

EFFECT OF WATER CONTAMINATION WITH USED ENGINE OIL ON GROWTH RATE, NUTRIENT INTAKE AND RUMEN STUDY OF WEST AFRICAN DWARF RAM

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Abstract

The study was to evaluate the effect of water contamination with used engine oil on growth rate, nutrient intake and rumen study of West African dwarf rams. Thirty (30) growing West African Dwarf Rams, age range between 14-16 months were randomly assigned into five treatment groups designated T0, T1, T2, T3 and T4 respectively, according to the graded levels of used engine oil contaminated water at the following varying percentage of 0.5, 1.0, 1.5 and 2.0 for treatment T1, T2, T3 and T4 respectively. While used engine oil-free water served as experimental material for the control (T0) in a completely randomized experimental design. Result showed that the decrease was according to the increasing levels of used engine oil contamination. The values (kg) are 19.43, 17.26, 15.45, 14.53 and 12.66 for treatment T0, T1, T2, T3 and T4 respectively. The Average weight gain, weight gain and final weight also follow the same trend in progressive decline ($p < 0.05$) as the quality of used engine oil increase among the treatments. The result for nutrient intake shows that, there was significant ($p < 0.05$) as there were decrease in the values from T0 to T4 as an increase level in the percentage inclusion of the water with engine oil contamination. However, the pH, Ammonia and microorganism in the rumen of West African WAD ram offered water contaminated with used engine oil showed significant ($p < 0.05$) difference among the treatment means. Animals on T0 has the highest values ($p < 0.05$) for rumen pH, followed by T1, T2 while T3 was compared significant ($p > 0.05$) with T4. The value for bacteria was found to be highest in T0 and T1 and lowest in T4. The value recorded for fungi, T0 had the highest values, followed by T1. Fungi for T2 was comparable ($p > 0.05$) with T3 while T4 had the least value. The value obtained for protozoa was highest in animals T2, followed by T0, T2 while T3 compared significantly ($p > 0.05$) with T4 as the lowest values. It was recorded from the results that sheep exposed to contamination of drinking water with used engine oil at varied percentage up to 10% can affect the water intake, feed intake, nutrient utilization and productivity of ruminants since the animal will not effectively utilize the feed.

Key Words: Water contamination, Used engine oil, Growth rate, Nutrient intake, Rumen study, WAD ram

Introduction

Water is an essential nutrient of all animals and total body water is the key component of living ruminants. It is important to be considered in nutrition and it should be available ad-libitum even when sheep are grazing or consuming feed with higher water content (Adolph, 1993). On a daily basis, animals interact with their environments and as a consequence, they are exposed to a broad range of chemical contaminants present in the food they eat, the air they breathe and the water they drink. However, uncontrolled discharges of used engine oil materials into environment is considered to be of great importance to small ruminant animals as they naturally move about while they graze or scavenge for food and drink from accumulated run-off water pools under extensive system of management (Tyson and Sawhney, 1985). For instance, environmental pollution is one of the major worldwide problem that affect human health and livestock productivity. Rajaganapathy *et al.* (2011) reported that heavy metal considers the main cause of environmental toxicity in farm animals. As it contaminates water, food and air with induction harmful hazard on animals by affecting physiological maintenance (i.e. electrolyte balance and osmotic regulation, nutrient transport, intermediary metabolism, lubrication, thermoregulation and excretion of urine and feces), growth, pregnancy and lactation Teresa *et al.*, 1997; Bojkovski *et al.*, 2010).

Digestion in ruminant is depending on ruminal microbes which have ability to synthesis enough amino acid and peptides from the inorganic nitrogen in ammonia (Faixova *et al.*, 2002) that supply 70-100% of amino acid requirement in the form of microbial protein and also have

ability to synthesis 70-85 of energy requirement in the form of short chain fatty acid (Thirumalesh *et al.*, 2013). Ammonia assimilation by rumen microbes depend on rumen pH (Veth *et al.*, 1999), rumen ammonia concentration (Mehrez *et al.*, 1977) and ruminal ammonia assimilating activity. Furthermore, a number of authors have showed that there were deleterious effects of crude oil contaminated forage in rabbit and goat (Berepubo *et al.*, 1994; Ebiegberi, 2009). However, there is paucity of information on the effects of used engine oil toxicity on sheep and goats in available literature. Since used engine oil is becoming uncontrollable, there is the need to evaluate the effect of used engine oil hydrocarbons on sheep and goat because they are at the risk of exposure to toxicity via farmland, feed crop, water and natural pastures. This study was therefore designed to evaluate the effect of water contamination with used engine oil on growth rate, nutrient intake, and rumen microbial population of West African dwarf ram.

Materials and Methods

Location of Study

The experiment was conducted at the Teaching and Research Farm, College of Agricultural Sciences, Olabisi Onabanjo University, Yewa Campus, Ayetoro, Ogun State, Nigeria. The university campus is located in a deciduous/derived savannah zone of Nigeria at latitude 7°15' N and longitude 3°3' E. Climate is sub-humid tropical with an annual rain-fall of 1,909.3 mm. Rainy season is between early April and late October. Rainfall pattern is bimodal with two peaks in June and September. Maximum temperature varies between 29°C during the peak of the wet season and 34°C at the onset of the

dry season and mean annual relative humidity is 81% (Onakomaiya *et al.*, 1992).

Management of the Experimental Animals

Thirty (30) growing West African Dwarf Rams, age range between 14-16 months with an average weight 8.40kg were used for the experiment. The experimental animals were purchased from a sheep market in Igan-Okoto, a town adjacent to the University Campus and acclimatized for 14 days. The animals were treated against endoparasites (dewormed) with levamisole injection at the concentration of 1ml/20kg body weight. Diazintol and ivermectin were used at the concentration of 2ml/litre of water for treating the animals against ectoparasites. During this period the animals were group fed with forage (*Panicum maximum*) in the morning and supplemented with whole cassava root meal based concentrate in the evening. Clean and cool water was also offered *ad libitum*.

At the commencement of the trial, the pens were fumigated with Izal (saponated creosol) at the rate of 1 part to 200 part of water to prevent external parasites. Animals were housed in individual pens (1.2mx1mx1.2m) which was equipped with both water and feed troughs. Wood shaving was used as bedding to absorb waste materials. The bedding materials were changed weekly and the pens were disinfected before supplying another bedding material.

Water Preparation and Experimental Design

Used engine oil was obtained from auto-mechanic workshops in Ayetoro, Ogun State, Nigeria. It was thoroughly

mixed together to obtain a homogenous mixture and then used to deliberately contaminate the animals drinking water at the following varying percentage of 0.5, 1.0, 1.5 and 2.0 for treatment T1, T2, T3 and T4 respectively. While used engine oil-free water served as experimental material for the control (T0). 0.5% water contamination contained 5ml of used engine oil per litre of water. 1.0% water contamination contained 10ml of used engine oil per litre of water, 1.5% water contamination contained 15ml of used engine oil per litre of water and 2.0% of water contamination contained 20ml of used engine oil per litre of water.

The experimental animals were randomly assigned into five treatments each of six growing rams in a complete randomized design. Diet consisted of *P. maximum*, forage offered in the morning around 9.00am and whole cassava root meal based concentrate as supplementary feed in the evening around 3.00pm. 4 litres of used engine oil contaminated water at 0ml/litre, 5ml/litre, 10ml/litre, 15ml/litre and 20ml/litre were offered to animals on T0, T1, T2, T3 and T4 respectively.

Quantity of feed offered and refused was measured daily to determine feed intake. Also, quantity of clean and cool water and contaminated water offered and refused were measured daily to determine the quantity of water consumed. Animals were weighted bi-weekly, feed and water adjusted accordingly. The growth trial lasted for 91 days. The behaviours and clinical signs of the animals were also monitored on a daily basis. The percentage compositions of experimental diets are shown in Table 1 below.

Table 1: Percentage composition of experimental diet

Ingredient	Percentage
Maize	36.8
GNC	8.6
Wheat Offal	46.6
Rice Husk	4.0
Bone Meal	2
Oyster Shell	1
Premix	0.5
Salt	0.5
Total	100

Collection of Rumen Content

Suction strainer was used to collect rumen fluid from the experimental animals according to the procedure used by Raun and Burroughs, (1962). The suction strainer was attached to a suction line of 3ft in length and ¼ inch outside-diameter tumbling to which a 50ml hypodermic syringe was connected. The suction line was passed inside a ¼ by 18 inches rubber guide tube. The rubber guide tube with the strainer projecting out of the end was passed over the tongue and epiglottis into the forward part of the oesophagus, from this point the strainer with the connected tumbling was released and allowed to pass on down the oesophagus into the rumen. The suction line was then used to draw out 30ml of rumen fluid was drawn and then discharged back into the rumen. This then served to flush the suction line prior to collecting the sample to be used for analysis. Following the collecting of the samples, the syringe was disconnected from the suction line; the line was blown out and drawn up the oesophagus until it butted against the guide tube at which time

the complete assembly was withdrawn from the animal. The sample was collected inside sterile sample bottles and stored in an ice spark for further analysis.

Microbial Analysis of the Rumen content

The total viable bacteria counts was determined using N.A while the fungal counts was determined using PDA. The media were prepared according to the manufacturer instruction and sterilized using autoclave at 121°C for 15minutes. The protozoa count was analysed microscopically using wet preparation method. A drop of the serially diluted sample was put on a slide and covered with cover slip. The protozoa present in the sample were done by observing the wet preparation under the microscope using X10 and X40 objective.

Chemical Analysis

Determination of proximate composition of forage and concentrate samples were carried out according to the official procedures (A.O.A.C., 2002), while Neutral detergent fibre, acid detergent fibre, and Acid detergent lignin measurements were carried out according to Van Soest *et al.* (1991). They include dry matter, organic matter, crude protein, crude fibre, ether extracts, ash, and nitrogen free extracts. The rumen content volatile fatty acids and ammonia were determined on thawed and centrifuged sample (3000Xg for 15minutes) using Conway (1962) techniques.

Statistical Analysis

Data obtained from the treatment were analysed variance using Duncan multiple range test (Duncan, 1995).

Results and Discussion

The chemical composition of the experimental diets is presented in Table 2. The nutritive value of the forage was in agreement with Eniolorunda *et al.* (2008), but were lower than the values reported by

Ajaji *et al.* (2005). This might be due to the soil, season and stages of maturity at the duration of harvest and attributed to the increases in the fibre content of the forage (Cleake and Bull, 1986).

Table 2 Chemical composition of experimental diet fed to West African dwarf Ram

Parameters	<i>Panicum maximum</i>	Concentrate
Dry Matter	75.25	87.26
Organic Matter	63.00	79.90
Crude Protein	7.09	18.20
Crude Fibre	31.20	8.75
C. Fat	3.20	4.50
ASH	12.25	7.30
NFE	46.26	61.25
NDF	91.82	49.62
ADF	37.30	30.45
ADL	9.21	14.99

Table 3: Performance characteristics of West African dwarf rams offered water contaminated with used engine oil

Parameters	T0	T1	T2	T3	T4	SEM
Forage (g/head/day)	792.86 ^a	642.86 ^b	607.14 ^b	585.71 ^b	542.86 ^b	5.11
Concentrate (g/head/day)	543.37 ^a	533.57 ^b	514.29 ^c	515.74 ^c	508.57 ^c	9.65
Total feed intake	1336.23 ^a	1176.43 ^b	1121.43 ^b	1101.45 ^b	1051.43 ^b	51.71
Initial weight (kg)	8.40	8.40	8.50	8.40	8.50	0.10
Final weight (kg)	19.43 ^a	17.26 ^b	15.45 ^b	14.53 ^c	12.66 ^d	1.75
Weight gain	11.03 ^a	8.86 ^b	6.95 ^c	6.13 ^c	4.16 ^d	1.87
Average weight	121.21 ^a	97.40 ^b	76.37 ^c	67.36 ^c	45.71 ^d	16.05
Feed efficiency	0.091 ^a	0.083 ^b	0.068 ^c	0.061 ^c	0.043 ^d	0.007
Water	2292.86 ^a	921.41 ^b	600.00 ^b	592.43 ^b	442.86 ^c	226.55
Water Intake (ml of UEO/H/D)	0.00 ^d	4.61 ^c	6.00 ^b	8.87 ^a	8.86 ^a	1.41

^{abcd} means the same row with different superscript are significantly ($p < 0.05$) different

The performance characteristics of West African WAD ram offered water contaminated with used engine oil is presented in Table 3. From the result final weight was significantly ($p < 0.05$) affected by the treatment between the treatment groups. The decrease was according to the increasing levels of used engine oil contamination. The values (kg) are 19.43, 17.26, 15.45, 14.53 and 12.66 for

treatment T0, T1, T2, T3 and T4 respectively. There was significant differences ($p < 0.05$) on the weight gain value between the treatment on the levels of used engine oil increment among the treatments. The animals on T0 (control) 11.03kg had higher weight gain than animals on contaminated water. As a result, there was a progressive decline ($p < 0.05$) on average daily weight gain as

the quality of used engine oil increase among the treatment. The animal on T0 (121.21kg) had the highest values than the animals on contaminated water with used engine oil (97.40, 76.37, 67.36 and 45.71) respectively.

Results obtained from performance characteristics are presented in (Table 3). Significant difference (P<0.05) existed in all parameters tested. Water intake significantly (p<0.05) decreased from T0 through T4 which indicates that water consumption decreased with an increase in the level of used engine oil in the water consumed. It suggests that the contaminated water may be unpalatable as the used engine oil changes the colour of the water and also gives it an unpleasant odour (OECD, 2000). Also, the forage, concentrate and total feed intake (g) decreased as the level of used engine oil increases. The result in the present study was consistent with the observation of (Aregheore and Ng'ambi, 2007) that water quality effects feed consumption, since

low quality water will usually reduce feed consumption. Gupta et al. (1968) observed similar detrimental effects in calves when Dichloro Diphenyl Trichloroethane (DDT) was included in their diets at levels of between 500 – 700 ppm, while lower concentrations did not exert any such adverse effect on feed intake. Crude oil ingestion in one form or another, during the critical development stages is known to depress growth (Rolling *et al.*, 2002). This study showed that since used engine oil are waste materials exposed by auto-technicians and get contacted with channel where animals are exposed to, cause an environmental stressor which might have stimulated ACTH and glucocorticoid synthesis in sheep to enhance these effects that are characteristic of the stress hormones, enzymatic properties, feed intake, body weight gain, feed conversion efficiency and the survival rate of goats were adversely affected by used engine oil contaminated water.

Table 4: Nutrient Intake on dry matter basis by body weight of West African dwarf rams offered water contaminated with used engine oil

Parameter	T0	T1	T2	T3	T4	SEM
Forage (DMI)	59.66 ^a	48.37 ^b	45.68 ^c	45.00 ^c	40.85 ^d	7.51
Concentrate (DMI)	47.41 ^a	46.56 ^b	44.88 ^c	44.07 ^c	44.38 ^c	0.82
Dry matter	107.07 ^a	94.93 ^b	90.57 ^c	89.07 ^{cd}	85.23 ^d	9.21
Organic matter	93.37 ^a	83.13 ^b	79.34 ^c	78.11 ^{cd}	74.84 ^d	8.09
Crude protein	15.15 ^a	14.27 ^b	13.67 ^c	13.54 ^c	13.11 ^c	0.71
Crude fiber	29.49 ^a	24.74 ^b	23.44 ^c	22.79 ^{cd}	21.44 ^d	1.61
Ether Extract	4.99 ^a	4.46 ^b	4.26 ^b	4.19 ^c	4.03 ^c	0.21
Ash	13.68 ^a	11.77 ^b	11.19 ^c	10.94 ^{cd}	10.36 ^d	1.31
Nitrogen free extract	69.96 ^a	62.42 ^b	59.58 ^c	58.68 ^{cd}	56.26 ^d	3.71
Neutral detergent fiber	99.76 ^a	85.50 ^b	81.27 ^c	79.37 ^d	75.08 ^d	15.07
Acid detergent fiber	46.12 ^a	40.23 ^b	38.31 ^c	37.55 ^{cd}	35.76 ^d	4.34
Acid detergent lignin	15.45 ^a	13.92 ^b	13.30 ^c	13.13 ^c	12.62 ^d	1.25

^{abcd} means the same row with different superscript are significantly (p<0.05) different

The nutrient intake of West African WAD ram offered water contaminated with used engine oil was presented in

Table 4. All measurements were significant (p<0.05) affected by dietary treatments. T0 tends to have the highest

values recorded in all treatment observed. While the reduction in DMI diets (forage and concentrate) in T2-T5 might be due to the increase in the percentage inclusion of the water with engine oil contamination. This was in agreement with (NRC 1974, 1997 and 2007) who reported that poor water quality can affect the water intake, feed intake, nutrient utilization, health and productivity of ruminants. However, the animals tend not to effectively utilize the feed, which might be due to the pollution in the water and unpleasant smell and

odour of the water. Veenhuizen and Shurson, (1992); Solomon *et al.* (1995) reveals that water quality typically encompasses physiochemical factors (e.g., turbidity, taste, smell), micro and macro mineral elements, organic matter and microbial contaminants as well as potential risk from anthropogenic pollutants and contamination. The T1 (control) having the highest values across the treatments, might be due to pollution free water offered to the experimental animals.

Table 5: pH, Ammonia and microorganism in the rumen of animals offered water contaminated with used engine oil.

Treatment	T0	T1	T2	T3	T4	SEM
Rumen pH	6.28 ^a	6.06 ^b	5.90 ^c	5.69 ^d	5.72 ^d	0.08
Bacteria x 10 ⁶	8.70 ^a	8.70 ^a	7.52 ^b	6.94 ^c	5.20 ^d	0.04
Fungi x 10 ⁶	1.90 ^a	1.80 ^b	1.69 ^c	1.68 ^c	1.52 ^d	0.12
Protozoa x 10 ⁶	0.57 ^b	0.60 ^a	0.55 ^c	0.54 ^c	0.51 ^d	0.04
Rumen ammonia	1.41 ^b	1.76 ^a	1.18 ^c	1.12 ^d	1.13 ^d	0.02

^{abcd} means the same row with different superscript are significantly ($p < 0.05$) different

The pH, Ammonia and microorganism in the rumen of West African WAD ram offered water contaminated with used engine oil were presented in Table 5. There were significant ($p < 0.05$) difference among the treatment means. Animals on T0 has the highest values ($p < 0.05$) for rumen pH, followed by T1, T2 while T3 was compared significant ($p > 0.05$) with T4. The value for bacteria was found to be highest in T0 and T1 and lowest in T4. The value recorded for fungi, T0 had the highest values, followed by T1. Fungi for T2 was comparable ($p > 0.05$) with T3 while T4 had the least value. The value obtained for protozoa was highest in animals T2, followed by T0, T2 while T3 compared significantly ($p > 0.05$) with T4 as the lowest values.

Rumen pH has as an important factor that measures the acidity and alkalinity of

rumen contents (Bowen, 2009). It was recorded that the rumen pH values for T0 and T1 where only values that falls within the ranges recorded by Koutsoumanis *et al.* (2007) who reported that the optimum microbial activity and growth lies between 6.00 and 7.00 for rumen pH. The relatively low in pH might probably due to the pollutants in the water that are not friendly to the rumen environment of the ruminant. It was also observed that the decline in the growth of microbes in the rumen was as a result of the increment in the level of pollutants in the water offered. The reduction might be due to refusal of the water by the animal after fed the experimental diets. According to Solomon *et al.* (1995) who reported that effect of water quality and physiochemical factors can bring about bad smell and odour. Akinlade and Ososanya (2019) concluded

that the growth of cellulolytic bacteria, rumen anaerobic fungi and protozoa is completely inhibited at pH values below 6.0.

Conclusion

It was recorded from the results that sheep exposed to contamination of drinking water with used engine oil at varied percentage up to 10% can affect the water intake, feed intake, nutrient utilization and productivity of ruminants since the animal will not effectively utilize the feed. However, it is therefore recommended that farmers and landless urban dwellers should confined or tether their animals in order for the animals not to be exposed to used engine oil contaminated water or other pollutants that can adversely affect the animals productivity, cause economic loss and food security.

Reference

Adolph, E.F. (1993). The metabolism and distribution of water tissues. *Physiol. Rev.*, 13: 336-371

Ajayi, D.A., Adeneye, J.A. and Ajayi, F.T. (2005). Intake and nutrient utilization of West African goats fed mango, Ficus and *Gliricidia* foliages and concentrate as supplement to a basal diet of guinea grass. *W. J. Agric. Sci.*, 1(2): 184-189.

Akinlade, A.T. and Osodanya, T.O. (2019) Diversity and physical properties of rumen microbial ecosystem of West African dwarf rams fed ammonium sulphate-fortified diets. *Nig. J. Amin. Prod.* 46(2): 209-218.

AOAC (2002). Association of Official Analytical Chemists 18th Revised Edition. In: Official methods of

Analyses, Washington DC pp. 210-240

- Berepubo, N.A., Johnson, M.C. and Sese, B.T. (1994). Growth potential and organ weights of weaner rabbits exposed to crude oil contaminated forage. *Int. J. Anim. Sci.*, 9: 73-76.
- Bojkovski, J., Relic, R., Hriustov, S., Stankovic, B., Savic, B., Pavlovic, I. and Petrujkic, T. (2010). Influence of biological and chemical contaminants on health status of small ruminant. *Bulletin UASVM, Veterinary Medicine*, 67(2):1-3
- Bowen, R. (2009). Rumen physiology and rumination. Retrieved from Colorado State University. <http://www.vivo.colosate.edu/hbooks/pathways/digestion/herbivores/ruminantion.html>
- Cleake, R.M. and Bull, L.C. (1986). Effect of forage maturity on ration digestibility and production by dairy cows. *J. Dairy Sci.*, 69: 1587-1594.
- Ebiegberi, M.N. (2009). Haematological characteristics and performance of West African Dwarf Goats fed crude oil contaminated forage. *African J. of Biotechnology*, 8(4): 699-702
- Faixova, Z. and Faix, S. (2002). Influence of metal ions on ruminal enzyme activities. *Acta Vet. Brno.* 71:451-455.
- Gupta, B.N., Mahadeven, V. and Singh, P. (1968). Studies on the effect of feeding DDT treated roughage on the biochemical functions of the rumen 1: Feed consumption and digestibility. *Indian Vet. J.*, 45: 1037- 1045.
- Koutsoumanis, K., Stamatiou, K., Skandamis, P. and G.J.E. (2007). Development of a microbial model for the combined effect of

- temperature and pH on spoilage of ground meat and validation of the model under dynamic temperature conditions. *Applied Environmental Microbiology*, 72(1): 124-134
- Mehrez, Z., Orskov, E. R., Mcgonald, I. (1977). Rates of rumen fermentation in relation to ammonia concentration. *Brit. Nutr.*, 38: 433-441
- National Research Council (1974). Nutrients and Toxic Substances in Water for Livestock and Poultry. Natl. Acad. Sci., Washington, DC.
- National Research Council (1997). Occupational Health and Safety in the Care and Use of Research Animals. Washington, DC.
- National Research Council (2007). Nutrient Requirements of Small Ruminants. Natl. Acad. Press, Washington, DC.
- OCED (2000). Guidance notes for the analysis and evaluation of repeat dose toxicity studies. Environment, Health and Safety publication series on testing and assessment. Environment Directorate Paris p.2772
- Rajaganapathy, V., Xavier, F., Sreekumar, D. and Mandal, P.K. (2011). Heavy metal contamination in soil, water and fodder and their presence in livestock and products: A Review. *Journal of Environmental Science and Technology*, 4(3):234-249.
- Raun, N.S. and Burroughs, W. (1962). Suction Strainer Technique in Obtaining Rumen Fluid Samples from Intact Lambs. *Journal of Animal Science*, 21(3): 454-457.
- Rolling, W.F., Milner, M.G., Jones, D.M., Daniel, F., Swannel, R.J. and Head, I.M. (2002). Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient enhanced oil spill bioremediation. *Appl. Environ. Microbiol.*, 68(11): 5537-5548.
- Solomon, R., Miron, J., Ben Ghedalia, D. and Zomberg, Z. (1995). Performance of high producing dairy cows offered drinking water of high and low salinity in the Arava Desert. *J. Dairy Sci.*, 78:620-624.
- Teresa, M., Vasconcelos, S.D. and Tavares, H.M. (1997). Trace element concentrations in blood and hair of young appendices of a technical-professional school. *Sci. Total Environ.* 205:189-199.
- Thirumalesh, T. and Krishnamoorthy, U. (2013). Rumen microbial biomass synthesis and its importance in ruminant production. *International Journal of Livestock Research*, 3(2):1-22.
- Van Soest, P.J., Robert, J.B. and Lewis, B.A. (1991). Method for dietary, fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74:3583-3597
- Veenhuizen, M. F., and G. C. Shurson. (1992). Effects of sulfate in drinking water for livestock. *J. Am. Vet. Med. Assoc.* 201:487-492.
- Veth, Mjde, Kolver, E.S., Veth, D.E. and Cottle, M.J. (1999). Pasture digestion in response to change in ruminal pH. 59th conference, Holy Cross College, Mosgiel, *Proceedings of the New Zealand Society of Animal Production*, 59:66-69.