutive periods bags were incubated 1, 2, 4, 6, 8, 12, 24, 48, and 72 h (assay 1), and 15, 30 and 45 min, and 1, 1.5, 2, 4, 6, 8, 12, 24 and 48 (assay 2). In assay 2 meals were ground (1 mm). In both assays, all bags (14 × 9 cm; polyester polyfilament; average pore size: 50 µm) were introduced at once in rumen immediately after morning meal and removed at designated times. Previous to incubation, bags were soaked in water (39°C, 15 min), and upon removal from rumen, bags were placed in cold water with ice and washed twice in a washing machine (30 l, 30 bags per washing batch, 3 min) and dried in an forced-air oven (60°C, 48). Bag contents of each blood meal sample were pooled per cow, incubation time and period, and stored for subsequent analysis. Another six bags per blood meal, were not introduced into the rumen and manipulated as the incubated ones (zero time, T_0).

2.3. Intestinal digestibility

Intestinal digestibility was estimated by the mobile bag technique (Peyraud et al., 1988). Fifteen nylon bags (6 cm × 7 cm, pore size 50 µm) per blood meal (sample size: 1.5 g; meals ground to 1 mm) were incubated (16 h) in the rumen of three dry Holstein cows (five bags per blood meal per cow; maximum: 30 bags per cow per day). After

rumen incubation, bags were placed (2.5 h) in acid pepsin–HCl solution (pH 2; 3 g pepsin/L 0.1N HCl) in a water bath (38.5°C) shaking bags every 5 min., and introduced (15 bags/cow/day,) into the small intestine during the evening meal (5 pm). Each bag was carefully washed by chime before introducing following one. Bags were recovered from feces beginning 8 a.m. to 5 p.m., bags appearing after 5 p.m. were discarded. Recovered bags were washed in a washing machine (30 l, 60 bags per washing batch, 45 min) and stored for nitrogen determination. Six bags per meal were incubated in the rumen of three cows (two bags per meal per cow) to estimate ruminal nitrogen disappearing after 16 h of incubation; bag characteristics and manipulation was as in sacco procedure.

In degradability and intestinal digestibility trials, animals were fed (10 kg DM per cow; 8:00 a.m. and 5:00 p.m.) alfalfa hay (17% CP, 42% NDF) and located indoors in individual pens with free access to water and mineral salt mixture (95% minerals, 12.5% Ca, 45.0% NaCl, 4.0 ppm F, Ca:P=2:1).

2.4. Chemical analysis

Blood meals were analyzed for dry matter (105°C), ash, EE and N (Kjeldahl) (AOAC, 1990), and buffer protein solubility (Licitra et al., 1996). N was measured in ruminal and intestinal residues. N pepsin insoluble residue was quantified by incubating bags (4/meal) in pepsin acid solution as in mobile bags procedure.

2.5. Calculations and statistical analysis

Rumen nitrogen degradation kinetics did not fit Orskov and Mc Donald's (1979) exponential model. Rumen degradable protein was estimated as average N disappearing from all incubated bags (conventional "in sacco" procedure), N disappearing from bags after 16 h incubation (mobile bag procedure) or pepsin soluble N. Rumen rapidly degraded N (soluble fraction) was estimated as N lost from zero time bags (T_0) (conventional "in sacco" procedure), T_0 values corrected by proportion of particles lost through sieve 50 μ (Top), T_0 values corrected by particle losses after 30 min incubation in water (Tow), and N soluble in buffer. Potential degradable N was calculated as difference between N degradability and T_0 , Top and buffer soluble N.

Results were statistically analyzed as a completely randomized design in a nested mixed model, an ANOVA was performed by GLM (SAS, 1982). Model used in crude protein degradability analysis was $Y_{ijk} = \mu + P_i + T_j + (PT)_{ij} + h_{(i)k} + (th)_{(i)jk} + e_{ijk}$, where Y_{ijk} is the response, μ the overall mean, P_i the mean effect of industrial process, T_j the mean effect of incubation time, $(PT)_{ij}$ the industrial process \times incubation time interaction, $h_{(i)k}$ the mean effect of blood meal nested within industrial process, $(th)_{(i)jk}$ the incubation time \times blood meal nested within industrial process interaction, and e_{ijk} the residual error. Fixed effects were industrial process and incubation times, and random effect was blood meal. Model used in CP soluble fraction and intestinal digestibility analysis was $Y_{ijk} = \mu + P_i + h_{(i)k} + e_{ijk}$, where Y_{ijk} is the response, μ the overall mean, P_i the mean effect of industrial process, $h_{(i)k}$ the mean effect of blood meal nested within industrial process, and e_{ijk} the residual error. Fixed effect was industrial process, and random effect was blood meal. Confidence intervals ($\gamma = 0.95$) were used to identify differences between fractions and experiments.

Table 1
Particle characterization of batch (BBM) and batch modified (BMBM) blood meals^a

	BBM	BMBM
Particles < 50µ		
Average (%)	15.25 a	8.72 b
CV (%)		10.03
Arithmetic mean (µm)		
Average (%)	283 b	445 a
CV(%)		3.69
Mean particle size (µm)		
Average (%)	89.9 b	130.5 a
CV(%)		6.46

^a Mean values with equal letter did not differ ($P > 0.05$).

3. Results and discussion

BBM average particle size (arithmetic mean) was smaller ($P < 0.05$), and proportion of small particles (mean particle size) and particles <50µ were higher ($P < 0.05$) than BMBM (Table 1). Results suggest BBM had higher number of particles scaping initially

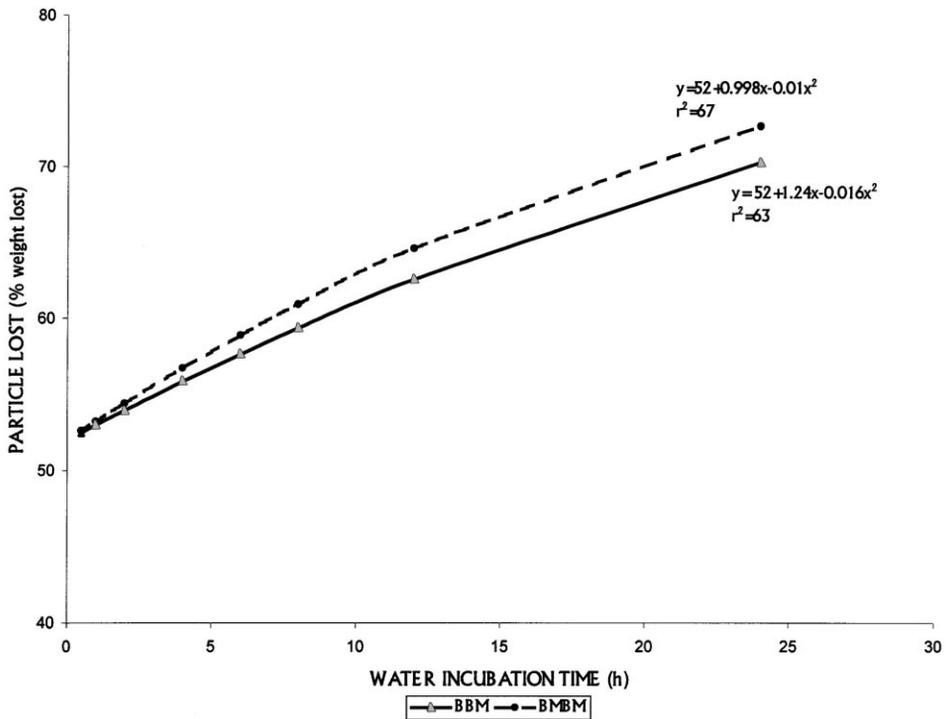


Fig. 1. Blood meals weight losses from bags incubated in water.

Table 2
Chemical composition of evaluated blood meals (DM basis)^a

Blood Meal	DM (%)	CP (%)	EE (%)	Ash (%)
BBM				
1	87.5	92.0	0.29	2.5
2	94.5	94.0	0.21	2.4
3	91.3	88.2	0.22	2.3
4	92.1	94.1	0.22	1.2
Avg.	91.3	92.0	0.24	2.1
S.D.	2.5	2.7	0.04	0.6
BMBM				
1	94.6	88.2	2.40	3.8
2	95.4	85.7	2.80	4.3
3	93.1	89.0	3.50	3.8
4	92.1	83.0	2.45	4.6
Avg.	93.8	86.5	2.79	4.1
S.D.	1.3	2.7	0.50	0.4

^a Avg.: average; S.D.: standard deviation.

from bags although as incubation time increased, washouts from bags would have been similar for BBM and BMBM (Fig. 1).

DM and CP contents of BBM and BMBM were similar; BMBM registered higher EE and ash which may have resulted from fat and bones added to avoid blood from sticking to tanks walls when drying (Table 2).

No time effect ($P > 0.3$) on nitrogen disappearing from rumen incubated bags was observed (Fig. 2), suggesting particle and/or nitrogen losses in rumen occurred at, or before, first incubation time. Particles washout dynamics indicate particles lost from bags might have increased as bags stay longer in rumen, suggesting particles retained in bags would had more N than washouts.

In both in sacco assays, N degradability was lower ($P < 0.05$) for BBM than for BMBM (Table 3). BBM degradability values were higher ($\gamma = 0.95$) in assay 2 (22%) than in assay 1 (14%). Differences in degradability could result from increased particle losses from bags in assay 2 when meals were ground before incubation, since BBM and BMBM T_0 values in assay 1 and 2 were similar ($\gamma = 0.95$). Degradability values agree with reported by Loerch et al. (1983a) (25%), Loerch et al. (1983b) (18%), Blasi et al. (1991) (12%), Palmquist et al. (1993) (15%), Wadhwa et al. (1993) (21 and 13%), Erasmus et al. (1994) (19%), Masoero et al. (1994) (20%), Calsamiglia et al. (1995) (12%) and NRC (1996) (20%). Meal grinding reduced variation (23 and 11%, assays 1 and 2) in N degradability among blood meals obtained from same processing method. Variation may be explained by contamination with urine, rumen contents and/or hair during blood collection, as well as lack of uniformity during processing.

When meals were ground, N degradability obtained from in sacco assays and from residue after 16 h incubation in rumen were similar ($\gamma = 0.95$) for BBM and BMBM, while values calculated from N pepsin insoluble residues were lower ($\gamma = 0.95$) for both groups of meals and no differences ($P < 0.05$) between processes were registered.

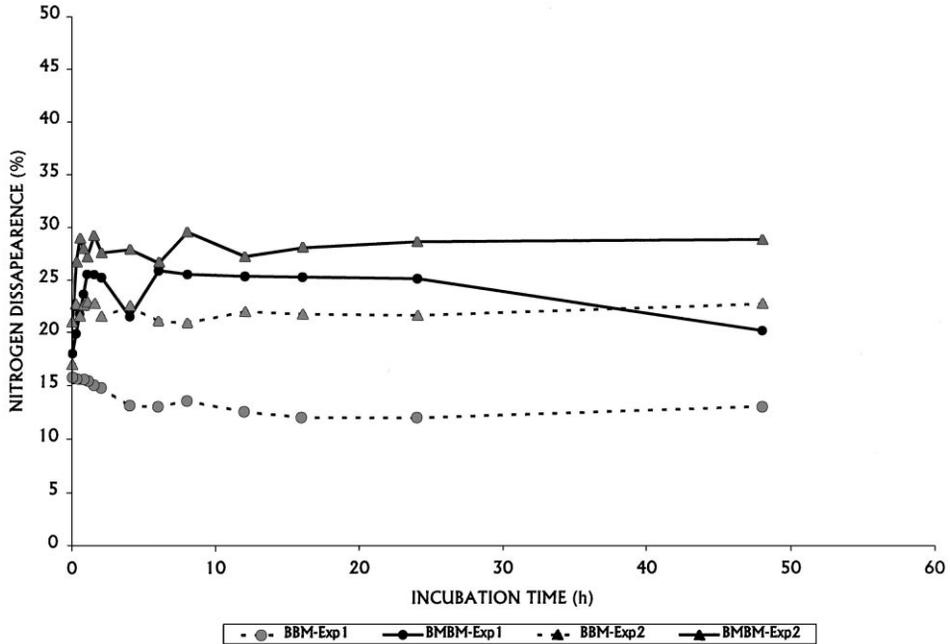


Fig. 2. Nitrogen kinetics of evaluated blood meals.

Differences between degradability estimators calculated from bag and pepsin insoluble residues could not be attributed to particle losses from bags since BBM had the smallest particle size and the lowest degradability. Pepsin insoluble N would not be a good estimator of rumen N undegradable fraction, suggesting intestinal enzymes may not degrade blood meal protein to same extent as microbial enzymes which agrees with results reported by Mgheni et al. (1994) and Hvelpund et al. (1992) on proteins from tropical roughages, grass silage, formaldehyde treated soybean meal and meat and bone meal.

Average rumen undegradable protein of BBM (83 and 75% of total CP, assays 1 and 2) was similar to reported by Howie et al. (1996) (84%) for seven batch dried blood meals. Batch drying modification evaluated in present work resulted in lower BMBM undegradable N (68 and 65%, assays 1 and 2) than reported (81%) by same author for seven ring dried blood meals. Differences may be partially explained by degree of protein modification by processing methods.

Estimates of N rapidly degraded in rumen (soluble fraction) were 3 (BBM) and 4% (BMBM) for buffer procedure, 3.8 (BBM) and 6.2% (BMBM) for Top, and 9 (BBM) and 11% (BMBM) Tow (Table 2), representing, respectively, 13, 20 and 39% of rumen degradable N. Differences between buffer and Tow could be explained by uneven N distribution among particles, with smaller particles containing less N than particles larger ones. Soluble N values agree with reported by Calsamiglia et al. (1995) (4%) and Clark et al. (1987) (3%).

Table 3

Nitrogen degradability and intestinal digestibility of batch (BBM) and batch modified (BMBM) blood meals^{a,b}

Item	BBM	BMBM
Nitrogen degradability (% CP)		
Conventional in sacco procedure		
Assay 1		
Avg. (%)	14.1 b	25.0 a
Confidence interval	12.2–15.9	23.2–26.8
Assay 2		
Avg. (%)	22.0 b	28.1 a
Confidence interval	20.4–23.6	26.4–30.0
Estimated after 16 h rumen incubation		
Avg. (%)	21.9 b	27.5 a
Confidence interval	20.3–23.6	25.8–29.1
Pepsin insoluble N		
Avg. (%)	17.5	18.2
Confidence interval	16.0–18.9	16.7–19.7
Nitrogen soluble fraction (% CP)		
Conventional in sacco procedure (T_0)		
Assay 1		
Avg. (%)	15.7	18.9
Confidence interval	11.8–19.7	14.9–22.8
Assay 2		
Avg. (%)	17.2	20.8
Confidence interval	12.3–22.1	15.9–25.9
To corrected by particle <50 μ (Top)		
Avg. (%)	3.8	6.2
Confidence interval	2.2–5.6	4.7–8.1
Buffer soluble protein		
Avg. (%)	2.9	3.8
Nitrogen potentially degradable fraction (% CP)		
Estimated from T_0 (in sacco procedure)		
Assay 1	0	6.1
Assay 2	0	7.3
Estimated from Top	18.2	21.9
Estimated from buffer soluble protein	19.1	24.2
Nitrogen intestinal digestibility (% RUP)		
Avg. (%)	20.4	27.0
CV (%)		42.9

^a Avg.: average, RUP: rumen undegradable protein.^b Mean values with equal letters did not differ ($P > 0.05$).

Potentially rumen degradable fraction (degradable N–N buffer soluble) was 19 and 24% for BBM and BMBM, representing approximately 85% of rumen degradable protein (Table 4).

BBM (20%) and BMBM (27%) digestibility of rumen undegraded N (Table 3) were not different ($P > 0.7$), lack of statistical response between processes resulted from high contribution of meal variation (74%) to total variation. Higher digestibilities were

Table 4

Average protein composition of batch (BBM) and batch modified (BMBM) blood meals

	BBM	BMBM
DM (%)	91.3	93.8
CP (% DM)	92.0	86.5
CP degradability (%)	18.0 ^a	26.5 ^a
Soluble fraction, (%) ^b	2.9	3.8
Potentially degradable fraction (%)	15.1	22.7
Degradation rate (%/h)	0	0
RDP (%) ^c	16.6	22.9
RUP (%) ^d	75.4	63.6
CP intestinal digestibility (%)	20.4	27.0
Intestinal digestible protein (%) ^e	15.4	17.2

^a Average assays 1 and 2.^b Buffer soluble N.^c Ruminant degradable protein = CP × CP degradability.^d Rumen undegradable protein = CP – RDP.^e RUP × CP intestinal digestibility.

detected in BMBM (60%) than in BBM (34%) indicating reduction of exposure of blood to heat could improve meals quality. Digestibility values were lower than reported by Palmquist et al. (1993) (99%), Erasmus et al. (1994) (56%) and Calsamiglia et al. (1995) (86%). Large variation on digestibility values was reported by Masoero et al. (1994) (76.57 ± 17.85 for roller dried blood meal) and Howie et al. (1996) (72.0–90.3 for ring, and 28.8–79.2 for batch dried blood meals).

Variation in N ruminal dynamics and intestinal protein digestibility within and between processes may be attributed to lack of uniformity in industrial procedures, particularly to heat applied to reduce blood water content. Protein damage associated with severely heated animal proteins of low carbohydrate content include physicochemical reduction on solubility (denaturation, coagulation, protein molecule shortage) and formation of indigestible protein–protein linkages involving lysine and, probably, cysteine, asparagine and/or glutamine (Hurrel and Carpenter, 1974; Waibel et al., 1977; Kramer et al., 1978; Clark et al., 1987; Cheftel, 1989; Van Varnevel, 1994; Van Soest, 1994).

A summary of average protein composition of batch and batch modified blood meals is presented in Table 4.

4. Conclusions

Evaluated modification of blood batch drying improved N degradability and allowed identify meals with higher digestibility of N undegraded fraction. Differences may have resulted from decreased protein damage due to less heat and length of heating on drying indicating processing methods must be uniform and lower exposures to heat used to increase blood meals quality. Estimates of rapidly degraded N by sample incubation in buffer and correction of zero time incubated bags by losses of particles of less than 50 μ

were similar indicating it should be advisable to sieve blood meals before rumen incubation.

References

- Association of Official Analytical Chemists, 1980. *Official Methods of Analysis*, 13th Edition. AOAC, Washington, DC.
- Blasi, D.A., Klopfenstein, T.J., Drouillard, J.S., Sindt, M.H., 1991. Hydrolysis time as a factor affecting the nutritive value of feather meal and feather meal-blood meal combination for growing calves. *J. Anim. Sci.* 69, 1272–1278.
- Calsamiglia, S.M., Stern, M.D., Firkins, J.L., 1995. Effects of protein source on nitrogen metabolism in continuous culture and intestinal digestion in vitro. *J. Anim. Sci.* 73, 1819–1827.
- Cheftel, J.C., 1989. *Proteínas alimentarias*. Ed. Acribia. Zaragoza, España.
- Clark, J.H., Murphy, M.R., Crooker, B.A., 1987. Symposium: alternate feed sources for dairy cattle. Supplying the protein needs of dairy cattle from by-product feeds. *J. Dairy Sci.* 75, 2304–2323.
- Erasmus, L.J., Botta, P.M., Cruywagen, C.W., 1994. Amino acid profile and intestinal digestibility in dairy cattle of rumen undegradable protein from various sources. *J. Dairy Sci.* 77, 541–551.
- Grant, R.J., Haddad, S.G., 1998. Effect of a mixture of feather and blood meals on lactational performance of dairy cows. *J. Dairy Sci.* 81, 1358–1363.
- Howie, S.A., Calsamiglia, S., Stern, M.D., 1996. Variation in ruminal degradation and intestinal digestion of animal by-product proteins. *Anim. Feed Sci. Technol.* 63, 1–7.
- Hurrel, R.F., Carpenter, K.J., 1974. Mechanisms of heat damage in proteins. 4. The relative lysine content of heat-damaged material as measured in different ways. *Br. J. Nutr.* 32, 589–603.
- Hvelplund, J.L., Wiesbjerg, M.R., Andersen, L.S., 1992. Estimation of the true digestibility of rumen undegraded dietary protein in the small intestine of ruminants by the mobile bag technique. *Acta Agric. Scand. Section A. Anim. Sci.* 41, 34–39.
- Israelsen, M., 1968. Determination of Particle Size in Wafers and Pellets of Dried Forages. *Bioteknisk Inst. Re., Kolding*.
- Kramer, S.L., Waibel, P.E., Behrends, B.R., Elkanhely, S.M., 1978. Amino acid in commercially produced blood meals. *J. Agric. Food Chem.* 24, 979–981.
- Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57 (4), 347–358.
- Loerch, S.C., Berger, L.L., Gianola, D., Fahey Jr., G.C., 1983a. Effects of dietary protein source and energy level on in situ nitrogen disappearance of various protein. *J. Anim. Sci.* 56, 206–216.
- Loerch, S.C., Berger, L.L., Plegge, S.D., Fahey, G.C., 1983b. Digestibility and rumen escape of soybean meal, blood meal, meat and bone meal and dehydrated alfalfa nitrogen. *J. Anim. Sci.* 57, 1037–1047.
- Masoero, F., Fiorentini, L., Rossi, F., Piva, A., 1994. Determination of nitrogen intestinal digestibility in ruminants. *Anim. Feed Sci. Technol.* 48, 253–263.
- Mgheni, D.M., Hvelplund, T., Wiesbjerg, M.R., 1994. Intestinal digestibility of rumen undegraded dietary protein from tropical roughages estimated by the mobile bag technique. *Acta Agric. Scand. Section A. Anim. Sci.* 44, 230–235.
- National Research Council, 1996. *Nutrient Requirements of Dairy Cattle*, 7th Edition. National Academy Press, Washington, DC.
- Orskov, E.R., De Hovell, F.D., Mould, F., 1980. The use of the nylon bag technique for the evaluation of feedstuff. *Trop. Anim. Prod.* 5, 195–213.
- Palmquist, D.L., Weisbjerg, M.R., Hvelplund, T., 1993. Ruminal, intestinal and total digestibility's of nutrients in cows fed diets high in fat and undegradable protein. *J. Dairy Sci.* 76, 1353–1364.
- Peyraud, J.L., Genest-Rulquin, C., Verité, R., 1988. Mesure de la digestion de l'azote des aliments dans l'intestin des ruminants par la technique des sachets mobiles. 1. Evaluation de la quantité de matières azotées indigestibles en sachet des principaux aliments. *Reprod. Nutr. Dévelop.* 27 (1), 129–130.
- SAS, 1982. *SAS User's Guide: Statistics*. SAS Institute, Cary, NC.

- Valentine, S.C., Bartsh, B.D., 1996. Blood meal as a protein supplement for dairy cows. *Proc. Aust. Soc. Anim. Prod.* 21, 349.
- Van Soest, P.J., 1994. *Nutritional Ecology of the Ruminant*, 2nd. Edition. Cornell University Press, Ithaca, USA.
- Van Varnevel, R., 1994. Heat damage in amino acid. *Br. J. Nutr.* 44, 1452–1463.
- Waldo, D.R., Smith, L.W., Cox, E.L., Weinland, B.T., Lucas, H.L., 1971. Logarithmic normal distribution for description of sieved forages material. *J. Dairy Sci.* 54, 1465–1469.
- Wadhwa, M., Makkar, G.S., Ichponani, J.S., 1993. Disappearance of protein supplements and their fractions “in sacco”. *Anim. Feed Sci. Technol.* 40, 285–293.
- Waltz, D.M., Stern, M.D., Illg, D.J., 1989. Effects of ruminal protein degradation of blood meal and feather meal on the intestinal aminoacid supply to lactating cows. *J. Dairy Sci.* 72, 1509–1518.
- Waibel, P.E., Cuperlovic, M., Hurrell, R.F., Kenneth, J.C., 1977. Processing damage to lysine and others aminoacids in the manufacture of blood meal. *J. Agric. Food Chem.* 25, 171–175.