Practical Application of Defaunation of Cattle on Farms in Vietnam: Response of Young Cattle Fed Rice Straw and Grass to a Single Drench of Groundnut Oil

Nguyen Thi Hong Nhan, Nguyen Van Hon, Nguyen Trong Ngu, Nguyen Tien Von¹, T. R. Preston* and R. A. Leng²

College of Agriculture, Cantho University, Vietnam

ABSTRACT: Farmers in the centre of Vietnam have a tradition of dosing young cattle with groundnut oil before fattening them on a diet of rice straw and road-side grass. These farmers claim the cattle grow faster. It was hypothesized that the effect of the oil could be to eliminate the protozoa from the rumen. This is known to increase the net microbial growth efficiency in the rumen and increase the protein supply to the animal. To test this hypothesis, two experiments were undertaken; one on-station with four cattle fitted with rumen cannulae and the second a growth trial with 25 young cattle in smallholder farms. When the cannulated animals were drenched with groundnut oil, the protozoa were eliminated from the rumen and animals could be kept free of protozoa by isolation. The ammonia concentration in the rumen fluid was decreased when the protozoa were eliminated and there was an indication of improved rumen dry matter degradability of the forage components of the diet. In the practical condition on the smallholder farms, the growth rates of cattle drenched with groundnut oil were increased considerably (65%) compared with untreated control animals. The laboratory results when taken together with the on-farm results indicate that these resource-poor farmers had been able to defaunate their cattle and to maintain the fauna-free state by isolation of their animals from extraneous stock. This traditional practice in Central Vietnam, whereby one family keeps only one or two animals that are hand fed and tethered, has quite a large potential for all of those countries where animals are fed agro-industrial by-products, as it is highly economic. The use of I litre of oil compared with 1 kg of rice polishing per day (300 kg over 300 days), would be highly profitable in all countries of South-East Asia. (Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 4: 485-490)

Key Words: Cattle, Groundnut Oil, Defaunation, Rumen Environment, Growth Rate

INTRODUCTION

Forages of low digestibility, such as rice straw and mature tropical grasses, are generally deficient in nutrients that are critical to the growth of microorganisms in the rumen of cattle. The efficient utilisation of these forages, therefore, requires a strategy of supplementation that ensures an efficient rumen ecosystem which in turn optimises microbial growth (Preston and Leng, 1987). In general, a source of ammonia and essential minerals are required for growth of the diverse groups of organisms in the rumen. Once the rumen ecosystem has been provided with optimal nutritional conditions, microbial growth is optimised and may approach the theoretical maximum per unit of organic matter fermented in the rumen for microbial growth under anaerobic conditions. However, the amount of microbial protein available post-rumen is somewhat less than that synthesised in the rumen

The rumen is an ecosystem in which the microorganisms interact both with positive effects, when as a consortium they degrade complex fibrous components of the feed, and negatively as some compete for nutrients and others are obligate or opportunistic predators on other organisms. Protozoa ingest and digest rumen bacteria (Coleman, 1975) and also graze on feed particles. Following engulfment and digestion of bacteria they release a proportion of the products to the medium and in this way appear to cause the lysis of many more bacteria than is necessary to meet their nutrient requirements. The potential of the protozoa to consume bacteria is enormous. Coleman and Sanford (1979) calculated that at high levels of protozoa in the rumen that 108 bacteria could be engulfed per hour per ml of rumen fluid and Nolan (1988) calculated this was enough to consume all the bacteria in the rumen in one day.

Protozoa are able, to a varying degree, to avoid washout from the rumen to the lower tract (Weller and Pilgrim, 1972). Since they have a finite lifespan, a proportion of the protozoa die in the rumen and are degraded by the resident microbes (Leng, 1982). The protein of their cells and the protein in the bacteria they consume are thus unavailable for digestion by the animal. In addition their ability to ingest feed particles

because of lysis and degradation of organisms within the rumen (Leng and Nolan, 1984).

^{*} Corresponding Author: T. R. Preston. University of Tropical Agriculture, Royal University of Agriculture, Phnom Penh, Cambodia. Tel: +855-23-365445, E-mail: trpreston@uta.edu.kh.

¹ College of Agriculture and Forestry, Hue University, Vietnam.

² University of Tropical Agriculture, Royal University of Agriculture, Phnom Penh, Cambodia.

Received September 4, 2000; Accepted December 2, 2000

486 NHAN ET AL.

and hydrolyse protein increases the percentage of the dietary protein that is degraded in the rumen (Ushida et al., 1990). It appears therefore that the presence of protozoa reduces the availability of both microbial protein and dietary protein for digestion in the lower gut (Veira et al., 1983).

Straw and mature tropical grasses are often low in total protein and almost totally devoid of dietary protein that escapes the rumen. It is now well recognised that on such diets the efficiency of growth of ruminants is limited by the low availability of protein absorbed from the small intestine (Chenost and Kayouli, 1997). Young cattle increase their rate of live weight gain when supplemented with protein meals that contain bypass protein under these circumstances (Preston and Leng, 1987; Leng, 1990).

Bird and Leng (1978) first demonstrated with cattle on low protein diets that growth rate and efficiency of growth were higher in fauna-free cattle then in animals on the same diet that were normally faunated. A number of studies have since confirmed that ruminants use their feed more efficiently when protozoa are absent from the rumen and there are sub-optimal levels of bypass protein in the diet (Bird, 1989). These studies were the stimulus to research to find methods to defaunate ruminants in production systems but none appears to have been successful to the present time.

The studies reported here refer to the effects of groundnut oil given as a single drench on rumen protozoa in young cattle. A method is described that removes protozoa from the rumen of cattle and maintains them free of rumen protozoa. Results of a growth trial under smallholder farmer management show the considerable growth advantage to cattle that are free of rumen protozoa. This research appears to be the first practical application of defaunation under field conditions.

MATERIALS AND METHODS

Experiment 1: Effects of groundnut oil on the rumen ecosystem

Treatments and design. Four local "Yellow" heifers (Bos taurus) of 150 to 200 kg live weight, fitted with permanent rumen cannulae, were allocated to four treatments according to a 2×2 factorial arrangement as follows:

- Two basal diets: rice straw or rice straw and grass (50:50 DM basis)
- · Drenched or not drenched with groundnut oil as anti-protozoal agent

The individual treatments were

- · RS: Rice straw ad libitum
- · RSO: Rice straw ad libitum plus 1000 ml of

groundnut oil given as a single drench at the beginning of the experiment

- · RSG: A 50:50 mixture DM basis of rice straw and native grass Brachiaria mutica ad libitum
- · RSGO: Same as RSG but with 1000 ml of groundnut oil given orally as a single drench at the beginning of the experiment.

The diets were given in consecutive periods. Thus all the four animals were fed rice straw for 20 days, beginning 5 days before administration of the oil (RSO and RSGO) and for 15 days afterwards. The diet was then changed to the mixture of rice straw and grass (50:50 DM basis) and the animals were infected with rumen protozoa and allowed a period of 2 weeks to re-faunate. The process of administering the oil was then repeated over a further 20 day period.

Diets: On the RS treatment the amount of straw offered was adjusted daily so as to minimise refusals. On the RSG treatment, the straw was ad libitum but the grass was restricted to maintain the 50:50 ratio (DM basis) of the straw and the grass.

Oil treatment: The animals on RSO and RSGO were fasted from 2.00 pm on day [-1] until 7.30 am on day [0]. The groundnut oil purchased in the local market was given at 7.00 am on day [0] in a single drench from a bottle.

Experimental procedures: The animals weighed on day [-5]. Feed offered and refused was recorded daily. Samples of rumen fluid 100 ml were taken by aspiration using a plastic tube and syringe every day at 07.00 am, 09.00 am and 01.00 pm. Protozoal numbers were counted immediately (0.2 mm deep chamber), pH was determined (digital pH meter) and ammonia measured by steam distillation of a sample of 20 ml of rumen fluid, with collection of the ammonia in boric acid solution and titration with 0.1 N H₂SO₄. Rumen degradability of the dietary ingredients was measured by a modification of the method described by Ørskov and Hovell (1980). Triplicate samples of the dried and ground (2 mm screen) forages (rice straw, Sacciolepis interrupta and Brachiaria. mutica grasses) were incubated in the rumen of each of the 4 animals at intervals in relation to the day the oil was administered: i.e. on days [-5, 5, 10 and 15]. Bags were removed at 12, 24 and 48 h after insertion in the rumen, washed in running water and dried at 100°C for 24 h.

Statistic analysis: Values of pH, protozoal numbers and ammonia were averaged over sampling times (07.00, 09.00 and 13.00h) and periods of 5 days ("before"=days -5 to -1; "5"=days 5 to 9; "10"=days 10 to 14; and "15"=days 15 to 19) and analysed by the General Linear Model of the ANOVA programme in the software of Minitab version 11. Variables were:

days, diets, oil treatment and error.

Experiment 2: Effects of groundnut oil on the growth of cattle fed rice straw in smallholder farms

Location: The trial was done in Angiang Province in the Mekong delta in Tinh Bien district, a mountainous area close to the border with Cambodia. Fifteen farm households participated in the study.

Treatments and design: Three treatments were compared in a population of 25 local "Yellow" cattle of mixed sexes (range of initial weight 100 to 180 kg) fed individually in separate pens (1 or 2 animals per household) on rice straw with restricted dry-season grazing (2 to 4 h/day).

The treatments were:

GNO: Administration of 1000 ml of groundnut oil as a single drench at the beginning of the experiment (5 female and 5 male cattle on 5 farms)

RB: Rice bran at 1 kg/day (4 female and 6 male cattle on 5 farms)

Control: No oil treatment or rice bran (2 female and 3 male cattle on 5 farms)

The administration of the groundnut oil followed the same procedure as outlined in experiment 1. The animals were allowed to graze in isolation from other livestock on native pasture for 2 to 4 h daily during the dry season and received rice straw ad libitum when confined in the household the remainder of the day. The animals were weighed individually at the beginning of the trial and subsequently after 30 and 60 days. Weighing was at 7.00 am prior to feed being offered but with free access to water.

RESULTS

Effects of groundnut oil on the rumen ecosystem

The numbers of protozoa in the rumen liquid decreased to zero at 12 h after administration of the oil and were still absent 15 days later. Rumen ammonia concentrations and rumen pH were significantly lower following oil treatment. These changes were consistently manifested on both the rice straw and the rice straw/grass diets.

Effect of groundnut oil on dry matter degradability of rice straw and grass

In the periods when there were no protozoa in the rumen, the dry matter loss from nylon bags in the rumen was significantly greater than in the period when protozoa were present. It was also apparent that the rate of dry matter loss was greater when 50% of the rice straw was replaced by fresh grass. Similar effects due to the addition of grass to a diet of low digestibility were reported by Guttierrez and Elliott (1978). They fed sheep a basal diet of sisal pulp with or without supplementation with African Star grass

(Cynodon nlemfuens).

Effects of a single drench of groundnut oil on the growth rate of cattle fed rice straw in smallholder farms

The cattle fed 1 kg of rice bran daily, or which were drenched once with 1 litre of groundnut oil at the beginning of the fattening period, grew faster (p<0.05) than the control animals that received only rice straw (figure 1). There were no differences between animals supplemented with rice bran and those drenched with groundnut oil.

DISCUSSION

The research published here was stimulated by observations of farmer practice in Vietnam. Farmers in Central Vietnam specialise in cattle fattening; they purchase store cattle and fatten them under hand feeding conditions using a combination of grass and straw usually supplemented with rice bran. It was observed by one of the co-authors (Nguyen Tien Von, 1999, unpublished observations) that some farmers would drench these animals on entry to the farm with one litre of vegetable oil. These animals appeared to grow at a much faster rate than other cattle in the area. Subsequent experimentation in the laboratory of the senior author in Cantho University has shown that young cattle drenched once with groundnut oil were free of protozoa in the rumen and that under smallholder farm conditions animals drenched with the

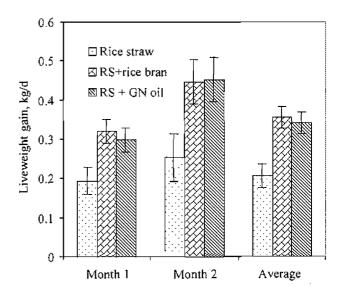


Figure 1. Mean values for growth rates over a period of two months of cattle fed rice straw (RS) with restricted grazing and supplemented with 1 kg/d rice bran, drenched with 1 litre groundnut oil (GN), or neither supplemented nor drenched (five farms per treatment)

Table 1. Mean values for ammonia, protozoal population and pH of rumen fluid in cattle before and after drenching with groundnut oil

Diets		Time, h	Before	5 days	10 days	SE/P1
Rice straw	N-NH ₃ mg/liter	0	114	96	74	7.38/0.016
		2	109	103	79	5.35/0.01
		6	101	98	77	2.22/0.001
	Protozoa,	0	0.45	0	0	0.05/0.001
	$\times 10^{.5}$ /ml	2	0.40	0	0	0.05/0.001
		6	0.40	0	0	0.032/0.001
	pН	0	7.4	7.32	7.3	0.036/0.03
	•	2	7.6	7.48	7.4	0.044/0.044
		6	7.5	7. <u>48</u>	7.4	0.029/0.015
	DM intake, kg/d		2.52	2.22	2.49	0.09/0.08
Rice straw+	N-NH ₃ mg/liter	0	193	178	133	11.25/0.013
Grass		2	242	201	· 1 78	12.99/0.028
		6	215	191	164	11.4/ 0.038
	Protozoa,	0	1.55	0	0	0.14/0.001
	$\times 10^{.5}$ /ml	2	1.22	0	0	0.12/0.001
		6	1.60	0	0	0.18/0.001
	<u></u> рН	0	7.37	7.23	7.15	0.07/0.13
. •	-	2	7.27	7.17	7.06	0.08/0.28
		6	7.32	7.03	7.0	0.07/0.019
	DM intake, kg/d		3.73	3.32	3.47	0.15/0.21

Standard error of mean/probability level.

oil grew significantly faster than animals that received no oil.

The results of this study are interpreted as confirmation that rumen protozoa reduce the efficiency with which ruminants use low digestibility forage for growth. In this case the animals under smallholder farm conditions that were drenched with groundnut oil grew faster than similar animals not given the oil and as fast as those fed a supplement of 1 kg of rice bran per day.

The laboratory studies with similar animals and diets showed that the oil completely eliminated the rumen protozoa, presumably by direct action of oil on the protozoa. Oil has been shown to have toxic effects on rumen protozoa (Newbold and Chamberlain, 1988) and high levels of oil in fibrous feeds may directly inhibit bacterial growth in the rumen (Devendra and Lewis, 1974) and on gas production either indirectly by reducing bacterial cell degradation in the rumen or directly by reducing bacterial fermentative capacity (Machmuller et al., 2000).

In cattle fed on low digestibility forage, supplementation with whole cottonseed containing 20% oil in increasing quantities will stimulate productivity until the oil content approaches a critical level when digestibility of fibre is reduced (Bird and Dicko, 1987). The effects of vegetable oils in the rumen are well known. The oils are rapidly hydrolysed to long chain fatty acids which either coat fibre, preventing

access to attachment sites for bacteria and perhaps the zoospores of the fungi, or they are directly toxic to all organisms.

The effects of the oil in the cattle in the studies reported here were obviously detrimental to the rumen organisms as it was observed that the bacterial populations were also reduced soon after drenching with oil (Nguyen Thi Hong Nhan, unpublished observations). However, the bacteria rapidly recovered as can be seen by the data for in sacco rumen digestibility which showed higher rates of dry matter loss from fibrous substrates in the periods 5-10 and 10-15 days following the oil treatment and when protozoa were shown to be absent. The effects of the protozoa-free state on digestibility of forage have been reported as: not changing, having been reduced and of being increased, compared with faunated animals on the same diets (Ushida et al., 1989). Soetanto et al. (1985) reported increased fungal activity in the rumen of sheep that were fauna-free compared to normal sheep suggesting that protozoa may reduce fungal infection of plant materials in the rumen by selectively ingesting their zoospores (Orpin, 1988). Anaerobic fungi play a major role in comminution of fibre particles in the rumen and any inhibition of their growth can reduce feed intake considerably (Gordon and Phillips, 1998). Anaerobic fungi increase in numbers in the absence of protozoa in the rumen (Orpin, 1977; Soetanto et al., 1985; Newbold and

Hillman, 1990; Romulo et al., 1989; Ushida et al., 1990) and this could account for the increased digestibility following defaunation with oil.

The results of the digestibility studies indicate that defaunation benefited the animal by increasing digestibility of the basal feed in addition to the well recognized effects of increased microbial protein availability to the lower tract and therefore an overall increase in availability of amino acids to the animal (Bird, 1989). This latter statement is well supported by the trends in rumen ammonia levels in the rumen which were much reduced in the absence of protozoa indicating an increased net uptake of ammonia by bacteria in the rumen. The ammonia levels in rumen liquid at 0, 2 and 6 h after feeding decreased significantly when measured at 5 and 10 days after oil administration. According to Hino and Russell (1987), activities have high deaminase eliminating them from the diet would be expected to lead to reduced ammonia levels. Similar reductions in ammonia concentrations due to defaunation of sheep and cattle on a variety of diets have been reported by Eadie and Gill (1971), Bird (1982) and Veira et al.

There has been a major research effort to find feed additives that control protozoa (Leng et al., 1992)

Table 2. Rumen DM loss (%) from rice straw, S. interrupta and B. mutica substrates in cattle before and after drenching with groundnut oil

Sustrate	Time,	Before	5	10	SE/P'				
	h		days	days					
Basal diet of rice straw alone									
Rice straw	12	8.9	9.9	11.3	0.56/0.051				
	24	11.2	12.8	16.0	0.50/0.001				
	48	17.5	22.3	25.9	1.43/0.01				
S. interrupta	12	15.6	15.8	17.3	0.45/0.061				
	24	17.4	17.6	21.1	0.71/0.011				
	48	24.4	28.6	31.1	1.24/0.015				
B. mutica	12	14.6	16.3	19.7	1.24/0.048				
	24	17.3	19.7	23.7	1.11/0.01				
	48	25.1	26.8	31.6	1.54/0.048				
Basal diet rice straw + grass									
Rice straw	12	9.4	12.3	14.8	0.76/0.004				
	24	15.4	22.1	26.5	2.07/0.016				
	48	33.8	39.8	45.3	1.33/0.001				
S. interrupta	12	19.5	20.0	23.8	1.19/0.066				
	24	26.0	30.7	33.2	1.7/0.045				
	48	46.2	50.4	57.4	2.64/0.046				
B. multica	12	19.8	22.4	24.7	1.35/0.093				
	24	26.7	33.0	36.5	2.27/0.044				
	48	41.6	47.6	54.9	2.87/0.033				

Standard error of mean/probability level.

and/or to develop immunological methods to create the fauna-free state which are being kept secret. However, a vaccine is not likely to be available until the distant future and almost certainly will be expensive and beyond the reach of smallholder farmers in developing countries. Furthermore, it may be inappropriate to maintain animals permanently in a fauna-free state as, in developing countries, there are times when the low N requirements of protozoa would be beneficial in maintaining the production of volatile fatty acids. This applies particularly to mature working animals that have a higher demand for energy substrates than animals in other productive states.

The need is to be able to induce the defaunated state to increase protein availability when animals are expected to be productive and are receiving a diet supportive of efficient rumen function but containing little bypass protein. The important issue is that in those countries where cattle depend on straw as their main feed supply, their productivity can be increased by the relatively simple procedures of drenching with oil to remove protozoa from the rumen, and hand feeding in relative isolation to prevent re-infestation.

Many methods are available to increase the feeding value of straw of which ammoniation is the most widely studied (Chenost and Kayouli, 1997). However, with the exception of China (Zhang Weixian et al., 1994), there has been little impact of this technology at smallholder farmer level. The results reported here, together with observations of farmer practice, suggest that it is economical to use the oil drench to defaunate ruminants fed mainly on rice straw as one drench (one litre of oil) gave the same benefits as feeding 1 kg per day of rice bran over a 100 day period.

ACKNOWLEDGEMENTS

We are grateful to the farmers in Angiang Province for their cooperation in carrying out the on-farm trial. We acknowledge the support of Cantho University in providing the facilities for the on-station experiment.

REFERENCES

Bird, S. H. 1982. Studies on the relationship between rumen protozoa and production in sheep and cattle. Ph. D. Thesis. University of New England: Armidale, Australia.

Bird, S. H. 1989. Production from ciliate free ruminants. In: The Roles of Protozoa and Fungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer). Penambul Books, Armidale, Australia. pp. 223-246.

Bird, S. H. and R. A. Leng. 1978. The effects of defaunation of the rumen on the growth of cattle on low protein high energy diets. Br. J. Nutr. 40:163-167.
Bird, S. H. and M. Dicko. 1987. Cottonseed supplements for

490 NHAN ET AL.

sheep. In: Recent Advances in Animal Nutrition in Australia (Ed. D. J. Farrell). University of New England, Armidale, Australia. pp. 82-83.

- Chenost, M. and C. Kayouli. 1997. Roughage utilisation in warm climates. FAO Animal Production and Health Paper 135, FAO, Rome.
- Coleman, G. S. 1975. Inter-relationship between rumen ciliate protozoa and bacteria. In: Digestion and Metabolism in the Ruminant (Ed. I. W. McDonald and A. C. I. Warner). University of New England, Armidale, Australia. pp. 149-164.
- Coleman, G. S. and T. Sandford. 1979. The engulfment and digestion of mixed rumen bacteria and individual bacterial species by single and mixed species of rumen ciliates protozoa grown in vivo. J. Agric. Sci. Camb. 92:729-740.
- Devendra, C. and D. Lewis. 1974. Fat in the ruminant diet; a review. Indian J. Anim. Sci. 44:917-935.
- Eadie, M. J. and J. C. Gill. 1971. The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage-concentrate diet. Br. J. Nutr. 26:155-167.
- Gordon, L. R. and M. W. Phillips. 1998. The role of anaerobic fungi in ruminants. Nutr. Res. Rev. 11:133-168.
- Guttierrez, E. and R. Elliott. 1984. Interaccion digestiva de la pulpa de henequen Agave fourcroydes y el pasto Estrella de Africa Cynodon plectostachyus. In Altenativas y Valor Nutritivo de Algunos Recursos Alimenticios Destinados a Produccion Animal. Informe Provisional No 16. Fundacion Internacional para la Ciencia. Stockholm. 16:229-246.
- Hino, T. and J. B. Russell. 1987. Relative contributions of ruminal bacteria and protozoa to the degradation of protein *in vitro*. J. Anim. Sci. 64:261-270.
- Leng, R. A. 1982. Dynamics of protozoa in the rumen of sheep. Br. J. Nutr. 56:917- 935.
- Leng, R. A. 1990. Factors affecting the utilization of "poor quality" forages by ruminants particularly under tropical conditions. Nutr. Res. Rev. 3:277-303.
- Leng, R. A., S. H. Bird, A. Klieve, B. S. Choo, F. M. Ball, G. Asefa, P. Brumby, V. D. Mudgal, U. B. Chaudry, N. Suharyano and C. Hendramo. 1992. The potential for tree forage supplements to manipulate rumen protozoa to enhance protein to energy ratios in ruminants fed on poor quality forages. In: Legume Trees and Other Fodder Trees as Protein Sources for Livestock. FAO Animal Production and Health Paper 102. FAO, Rome. pp. 177-191.
- Leng, R. A. and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072-1089.
- Machmuller, A., D. A. Ossowski. and M. Kreuzer. 2000. Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. Anim. Feed Sci. Tech. 85:41-60.
- Newbold, C. J. and D. G. Chamberlain. 1988. Lipids as rumen-defaunating agents. Proc. Nut. Soc. 47:154A.

- Newbold, C. J. and K. Hillman. 1990. The effects of ciliate protozoa on the turnover of bacterial and fungal protein in the rumen of sheep. Letters in Appl. Microbiol. 11:100-102.
- Nolan, J. V. 1988. Implications of protozoa and fungi for the nutrition of ruminants. In: The Roles of Protozoa and Fungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer). Penambul Books, Armidale, Australia. pp. 211-222.
- Orpin, C. G. 1977. Studies on the defaunation of the ovine rumen using dioctyl sodium sulphosuccinate. J. Appl. Bact. 43:309-318.
- Orpin, C. G. 1988. Ecology of rumen anaerobic fungi in relation to the nutrition of the host animal. In: The Roles of Protozoa and Tungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer). Penambul Books, Armidale, Australia. pp. 29-38.
- Ørskov, E. R., F. D. DeB. Hovell and F. Mould. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5:195-213.
- Preston, T. R. and R. A. Leng. 1987. Matching Livestock Systems with Available Resources in the Tropics and Subtropics. Penambul Books, Armidale, Australia.
- Romulo, B., S. H. Bird and R. A. Leng. 1989. Effects of defaunation on intake, digestibility, N retention and fungal numbers in sheep fed straw-based diets. In: The Roles of Protozoa and Fungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer). Penambul Books, Armidale, Australia pp. 285-288.
- Soetanto, H., G. L. Gordon, I. D. Hume and R. A. Leng. 1985. The role of protozoa and fungi in fibre digestion in the rumen of sheep. In: Efficient Animal Production for Asian Welfare. AASAP. Society, Seoul, Korea. Volume 2, pp. 805-807.
- Ushida, K. C. Kayouli, S. de Smet. and J. P. Jouany. 1990. Effect of defaunation on fibre and protein digestion in sheep fed on ammonia-treated straw with or without maize. Br. J. Nutr. 64:765-775.
- Ushida, K. J. P. Jouany and D. I. Demeyer. 1989. Effects of presence or absence of rumen protozoa on the efficiency of utilisation of concentrate and fibrous feeds. In: Physiological Aspects of Digestion and Metabolism in Ruminants (Ed. T. Tsuda, Y. Sasaki and R. Kwashima). Academic Press Inc.
- Veira, D. M., M. Ivan and P. Y. Jul. 1983. Rumen ciliate protozoa: effects on digestion in the stomach of sheep. J. Dairy Sci. 86:1015-1022.
- Weller R. A. and A. F. Pilgrim. 1974. Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous in vitro fermentation system. Br. J. Nutr. 32:341-351.
- Zhang Weixian, Gu Chuan Xue, F. Dolberg and P. M. Finlayson. 1994. Supplementation of ammoniated wheat straw with hulled cottonseed cake. Livestock Research for Rural Development. 6:1 http://www.cipav.org.co/lrd/lrd6/1/dolberg61.