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THE EFFECTS OF A HIND-GUT FERMENTATION ON ENDOGENOUS URINARY NITROGEN EXCRETION IN SHEEP NOURISHED BY INTRAGASTRIC INFUSION[1]

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Mide içi infüzyon ile beslenen koyunlarda kalın barsak fermentasyonunun endojen idrar azotu atımına etkileri

Özet: *Dört dişi koyuna rumen kanülü, abomasum ile ileum infüsyon kateteri, ayrıca bir koyunun sekumunada kanül takıldı. Bütün hayvanlar tamamen mide içi infüzyon ile beslendiler. Uçucu yağ asitleri, tampon ve makro mineraller rumen'e kazein abomasum'a infüze edildi. Değişik seviyelerde kalın barsak fermentasyonu oluşturmak için üç ayrı seviyede besin maddesi terminal ileum'a infüze edildi. İleum'a yapılan infüzyon uygulaması* (1) *su infüzyonu;* (2) 25 *g / gün nişasta ve* 50 *g / gün sellüloz infüzyonu,* (3) 50 *g / gün nişasta ve* 50 *g / gün sellüloz infüzyonu. İlk 7 gün hayvanlar tesbit edilen kalın barsak infüzyonuna alıştırıldılar. Sindirilebilirlik ve azot dengesi ölçümlerinden sonraki* 15 *ve* 19*'uncu günlerde azotsuz besleme uygulandı ve endojen idrar azotu atımı tesbiti için latin kare metodu uygulandı. Endojen idrar azotu atımı bütün uygulamalar ortalaması alındığında* 206 *mg / kg* $^{0.75}$ */ gün bulundu ve kalın barsak fermentasyonunun endojen idrar azotu atımı üzerinde önemli etkisi olmadığı saptandı. Azotsuz besleme sırasında gaita azotunun ve toplam azotun kalın barsak fermentasyonu varlığında yükseldiği gözlendi.*

Summary: *Four female sheep were fitted with rumen cannulas and abomasal and ileal infusion catheters; one of the sheep was also fitted with a cannula at the caecum. All animals were nourished wholly by intragastric infusion of nutrients. Solutions of volatile fatty acids, buffer and major minerals were infused into the rumen and casein infused into the abomasum. All animals were received three levels of nutrient infusion into the terminal ileum in order to achieve different levels of hindgut fermentation. The ileal infusion treatments were* (1) *water infusion;*

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(2) 25 g / d starch and 50 g / d cellulose infusion; (3) 50 g / d starch and 50 g / d cellulose infusion. The first 7 days served as the preliminary period in which animals were adjusted to the prescribed level of hind-gut infusion after digestibility and N-balance measurement days 15—19 inclusive constituted a nitrogen-free period in which faeces and urine were collected and analysed on a daily basis to establish endogenous urinary nitrogen excretion. 3×3 Latin square design was used with treatment periods of 3 weeks durations Overall mean value of endogenous urinary nitrogen (EUN) excretion of the three treatment groups was 206 mg N / kg $^{0.75}$ / d and there was no significant effect of hind-gut fermentation on EUN excretion. During N-Free regime faecal N and total excretion of N increased in the presence of a hind-gut fermentation.

Introduction

The requirement for protein by ruminant animals is a combination of the needs for the rumen microorganisms and of the host animal. The animal's requirement for amino acid-N (tissue N; TN) is defined as the sum of the N needed to maintation N as hair and shed epithelial cells, and the amino acids retained in the body, the foetus, and secreted as milk (17). When dietary energy is supplied at a level close to that needed to maintain the energy equilibrium of the host animal (maintenance), Roy et al. (17). Concluded that for an animal neither lactating, nor pregnant, TN would be met and even exceeded by the protein synthesised in the rumen by the microorganisms.

At energy intakes close to maintenance, the main component of TN requirement will be the N needed to offset the endogenous losses in the urine (endogenous urinary N, EUN) (7).

In the proposals formulated in 1976 and published in ARC (1) EUN was considered to represent the nitrogen requirement for tissue maintenance of ruminants. The EUN had been determined as the urinary N loss in experiments where N-free diets had been fed to ruminants or by extrapolation to zero dietary N intake or to zero apparently digested N intake from experiments where a series of different intakes of nitrogen have been used.

This is difficult to measure in ruminants, due to the fact that N has to be supplied to satisfy the requirements of the rumen microorganisms, and therefore the technique of using N-free diets (as with single-stomached animals) cannot be applied (7). A further comp-

lication with ruminants is that endogenous losses may be partitioned between the urine and faeces, dependent on the amount for fermentation taking place in the hind gut, and the consequent excretion of N in the debris of the microorganism participating in the fermentation (13). This faecal N will be in addition to the undigested microbial debris originating from the rumen which can also contain recycled N of endogenous origin. However, ARC (1) used values for EUN derived from experiments in which therefore underestimated true endogenous losses. All these problems stem from the difficulty of measuring endogenous losses of N in the normally-fed ruminant, in which these losses are partitioned between the urine and the faeces (17).

The development of an technique (15) by which functional ruminants were maintained by intragastric nutrition made it possible to infuse N-free nutrients and to measure urinary N almost uncomplicated by rumen microbial activity. The first observation on urinary N excretion under these conditions was reported by Orskow&Grubb (14) and the early observations with sheep have been extended to steers and dairy cows (16). In general the total excretion of N is lower when N-free diets are ingested that when an N-free infusion is given. This is to be expected since microbial protein will be produced from the recyling of urea as long as the animals are consuming some feed and rumen fermentation is sustained.

Early experiments show that if the digesta arriving at the caecum and large intestine contains fermentable carbohydrate, but is deficient in N (relative to the requirements of the microorganisms of the caecum and large intestine), then urea can pass from the blood and be trapped in the microbial biomass. However, since there is no subsequent digestion of the biomass and return to the host animal, the N is lost as microbial debris in the faeces (8). This point was demonstrated by Orskov&Food (12) in an experiment in which starch was infused into the caecum of a lamb given a constant amount of dried grass as feed. As the amount of starch infused was increased, so did faecal N, which rose from 5.8 to 9.6 g / d. Due to this diversion of N excretion from the urine to the faeces, the apparent digestibility of N fell from 0.69 to 0.48. Orskov—Grubb (14) also showed that infusion of substrate into the caecum had no effect on total endogenous nitrogen excretion (TEN); it only increased faecal N and reduced urinary N. It is also interest that consumption of 1 kg / d of indigestible fibre by steers and dairy cows did not alter TEN but only increased the N excreted in faeces (16).

Materials and Methods

Four female lambs of Suffolk Scottish Blackface breeding were used. The lambs were 7 months of age at the start of the experiment and had an average liveweight of 37 kg. Each animal was fitted with a rumen cannula, an abomasal infusion catheter and an ileal infusion catheter as described by Orskov et al. (15). One of the sheep was fitted also with a cannula at the caecum as described by MacRae et al. (11).

Lambs were housed indoors in metabolism Crates under continuous lighting. After surgery recovery all animals were transferred from solid food (pelleted barley diet) to total intragatsric nutrition during the introductory stage of the experiment. The procedure was to increase the amount of infusate in steps of multiples (0.25) of maintenance requirement for energy and maintain the new level for one or two days. At the same time the amount of food given by mouth was reduced over 6 days.

The methods used to maintain animals by intragastric infusion were essentially those described by Orskov et al. (15), Macleod et al. (10) and Hovell et al. (7) in which solutions of Volatile fatty acids (VFA), buffer and major minerals were infused into the rumen and casein infused into the abomasum. In the present experiment animals received infusions of starch and cellulose into the terminal ileum.

The calculation of total energy to be supplied was based on the assumption that the maintenance requirement for energy was 450 kJ / kg $^{0.75}$ per day (7) and Nitrogen requirement for maintenance taken to be 350 mg N / kg $^{0.75}$ per day (2). Vitamin A, D and E were given i.m injection. N intake was maintained throughout at level of (1) maintenance except for 5 days in each treatment period when animals were maintained on a nitrogen free intake to allow measurement of endogenous urinary nitrogen excretion. The casein infusion was stopped during this 5 days and replaced with an equal volume of water.

Animals received three levels of nutrient infusion into the terminal ileum in order to achieve different levels of hind-gut fermentation. The ileal infusion treatments were (1) water infusion (2) 25 g / d starch and 50 g / d cellulose infusion (3) g / d starch and 50 g / d cellulose infusion. These quantities based on estimates in the literature for the amounts of these constituents of normal diets, which might be expected to reach the terminal ileum in sheep given conventional feeds (13).

In order to help establish the initial fermentation, an inoculation of rumen fluid (50 ml) was given into the hind-gut via ileal infusion catheter.

In each period, the first 7 days served as the preliminary period, in which animals were adjusted to the prescribed level of hind-gut infusion. Days 8—12 inclusive (5 days) were used for quantitative collection of faeces and urine for digestibility and N-balance measurement. Days 15—19 inclusive (5 days) constituted the N-free period, when casein infusion into the abomasum was discontinued and faeces and urine were collected and analysed on a daily basis to establish endogenous N excretion.

The metabolism crates were fitted with a PVC-coated expanded metal floor. Urine and faeces caught in a fibre glass separator funnel which covered the entire floor area. Urine passed directly into a collection tray containing 10 % sulphuric acid (300 ml per 12 h collection) to prevent loss of ammonia. Urine volume was weighed sampled and stored at —20° until analysed. Faeces material was separeted from the urine by means of a nylon mesh which covered the urine collection tray and this was bulked with any faeces caught in the separator funnel. The faeces sample was collected over 5 days, bulked together, weighed and stored in sealed containers in a refrigerator until required for analysis.

Faeces for bacterial examination were obtained per rectum of each animal.

Caecal digesta were sampled into containers. It proved difficult to obtain samples from cannulated sheep and on occasion samples were too small for analysis. Rumen samples were taken routinely twice a day for pH and osmotic pressure of rumen fluid.

Urine samples were analysed for total nitrogen using the automated kjeldalh method of Davidson et al. (5). Faecal samples was 600°C for organic matter determination (3). Faeces were analysed for starch as described by Bergmeyer (4) and for ADF as described by AOAC (3). Estimation of the number of total viable bacteria were made as described of Hobson (6) using M 8 roll tubes and cellulose roll tubes. Aerobic bacteria were counted using plate count agar as descibed by Leininger (9).

pH was determined electrometrically and osmotic pressure by freezing point depression.

A 3×3 Latin square was used. The experimental desing was therefore treated as a randomized black in which the 3 treatments were unequally represented in each period and the 12 observation were subjected to an analysis of variance for nonorthogonal data which allowed treatment means to be adjusted for animal and period effects. In addition to the 3 animals allocated to treatment within the Latin square, the animals fitted with a cannula in the caecum was also taken through the same sequence of treatments.

Results and Discussion

The health of experimental animals remained good throughout the experiment. Mean energy and nitrogen intakes of animals are shown in Table 1 and there was no difference between treatments.

The mean daily excretion of N in faeces and urine over the five days of N-free intake are shown for each treatment in Figs 1 a, b and c. For comparison the mean daily excretions of N over the 5-day digestibility trial, when N intake was sufficient for maintenance are also shown. The values for faecal excretion on the N-free regimen were derived from the composite sample collected over days 1, 2 and 3 the individual values for days 4 and 5. All values for urine N were the mean excretion for the individual days.

In all three treatments urinary N excretion declined steadily over first 3 days of N-free intake and then reached an approximate plateau at about 200 mg N / kg $^{0.75}$ / d. This compares with a mean value of about 350 mg N / kg $^{0.75}$ / d observed during the days when adequate N was included in the infusates (Table 2). Statistical analysis of the changes in N excretions in faeces and urine between day 3 and day 5 of N-free intake showed that these did not differ significantly from zero and the mean excretions over days 3-5 inclusive were therefore examined for the presence of treatment effect. The mean excretions of urine N, faecal N and total N over these 3 days are shown in Table 3.

Mean urinary N excretion on the N-free intakes (the EUN excretion) did not differ between the three treatment groups and had an overall mean value of 206 mg N / kg $^{0.75}$ / d. Faecal N excretion, in contrast, increased progressively from treatment 1 to Treatment 3 and was significantly higher in the animals receiving infusions of starch

Table 1. Mean intakes and faecal excretion of DM, OM, starch and cellulose, apparent digestibility coefficient and faecal bacterial counts in sheepgiven infusions of starch and cellulose into the terminal ileum (each value is the mean of 4 observations)

Treatments †	Mean liveweight ($kg^{0.75}$)	Intakes					Faecal Excretion				Apparent digestibilities			Faecal bacteria	
		Energy (kJ / $kg^{0.75}$ / d)	Nitrogen (g / $kg^{0.75}$ / d)	Starch (g / d) ⁂	Cellulose (g / d) ⁂	OM (g / d)	DM (g / d)	Starch (g / d)	Cellulose ⌀ (g / d)	OM (g / d)	Starch	Cellulose	OM	Aerob, Anaerob (log count / g)	
1	14.98	437.7	0.448	—	—	398.7	12.2	0.08	1.70	5.90	—	—	0.98	8.17	8.69
2	14.96	443.5	0.654	19.35	43.79	467.7	58.9	0.96	35.70	50.10	0.94	0.18	0.89	9.00	9.83
3	14.83	450.6	0.446	41.29	45.92	497.4	65.5	0.55	37.50	54.70	0.98	0.11	0.88	9.49	10.12
SED	0.240	14.69	0.011	–	·	11.09	8.38	0.25	3.73	6.28	0.003	0.052	0.015	0.482	0.912
Statistical significance	NS	NS	NS	—	· ·	**	**	NS+	**	**	NS	NS	**	NS	NS

DM : Dry matter
OM : Organic matter
NS : Not significant
NS+ : $P \sim 0.1$
* : $P < 0.05$
** : $P = 0.01$
† : Treatments 1, 2 and 3 refer to the levels of starch and cellulose infused: 1 = nil; 2 = 25 g starch, 50 g cellulose; 3 = 50 g starch, 50 g cellulose
⁂ : Infused at terminal ileum: intakes expressed on DM basis
⌀ : Estimated as acid-detergent fibre (ADF)

Table 2. Nitrogen intakes, excretion and retention expressed per unit metabolic bodyweight ($kg^{0.75}$)

Treatment T	Mean liveweight ($kg^{0.75}$)	N intake (mg / $kg^{0.75}$ / d)	Faecal N (mg / $kg^{0.75}$ / d)	Urinary N (mg / $kg^{0.75}$ / d)
1	14.98	448.4	21.8	357.5
2	14.98	465.0	54.3	361.9
3	14.83	446.9	74.7	342.8
SED	0.240	11.93	8.29	16.14
Statistical significance	NS	NS	**	NS

N : Nitrogen
NS : Not significant
* : P—0.05
** : P—0.01
T : See Table 1 for description of treatments

Table 3. Mean daily excretion of nitrogen during days 3—5 inclusive of N-free infusion. Each value is the mean of 4 observations (Treatments 1 and 3) or 3 values (Treatment 2)

Treatments	Mean liveweight ($kg^{0.75}$)	Energy intake (kJ / $kg^{0.75}$ / day)	Urinary N (mg / $kg^{0.75}$ / day)	Faecal N (mg / $kg^{0.75}$ / day)	Total N (mg / $kg^{0.75}$ day)
1	14.94	351	209	22	227
2	14.90	354	222	54	274
3	14.78	377	188	76	265
SED	0.371	18	24	13	13
Statistical significance	NS	NS	NS	*	NS+

N : Nitrogen
NS : Not significant
NS+ : $P<0.1$
* : $P<0.05$

and cellulose into the ileum than in those given the control treatment ($P<0.05$). In consequence, total daily N excretion was also higher in the animals given infusion into the hind gut ($P<0.1$).

Mean values for urinary urea-N excretion each day of the 5-day N-free period are given in Table 4.

Urine urea-N tended to be lower on Treatment 3 on days 1 and 2 of N-free intake ($P<0.1$, $P<0.05$) but thereafter did not differ significantly between treatments on successive days, although Treatment 3 values remained consistently lower than those of the other 2 treat-

Table 4. Mean daily excretion of urea-N in urine over 5 successive days in sheep maintained by infusion under N-free conditions and given infusins of starch and cellulose into the termiral ileum. Each value is the mean of 4 observations (Treatments 1 and 3) or 3 values (Treatment 2)

Treatments †	Mean liveweight ($kg^{0.75}$)	Urine urea N ($mg / kg^{0.75} / d$) Days 1	2	3	4	5
1	14.94	195	172	149	135	121
2	14.90	288	190	134	136	138
3	14.78	179	114	103	104	099
SED	0.371	0.032	0.014	0.018	0.017	0.021
Statistical Significance	NS	NS+	*	NS	NS	NS

N: Nitrogen NS: Not significant NS+: $P<0.1$ *: $P<0.05$ †: See Table 1 for description of treatments.

ments. The mean excretion of urea-N as a portion of total urinary N over days 3—5 inclusive was 0.65 and 0.55 for Treatments 1,2 and 3, respectively, but these values did not differ significantly between treatments.

In the proposals formulated in 1976 and published in ARC endogenous urinary nitrogen (EUN) was considered to repsresent the nitrogen requirement for tissue maintenance of ruminants. The EUN may be determined as the urinary nitrogen in experiments where N-N-free diets are given or more precisely under intragastric infusion conditions when infusion of N-free nutrients are given into the rumen and abomasum.

In the present experiment effect of different levels of hind-gut fermention on EUN excretion were examined in sheep nourished by total infusion. Nearly all the nitrogen excretion in these animals was via the urine (Table 3) and EUN was not affected significantly by changing hind-gut fermentation. Faecal nitrogen excretion, in contrast, increased progressively from treatment 1 to treatment 3. Thus the observations of Orskov&Grubb (14) that increases in faecal N excretion in response to infusions of nutrients to the caecum were balanced by reductions in EUN excretion, leaving total N excretions unchanged, were not confirmed in the present work.

Overall mean value of EUN excretion of the three treatment was 206 mg N / kg $^{0.75}$ / d and was higher than the recommendation of ARC (1) in which EUN excretion of sheep is estimated from the rela-

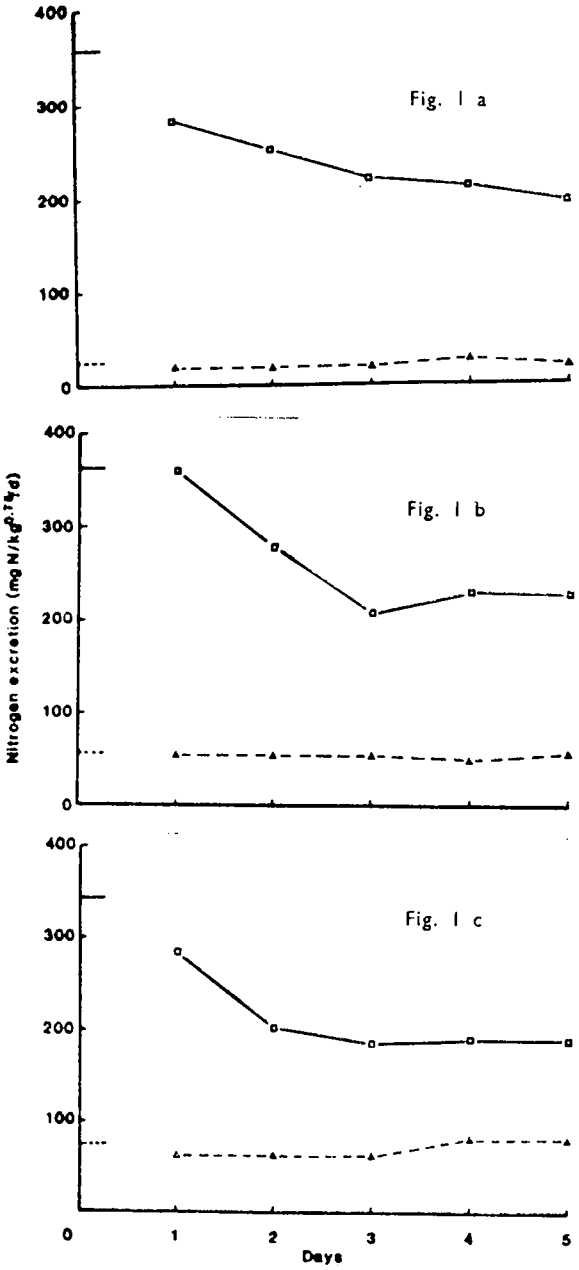

Fig 1: Mean daily excretions of nitrogen in urine (□) and faeces (△) in sheep given N-free intakes over 5 successive days and infusion of starch and cellulose into terminal ileum. The levels of hind gut infusion were nil (Fig. 1 a), 25 g starch and 50 g cellulose (Fig. 1b) and 50 g starch and 50 g cellulose (Fig. 1c). The mean daily excretion of N in urine and faeces over 5 days when N intake was sufficient for maintenance (Table 2) are also shown against the left hand axis.

tionship EUN = 0.2348 W + 0.54 g / d where W is the liveweight (kg) of the animal. Using this relationship in this experiment, the EUN excretion in sheep of 37—39 kg liveweight calculated to be 140—145 mg N / kg 0.75 / d. In contrast, the EUN reported here was considerably less than the values reported by Orskov&Grubb (14), and by Hovell et al. (7) using totally infused sheep (427 and 429 mg N / kg $^{0.75}$ / d, respectively) and is lower also than the mean value of 350 mg N / $^{0.75}$ / d recommended mean value of ARC (2). It is concluded from the results of this experiment that endogenous urinary N excretion showed a mean overall value of 206 mg N / kg $^{0.7}$ / d and did not change significantly in response to change in hind-gut fermentation.

References

1. **Agricultural Research Council** (1980): *The Nutrient Requirement of Ruminant Livestock.* Commonwealth Agricultural Bureaux, Slough.

2. **Agricultural Research Council** (1984): *The Nutrient Requirements of Ruminant Livestock, Suppl.* No. 1. Commonwealth Agricultural Bureaux, Slough.

3. **Association of Official Agricultural Chemists** (1975): *Methods of Analysis.* Washington, D.C.

4. **Bergmeyer H.U.** (1963): *Methods of Enzymatic Analysis,* P. 123. Academic Press, New York London.

5. **Davitson, J., Mathieson, J. & Boyne, A.W.** (1970): *The use of automation in determining nitrogen by the Kejldahl method, with final calculation by computer.* Analyst. 95, 181—193.

6. **Hobson, P.N.** (1969): *Rumen Bacteria. Methods in Microbiology* 3B, 133—149.

7. **Hovell ,F.D. DeB., Orskov, E.R., Grubb, D.A. & Macleaod, N.A.** (1983): *Basal urinary nitrogen excretion and growth response to supplemental protein by lambs close to energy equilibrium.* Br. J. Nutr. 50, 173—187.

8. **Hovell, F.D. DeB** (1985): *Protein digestion by ruminants.* In: Feedingstuffs Evaluation (Livingstone, R.M., ed.), p. 49. Rowett Research Institute. Aberdeen. (Feeds publication No. 1).

9. **Leininger, H.V.** (1976): *Equipment, media, reagents, routine tests and stains.* In: Compendium of Methods for the Microbiological Examination of Foods (Speck, M.L., ed), p. 57, American Public Health Association.

10. **Macleod, N.A., Corrigal, W., Stirton, R.A. & Orskov, E.R.** (1982): *Intragastric Infusion of nutrients in cattle.* Br. J. Nutr. 47, 547—554.

11. **MacRae, J.C., Reid, C.S.W., Dellow, D.W., Wyburn, R.S.** (1973): *Caecal cannulation in the sheep.* Res. Vet. Sci. 14, 78—85.

12. **Orskov, E.R., Foot, M.H.** (1969): *The Influence of caecal starch infusion in sheep on faecal output of nitrogen, starch and dry matter.* proc. Nutr. Soc. 28, 31A.

13. **Orskov, E.R., Fraser, C., Mason, V.C., Mann, S.O.** (1970): *Influence of Starch digestion in the large intestine of sheep on ceacal fermentation, caecal microflora and faecal nitrogen excretion.* Br. J. Nutr. 24, 671–682.

14. **Orskov, E.R., Grubb, D.A.** (1978): *The minimal nitrogen metabolism of lambs.* Proc. Nutr. Soc. 38, 24A.

15. **Orskov, E.R., Grubb, D.A., Wenham, G., Corrigall, W.** (1979): *The sustenance of growing and fattening ruminants by intragastric infusion of volatile fatty acids and protein.* Br. J. Nutr. 41, 553–558.

16. **Orskov, E.R., Macleod, N.A.** (1982): *The determination of minimal nitrogen excretion in steers and dairy cows and its physiological and practical implication* Br. J. Nutr. 47, 625–636.

17. **Roy, J.H.B., Balch, C.C., Miller, E.R., Smith, R.H.** (1977): *Proceedings of 2nd EAAP Symposium on Protein Nutrition and Metabolism.* European Association for Animal Production Publ. No. 22, Wageningen, Puduc., pp. 126–129.