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REPORT

ON

POST DOCTORAL F'ELLOWSHIP

YEARLY ACTIVITIES

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INTRODUCTION

In mid-June 1994 the Senegalese government received an application form for KOSEF (Korea Science and Engineering Foundation) post doctoral fellowship for foreign researchers from the government of the Republic of Korea, and transmitted it to all research institutes and universities for applicants. Our institute (the Senegalese Agricultural Research Institute) selected candidate for the fellowship and 1 was chosen. 1 filled out the application form and put together a research plan which outlined the area I wanted to be involved in and have research undertaken on . The plan stated:

" a - undertake experiments and courses on nutritional physiology for small ruminants to better understand how to analyze and make inference on feeding behavior of short cycle reproductive animals (small ruminants and poultry) and on response under feeding with agricultural by-products under extensive or intensive feeding";

" b -finding ways to optimize the value of those by-products in the feeding strategy to be recommended".

In December 1994, we received confirmation that the application sent was accepted and that I will be working with Dr. J. K. Ha (my host scientist) from the Seoul National University College of Agriculture and Life Sciences. 1 made contact with him and proposed to put together a program and schedule of activities upon my arriva1 in Korea.

Upon my arriva1 in Korea on the 24th of February 1995 and after a first meeting with my host scientist and a brief visit to the members (graduate students and post doctorate assistant) of his laboratory, we decided that 1 will take some times to hand out the proposal on the experiments I intended to work on for the duration of my stay. A month later I came up with two topics:

"study of the nutritive value of a non conventional foodstuffs: cardboard" and

"test on potential for native chicken to use high fiber ration".

ACTIVITIES UNDERTAKEN

Upon my arrival, 1 was sheltered during the first month at the guest house of the College of Agriculture and Life Sciences then moved to a room at the farm station.

The day of my arrival, 1 had a meeting with my host scientist and the graduate students and post doctorate assistant of his laboratory during which 1 was given a summary on their works; after the meeting 1 was introduced to the head of the Animal Science and Technology Department dr. I. K. Han with whom I took about my work and my expectations then to the Dean of the College and his staff.

After settling down, 1 soon was faced with the fact that all activities were undertaken in Korean language which 1 could not read, write or speak; this was and still is an handicap in the pursuit of my activities because 1 could not use my files brought with me and communicate or search for references on research works done by my fellows Korean researchers in my field. 1 did overcome some of it by buying a computer and some software in English which did allow me to write my proposa1 and be able to perform the analysis needed on the data 1 gather in the course of my experiments here.

The proposal on "study of the nutritive value of a non conventional foodstuff: cardboard" was carried out on five experiments:

1) comparative digestibility of treated cardboard (sodium hydroxide: NaOH) and rice straw using "rumen collection" and "KNGF-SNU" a fungi isolated by the research team of the ruminant nutrition laboratory from the rumen of Korean native goat;

2) effect of chemical treatment i.e. sodium hydroxide, ammonium hydroxide, hydrogen peroxide and soaked with running water) on in vitro dry matter digestibility (IVDMD) of cardboard;

3) effect of protein supplement (cottonseed meal) on in vitro dry matter digestibility (IVDMD) of cardboard;

4) effect of chemical treatment (i.e. NaOH or NH_4OH) on in situ dry matter digestibility (ISDMD) of cardboard;

5) biochemical rumen characteristics (volatile fatty acids and ammonia concentration of sheep fed diets with different levels of substitution of rice straw by cardboard.

Recycling waste products has received attention in recent years due to shortages of food (roughage, cereal and cereal by-products) for feeding of animal especially in developing countries where there is competition between humans and animals in the use of some foodstuffs. This lead to the tria1 of non conventional foodstuffs. Wood products and residues have been receiving consideration as possible energy sources for ruminants. Their use could free more additional foodstuffs for man and allow recycling of wood products such as waste paper into productive use (meat and milk) in ruminants, Feeding waste paper to ruminants has created interest because it utilizes a waste product which has the potential to become an economically feasible roughage substitute in ruminants diets.

Early works have shown that varieties of waste papers can be consumed and digested by ruminants, that dry matter disappearance (DMD) rate varies depending on the nature of waste papers(i.e. brown cardboard and brown wrappings paper have higher DMD than glossy or silk magazine which have higher DMD than newsprint paper). The variation could be the effect of difference in manufacturing process. Chemical treatment (mainly NaOH) of cardboard have shown to improve IVDMD and the same is true for nitrogen supplement. Complete diets incorporating cardboard have been well digested by ruminants.

TO be successful in the promotional use of cardboard, one may show how well it can be a substitute to low quality forage and roughage. Our objective in the different trials done was to evaluate the digestibility value of cardboard and its ability to be a good substitute to low quality forage as basa1 diet in a feeding strategy of areas where there is shortage of conventional foodstuffs for animals.

Experiment 1

200 g of cardboard were treated with a 2000 ml solution of 4% sodium hydroxide (NaOH) into a becher, sealed for 96 hours at room temperature, then air dried for 48 hours. The treated cardboard and a same amount of rice straw and non treated cardboard were grinned (1 mm) separately. 2 g of triplicates samples of each treatments were put into test tubes and 10 ml of Lowe's medium were added under CO_2 and autoclaved for 45 mn. Rumen content from a fistulated sheep fed rice straw and concentrate (ratio 30:70) was collected by aspiration through a rubber tube and under anaerobic conditions (Hungate anaerobic system CO_2) was filtered using a four layer cheesecloth, the rumen fluid was them centrifuged at 10000 rpm for 15 mn at $10^{O}C$, the supernatant and the upper white layer were collected and the "'rumen collection" which was homogenized using a vortex and then diluted at 10% with a Bryant's solution under CO_2. Culture of "KNGF-SNU" fungi was also homogenized using a vortex and then diiuted at 10% with a Bryant's solution under CO_2. Samples were then inoculated with 1 ml of one of the treatments ("rumen collection" or "KNGF-SNU") and incubated for 7 days at $39^{O}C$. Replicates were taken out at day 4 and 7, dried for 48 hours at $70^{O}C$ then weighed for dry matter disappearance (DMD).

The results show at 4 days of incubation a DMD rate of 8.8, 16.5 and 30.0 by "rumen collection" for treated cardboard, non treated cardboard and rice straw respectively 10.0, 20.5 and 29.2 by "KNGF-SNU" for treated cardboard, non treated cardboard and rice straw respectively. At 7 day incubation the DMD rates were 13.2, 17.0 and 32.5 by "rumen collection" for treated cardboard, non treated cardboard and rice straw respectively and 8.9, 21.9 and 31.8 by "KNGF-SNU" for treated cardboard, non treated cardboard and rice straw respectively.

These data suggest that rice straw is more digested than cardboard and that chemical treatment did not improve the digestibility of cardboard. The "KNGF-SNU" shows higher ($p < 0.05$) potential for digesting cardboard than "rumen collection" but no difference in digestibility potential between "KNGF-SNU" and "rumen collection" was found for rice straw. This may be due to the fact that "KNGF-SNU" is cultured and

conserved under filter paper which may increase its potential for wood by-product degradation.

Experiment 2

200 g of cardboard and 2000 g of a solution of one of the chemical treatments (4% NaOH, 3% NH_4OH, 1% H_2O_2 and running water) were put into a becher which was then sealed and allowed the chemical treatments to react for 96 hours, then air dried for 48 hours, then grinned (1 mm). The rumen fluid was obtained in the same way than in experiment 1 without the centrifugation. The first phase of the Tilley and Terry in vitro digestibility method was then undertaken which consists of a 48 hours incubation of 2 g sample with 20 ml rumen fluid at $39^{O}C$.

The in vitro dry matter disappearance (IVDMD) rates were 65.5, 59.3, 40.7, 52.2 and 54.4 for non treated cardboard, NH_4OH treated cardboard, NaOH treated cardboard, H_2O_2 treated cardboard and soaked cardboard (running water treatment) respectively.

Non treated cardboard has higher ($p < 0.05$) IVDMD rate than NaOH treated cardboard, but no significant difference in IVDMD rate was found between non treated cardboard and the other chemical treatments. The overall finding is that chemical treatment does not improve the IVDMD rate of cardboard.

Experiment 3

Cottonseed meal (CSM) was use as protein source in a trial to evaluate the effect of protein supplement level in the digestibility improvement of cardboard. Three levels were used: 10, 15 and 20%. The rumen fluid was obtained in the same way than in experiment 2 and the Tilley and Terry in vitro digestibility method done also in the same way than in experiment 2.

The IVDMD rates at 24 hour incubation were 25.7, 25.2 and 32.7 for non supplemented cardboard, 10%, 15% and 20% CSM supplemented cardboard respectively, and 45.8, 48.3, 46.4 and 44.5 at 48 hour incubation for non supplemented cardboard, 1 0%, 15% and 20% CSM supplemented cardboard respectively.

The results suggest that supplemented CSM at 15 and 20% levels improves IVDMD rate of cardboard at 24 hour incubation but not at 48 hour incubation.

Experiment 4

The treated cardboard (NaOH and NH_4OH) from experiment 2 were used in the in situ trial conducted using the facilities of the National Livestock Research Institute. Triplicates of nylon bags containing 2 g of samples of one of the treatments (non treated cardboard, NaOH treated cardboard and NH_4OH treated cardboard) were incubated into the rumen of a four years old fistulated Holstein cow fed concentrate and alfalfa hay. Samples were removed at 0, 3, 6, 9, 12, 16, 24, 48, 72 and 96 hour; after removal nylon bags are washed gently under warm running water then agitated for more washing into a bucket full with running water for 30 to 35 mn until the water is clear. the bags are then dried for 48 hours at $70^{O}C$ and weighed for dry matter disappearance rate. all replicates of a given removal time and treatment are then mixed and sent for ADF and NDF analysis.

The in situ dry matter disappearance (ISDMD) rate were 29.7, 38.1 and 41.5 for non treated cardboard, NaOH treated cardboard and NH_4OH treated cardboard respectively at 24 hour incubation time and 48.7, 47.9 and 44.4 for non treated cardboard, NaOH treated cardboard and NH_4OH treated cardboard respectively at 48 hour incubation time.

The results suggest that chemical treatments (mainly NaOH or NH_4OH) improves ($p < 0.05$) ISDMD rate at 24 incubation but not at 48 hour incubation.

Experiment 5

Three fistulated adult sheep were used in a Latin square design 3 x 3 with 3 diets: rice straw and concentrate (T1)(ratio 40:60), cardboard, rice straw and concentrate (T2) (ratio 20:20:60) and cardboard and concentrate (T3)(ratio 40:60). The adjustment period was 4 days and the collection period 3 days. Every 7 days each sheep was fed a new diet different from the one for the last week. Rumen content samples were taken using a rubber tube 4 hours after the morning feeding of every day of the collection period. Upon

arriva1 at the laboratory, the samples are filtered using a four layer cheesecloth and 10 ml of the rumen fluid are collected into test tubes where are added 1 ml of a 2% $HgCl_2$ solution and 2 ml of a 25% HPO_3 solution, then the mixture is centrifùged at 3000 rpm for 15 mn at $10^{O}C$. 2 ml of the supernatant were collected for further ammonia analysis using the Chaney and Marbach method with a spectophotometer at 630 nm. Duplicates of 1 ml of the supernatant are then centrifùged again at 14000 rpm for 15 mn at $10^{O}C$ and the new supernatant collected for VFA analysis at Kon Kuk university using a Gas Chromatograph (Hewlett Packard Gas Chromatograph 5890A series II).

The ammonia concentration (mg per 100 ml rumen fluid) was 5.7, 2.3 and 2.6 for diet T1, diet T2 and diet T3 respectively.

The total VFA concentrations (mmol per liter of rumen fluid) were 6.4, 9.1 and 8.5 for diet T1, diet T2 and diet T3 respectively.

The acetic acid concentrations were 3.6, 5.1 and 4.8 and represented 57, 56 and 56% of the total VFA for diet T1, diet T2 and diet T3 respectively.

The propionic acid concentrations were 1.7, 2.6 and 1.9 and represented 26, 28 and 22% of the total VFA for diet T1, diet T2 and diet T3 respectively.

The butyric acid concentrations were 1 .0, 1.4 and 1.8 and represented 16, 15 and 21% of the total VFA for diet T1, diet T2 and diet T3 respectively.

The ammonia concentration was lower ($p < 0.05$) for the diets containing cardboard which show higher ($p < 0.05$) concentration of total VFA.

Although the acetic acid concentration was higher ($p < 0.05$) for the diet containing cardboard, there was no significant difference ($p < 0.05$) between diet by looking at the acetic acid concentration as percentage of the total VFA. The propionic acid concentration as a percentage of total VFA was higher ($p < 0.05$) for diet containing rice straw. the butyric acid was higher ($p < 0.05$) for diet containing cardboard but was higher ($p < 0.05$) as a percentage of total VFA only for the diet without rice straw.

All diets in the experiment show lower ($p < 0.05$) acetic acid concentration expressed as percentage of total VFA than usual (63). Diets with rice straw show higher ($p < 0.05$) propionic acid concentration expressed as percentage of total VFA than usual (21). Diet with cardboard as sole source of roughage (without rice straw) shows normal

propionic acid concentration and higher ($p < 0.05$) butyric acid concentration expressed as percentage of total VFA than usual (16).

It seems that there is a shift in VFA production from acetic acid to propionic acid for the diet containing rice straw and from acetic acid to butyric acid for diet with cardboard as sole source of roughage (without rice straw). The higher level of total VFA for diet containing cardboard may be due to the fact that cardboard is more easily and rapidly degraded than rice straw, and the same fact may explain the low level of ammonia by a rapid incorporation of peptides by microbes because of the already available VFA from cardboard degradation or by the sparing effect on protein degradation because of the readily available carbon skeleton fi-om cardboard degradation.

The overall findings of theses triais suggests that cardboard is suitable to be a ruminant feed and that it can be a good substitute for low quality forage as source of energy in ruminant feeding.

A growth tria1 involving the evaluation of weigh gain, body composition and weigh gain composition was on schedule but could not be done for lack of fund to buy the number of animals necessary for the experiment.

The proposa1 on: "test on potential for native chicken to use high fiber ration" consisted of two trials: a digestibility one and another on growth which would have evaluate three levels of fiber content of the diet (3, 5 and 7) and would have look at the level of intake, digestibility, weigh gain, composition and nature of gain and the microbial composition of digestive tract.

Although we had all the help needed for the feed from Dr. 1. K. Han, the animals we had at our disposa1 were from a breeding stock and part of the experiment could not be performed. The alternative we had to but the native chicken from outside and manufacturing a new set of digestibility cages could not be made for lack of fund.

On May 1995, 1 attended the symposium on "supply of livestock products to rapidly expanding urban population".

On September 1 made a presentation for the graduate students of the Department of Animal Science and Technology on "Senegal: an overview" in which I underline Senegal main characteristics: type of government, constitution, culture, politic, economy, education system, population and geography.

1 also have the privilege to be present at two presentations by:

- Dr. Cheng (Livestock Sciences Section: Research Station, Lethbridge, Alberta, Canada.) on :

a) the utilization of rumen fungi enzymes in biotechnology: exploitation of rumen microbial enzymes to the benefit of the feed industry,

b) microbial attachment to feed and mode of action.

- Dr. Ushida (Kyoto Prefectural University Japan) on:

a) understanding of microbiological physiology of rumen and hind gut anaerobic bacteria, fungi and protozoa,

b) inter-species hydrogen transfer in anaerobic microbial ecosystems.

My host scientist laboratory being mainly a rumen microbiology one, I did learn a great deal about rumen microbes studies techniques (isolation and characterization, enzyme activities determination and evaluation). The ruminant nutrition being the understanding of the metabolism of the microbes of the rumen and finding way to açhieve a more efficient way to improve the production by the microbes of the end products needed by the host animals for its own production, one can understand the importance of rumen microbiology studies and its impact on the development of future strategies in feeding ruminants. The metabolism of the microbes being the end result of the enzyme activities, knowing and characterizing the different enzymes (and their mode of action) involved in the process of degradation of the different constituents of the feed (carbohydrates, protein, lipids and minerals) are essential in the improvement of the end products production the host animal needs for its production (meat and milk).

What I have learn in these areas will be of great importance and help in implementing new topics of research in our laboratory and will surely improve the use of low quality forage and non conventional foodstuff.

ACKOWNLEDGMENTS

1 wish to thanks the Korea Science and Engineering Foundation (KOSEF) by creating this new program of post doctoral fellowship for a better exchange of information and cooperation between Korean scientists and foreigners which have allowed me to come to Korea and work with some of my colleagues of my field, and giving me the opportunity to increase my knowledge in ruminant nutrition.

My thanks to Dr. J. K. Ha for allowing me to work with him in his laboratory, for all the support and help he gave and for facilitating the execution of all the experiments.

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RECOMMENDATIONS

The language barrier is a great handicap in the communication process and more efficient work by the foreigners con-ring to Korea, it will be therefore of great interest for the future post doctoral fellowship participants of this program to have a two to three months intensive language course. 1 did have a lot of problem communicating (understand and being understood) and think that 1 would have gain more in term of know-how from my host scientist if I was able to speak, write and read the Korean language and also it is the best way to understand the Korean culture which also is part of the aim of this program.

Although we as participants of this program have an allowance sufficient for our expenses, the host scientist needs more financial incentives to support the experiments the participants are undertaking but also it may be a way to help upgrade the laboratory or new apparatus needed for the work they are doing