

碩士學位論文

低質粗飼料의 飼料價値 增進에 關한 研究

Studies on Improving the Nutritive Value of Low
Quality Roughages



濟州大學校大學院

畜産學科

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STUDIES ON IMPROVING THE NUTRITIVE VALUE OF LOW QUALITY ROUGHAGES

BY

Jai Jun Choung

Thesis submitted in partial fulfilment for the
Degree of Master Science at the
Graduate School,
Cheju National University.



SUPERVISOR : Professor Chang Cho Choung

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I certify that the work reported in this
thesis has not been submitted to any university
or institution for a degree or similar award.



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摘 要

本 研究은 보리와 油菜의 副産物인 보리짚, 보리가락, 油菜대와 油菜각지 등의 低質粗飼料源의 飼料利用 方案으로 放射線照射 및 理化學的處理, 酵素添加와 放射線照射의 複合處理가 飼料價値增進에 미치는 影響을 究明하기 위하여 施行하였다.

實驗 I 은 보리짚, 보리가락과 油菜각지에 0 - 25 Mrad 의 r - ray 를 照射하였고, 實驗 II 에서는 보리짚, 보리가락, 油菜대와 油菜각지에 粉碎, NaOH, NH₄OH, 酵素添加와 放射線照射 (2.5 Mrad) 의 複合處理를 하여 다음과 같은 結果를 얻었다.

1. 放射線照射는 모든 供試試料의 NDF 와 Hemicellulose 의 含量을 減少시켰으나 ADF, Cellulose 와 Lignin 含量은 變化가 없었다. ADF - N 含量과 還元糖生成量은 處理物質에 따라 相異한 效果를 나타내었다.

2. Cellulose 의 結晶度는 보리짚에서는 25 Mrad 照射區에서, 보리가락에서는 5 Mrad 照射區에서 현저히 增加하였으며 油菜각지의 Cellulose 結晶度는 放射線照射에 依하여 減少되었다. 10 과 25 Mrad 照射區는 Cellulose 에 依한 試料의 加水分解를 培養 30 分까지 급격히 增加시키나 그 이후 培養 1 時間까지는 分解가 減少되거나 增加가 없었다.

3. Dacron bag DM 消化率에 대한 放射線 照射는 72 時間 培養後, 보리짚은 2.5 와 10 Mrad 照射區에서, 보리가락은 2.5 와 5 Mrad 照射區에서 消化率이 減少되었다. 油菜각지는 全照射區에서 消化率이 增加되었으나 5 Mrad 照射區는 2.5 Mrad 照射區에 比하여 낮았다.

4. 放射線照射는 供試試料의 細胞壁消化와 消化時間을 增加시켰으며, 12 時間 培養期間에서 가장 낮은 消化率을 나타낸 25 Mrad 照射區의 보리짚과 5 Mrad 照射區의 보리가락은 Cellulose 結晶度の 增加와 밀접한 關係를 나타내었다.

5. *In vitro* DM 消化率은 보리짚에 있어 5 와 25 Mrad 照射區에서, 보리가락은 2.5 와 5 Mrad 照射區에서 減少하였으며 油菜각지는 全照射區에서 消化率이 增加하였으나 5 Mrad 照射區는 2.5 Mrad 照射區보다 消化率이 낮았다.

6. *In vitro* total VFA 生成量은 培養時間이 增加함에 따라 增加하였으며 보리짚은 5 와 10 Mrad 照射區에서, 보리가락은 2.5 와 5 Mrad 照射區에서 그 生成量이 減少되었다. 油菜각지는 放射線照射에 依하여 total VFA 生成量이 增加

되었으나 5 Mrad 照射區는 2.5 Mrad 照射區보다 낮았다.

7. Acetic acid의 生成量은 照射線量에 따라 差異를 보였으며 2.5 Mrad 照射區가 Acetic acid生成에 좋은 效果를 나타내었다. 放射線照射는 Propionic acid의 生成量을 增加시켰으며 Acetic/Propionic acid ratio를 變化시켰다.

以上の 結果로 볼때 高線量의 放射線照射는 纖維素物質을 溶解시키나 微生物의 활동을 抑制하는 物質을 生成시키는 것으로 思料되며 放射線의 適正照射線量은 2.5 Mrad로 推定된다.

8. 實驗 II에서, NaOH處理는 處理水準이 增加함에 따라 消化率은 比例的으로 增加하였고 NDF, Hemicellulose 含量은 減少하였다. 보리짚과 보리가락의 ADF 含量은 變化가 없었으나 油菜대와 油菜각지에 있어서는 減少되었다. NaOH의 適正處理水準은 보리짚, 油菜대와 油菜각지는 6%, 보리가락은 4.5%로 推定된다.

9. NH₄OH處理時 3%處理水準까지 消化率이 增加되었으며 보리짚과 보리가락의 NDF, Hemicellulose 含量은 NH₄OH處理에 依해 減少되나 油菜副產物에 있어서는 變化가 없었다. Total N의 含量은 3 또는 4.5% 處理水準까지 현저히 增加하였고 油菜각지의 Total N 含量은 다른 試料에 比해 낮았다. NH₄OH의 適正處理水準은 보리짚과 油菜대는 3%, 보리가락과 油菜각지는 4.5%로 推定된다.

10. 供試試料에 대한 粉碎는 消化率을 현저히 增加시켰으며 2 mm screen grinding은 보리짚과 油菜대의 消化率 增加에, Fine grinding은 보리가락과 油菜각지의 消化率 增加에 좋은 效果를 나타내었다. 粉碎와 放射線照射의 複合處理는 粉碎의 단독처리에 相補效果가 있었으며 油菜대와 油菜각지에 效果가 있었다.

11. 物理化學的處理와 放射線照射의 複合處理는 NaOH와 NH₄OH處理區 모두 油菜대와 油菜각지의 消化率 向上에 좋은 效果가 있었고 NDF와 Hemicellulose 含量은 NaOH나 NH₄OH 단독처리보다 현저히 減少되었다. NaOH와 放射線照射의 適正複合處理水準은 6%, NH₄OH와 放射線照射의 適正複合處理水準은 보리짚이 4.5%, 보리가락은 6%, 油菜대는 1.5% 그리고 油菜각지는 3%로 推定된다.

12. 放射線照射와 酵素添加의 複合處理는 보리가락과 油菜각지의 消化率을 增加시켰으나 보리짚에서는 25 Mrad 照射區에서만 消化率이 向上되었다.

SUMMARY

The studies were conducted on gamma irradiation effect of low quality roughages having potential as ruminant feedstuffs.

The studies falls into 2 major categories: effect of gamma irradiation on low quality roughages (Experiment I), and Mixed treatment effects of gamma irradiation with grinding, chemical treatment and enzyme addition (Experiment II).

In Experiment I, effect of gamma irradiation on the fibrous material degradation, ADF-N content, reducing sugar formation, crystallinity of cellulose and its susceptibility, dacron bag dry matter disappearance, in vitro dry matter disappearance and VFA production of barley straw (BS), barley hull (BH) and rape husk (RH) were reported.

Four levels of irradiation were applied: 2.5, 5, 10 and 25 Mrad. Three adult sheeps fed high quality Italian ryegrass hay ad libitum. Dacron bag dry matter disappearance was measured when suspended for 12, 24, 36, 48 and 72 hours in the rumen. Four incubation periods were used (12, 24, 36 and 48 hours) to determined in vitro dry matter disappearance, and individual VFA production was determined at 1, 2, 4 and 6 hours fermentation.

Gamma irradiation decreased the NDF and hemicellulose content but contents of ADF, cellulose and lignin were not changed. Reducing sugar content and ADF-N content varied with test samples;

ADF-N content of BS was decreased, however, the ADF-N of BH was increased by irradiation. ADF-N content of RH was not changed. Gamma irradiation increased reducing sugar formation of BH and RH, but reducing sugar formation of BS was increased at 2.5 and 10 Mrad levels.

Cellulose crystallinity of BS was markedly increased at the 25 Mrad level, and 5 Mrad irradiation also significantly increased cellulose crystallinity of BH. Higher levels (10 and 25 Mrad) of irradiation increased enzyme hydrolysis of low quality roughages in a short time. But the relationship between degrees of crystallinity of cellulose and its susceptibility to cellulose was found only at a dose of 2.5 Mrad.

Dacron bag dry matter digestibility (DMD) of gamma irradiated BS, BH and RH varied with samples types and irradiation doses; BH showed a more positive effect to gamma irradiation than BS and RH. In 72 hours suspension, DMD of BS decreased at 2.5 and 10 Mrad levels, and lower irradiation levels (2.5 and 5 Mrad) decreased the DMD of BH. All levels of irradiation increased the DMD of RH, while DMD of irradiated with 5 Mrad was lower than that of 2.5 Mrad.

Gamma irradiation increased the cell wall digestion and lengthen the cell wall digestion time up to 48 hours. A level of 25 Mrad decreased cell wall digestion of BS, and 5 Mrad irradiated BH showed lower cell wall digestion at 12 hour suspension.

The 5 and 25 Mrad irradiation reduced in vitro dry matter

digestibility (DMD) of BS, and low irradiation levels (2.5 and 5 Mrad) decreased DMD of BH at 12 hours incubation. Gamma irradiation increased DMD of RH, whereas 5 Mrad level was lower than that of 2.5 Mrad level.

In vitro total VFA production of all test samples was increased by prolonging incubation time. Total VFA production of BS was decreased at 5 and 10 Mrad levels, and 2.5 and 5 Mrad levels decreased VFA production of BH while VFA production of RH was decreased at the 5 Mrad irradiation level.

Gamma irradiation changed the acetic/propionic acid (C2/C3) acid ratio and increased the propionic acid production.

In experiment II, effects of grinding (2 mm screen grinding and fine grinding), chemical treatment (NaOH and NH₄OH) and gamma irradiation (2.5 Mrad), and its combination on improving the nutritive value of barley straw (BS), barley hull (BH), rape stem (RS) and rape husk (RH) were studied.

NaOH treatment increased DMD of BS, BH, RS and RH by increasing the NaOH level. Physical treatment also enhanced the effect of alkali treatment. Fine grinding was more effective in improving DMD of NaOH treated BH, and 2 mm screen grinding was more effective in improving the DMD of NaOH treated RS and RH. NaOH treatment markedly decreased NDF and hemicellulose content of RS and RH by increasing NaOH levels. The optimal treatment level of NaOH was 6% in BS, BH and RH but 4.5% in RS.

NH_4OH treatment improved DMD of BS, BH, RS and RH by increasing the NH_4OH level up to 3%. The NDF and hemicellulose content of BS and BH were decreased by NH_4OH treatment. Total N content of BS, BH, RS and RH markedly increased by ammonia treatment, however, the lowest total N content was found in RH. The highest total N content of BS, RS and RH was obtained at 4.5% level of NH_4OH level, but the highest total N content of BH was obtained at 3% NH_4OH level. The optimal treatment level of NH_4OH was 3% in BS, RS and RH, but 4.5% in BH.

Grinding increased dry matter digestibility (DMD) of BS, BH, RS and RH. 2 mm screen grinding was effective to improving the DMD of BS, and RS, and fine grinding was more effective in BH and RH.



Combination of grinding and gamma irradiation showed compensation effect in improving the DMD of low quality roughages.

The effect of mixed treatment with chemical and gamma irradiation varied with sample types. Mixed treatment of grinding, NaOH and irradiation on the BS and BH were less effective than only grinding and NaOH treatment. The positive effect of mixed treatment with grinding, NaOH and irradiation was found in BH and RS at 6% NaOH level. Mixed treatment with NaOH and gamma irradiation linearly decreased the NDF and hemicellulose contents BS, BH and RH than only NaOH treatment. But, ADF content was not changed by mixed treatment with NaOH and irradiation.

Mixed treatment with grinding, NH_4OH and irradiation on BS and BH was less effective than NH_4OH treatment. The positive effect was obtained only treated with 4.5 and 6% NH_4OH in BS and irradiation was effective in improving the DMD of RS and RH than only NH_4OH treatment. The NDF and hemicellulose content were decreased by mixed treatment with NH_4OH and irradiation than only NH_4OH treatment. But, ADF content was not changed by mixed treatment with NH_4OH and irradiation. The optimal combination level of NH_4OH and irradiation was 4.5% in BS, 6% in BH, 1.5% in RS and 3% in RH.

Enzyme addition increased the DMD of gamma irradiation BS only at the 25 Mrad irradiation level. Enzyme addition increased the DMD of gamma irradiated BH and RH at all levels.



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I. INTRODUCTION

It is increasingly evident that unless effective restraint is placed upon the rate of population increase, the world is moving into a serious ecological imbalance between food supply and demand.

This ecological imbalance is further exacerbated by the fact that in the developing countries of the world the animal protein consumption per head is increasing. The improvement of living standards in developing countries will lead to further increase in animal protein consumption and a growing demand for high quality red meat.

The increase of animal protein consumption is accompanied by a rapid rise in the demand for feedstuffs and animal stock. Grain is an important food for both man and the production of animal protein. Consequently there exists competition between man and animal production. In many areas of the world, agricultural by-products are becoming important feedstuffs for ruminants as competition for grains and the needs for high protein meals increases.

The ruminant has biological interest because it is the most developed form of herbivore. Ruminants manage to extract more nutrient from coarse fibrous feedstuffs than any other herbivore. Symbiotic microbial population of the ruminant digestive tract allows the host animal to derive energy from cellulose and hemicellulose in the form of volatile fatty acids, which are metabolised

by the animal tissues.

Large quantities of low quality roughages are available such as straw, grain hulls, corn stover, corn cobs and other agricultural by-products. These can be efficiently utilized by the ruminants as livestock feed. The potential use of low quality roughages, as animal feeds is worthy of consideration since ruminants are uniquely adapted to utilize the cellulose in high fibrous materials.

In KOREA, about 765 M/T of rice straw and 200 M/T of barley straw are produced annually. Rice and barley straw are most important sources of feedstuffs for ruminants. However, only a small proportion of this straw is fed to livestock. Most of the straw, an important plant nutrient, is either plowed into the soil to improve fertility or burnt in the field.

Although these materials contain enough cellulose to make them a rich source of energy for ruminants, they are poor-quality feeds in their natural state. However, they have several shortcomings : low digestibility, low protein content, poor paratability and bulkiness.

Many studies using chemical, physical and gamma irradiation treatment have been conducted to improve the nutritive value and digestibility of these materials. Reliable technical solutions of the best ways to utilize low quality roughages are lacking and of those practices employed there is no consistent rationale as to why they sometimes work and sometimes fail.

The present studies were carried out to measure the effects of different treatments on improving the nutritive value of low quality roughages.

The examination of different treatment :

- 1) effect of gamma irradiation on the properties of roughages and on the nutritive value of low quality roughages.
- 2) effect of chemical treatment on improveing the nutritive value of low quality roughages.
- 3) effects of mixed treatment by grinding, chemical treatment, enzyme addition and gamma irradiation on improving the nutritive value of low quality roughages.

This review of the literature assesses the carbohydrate metabolism, especially structural carbohydrate-metabolism in the rumen. Consideration is given to the properties of low quality roughages and to the effects of different treatments applied to improve their nutritive value and techniques to measure their nutritive value.

II. REVIEN OF LITERATURE

2. 1. Carbohydrate metabolism in the rumen

2. 1. 1. Fermentation of carbohydrate

Carbohydrates are the main source of energy for productive processes associated with growth, pregnancy and lactation in the ruminant animal.

The bulk of carbohydrate in low quality roughages is the polymer : Cellulose, hemicellulose, pectin and starch. The first step in fermentation of carbohydrates is hydrolysis to form mono, di, tri, et cetera, saccharides and oligosaccharides (figure 2.1).

Cellulose, the chief constituent of the cell wall in the most plants, is one of the most abundant organic substances. Cellulose molecules are long, unbranched chains of β -1, 4 linked with glucose units. Cellulose in plants exists in a loosely associated (amorphous) form along with hemicellulose and other polymers as well as in a highly crystalline form.

Manta (who quoted from Phillipson and Cuthbertson, 1956) summarized the process of degradation of cellulose to simple sugars in three stages :

- a) the breaking down of cellulose into smaller polysaccharides, resulting in the production of glucose and cellobiose;
- b) a second stage that seems to be analogous to the enzymic

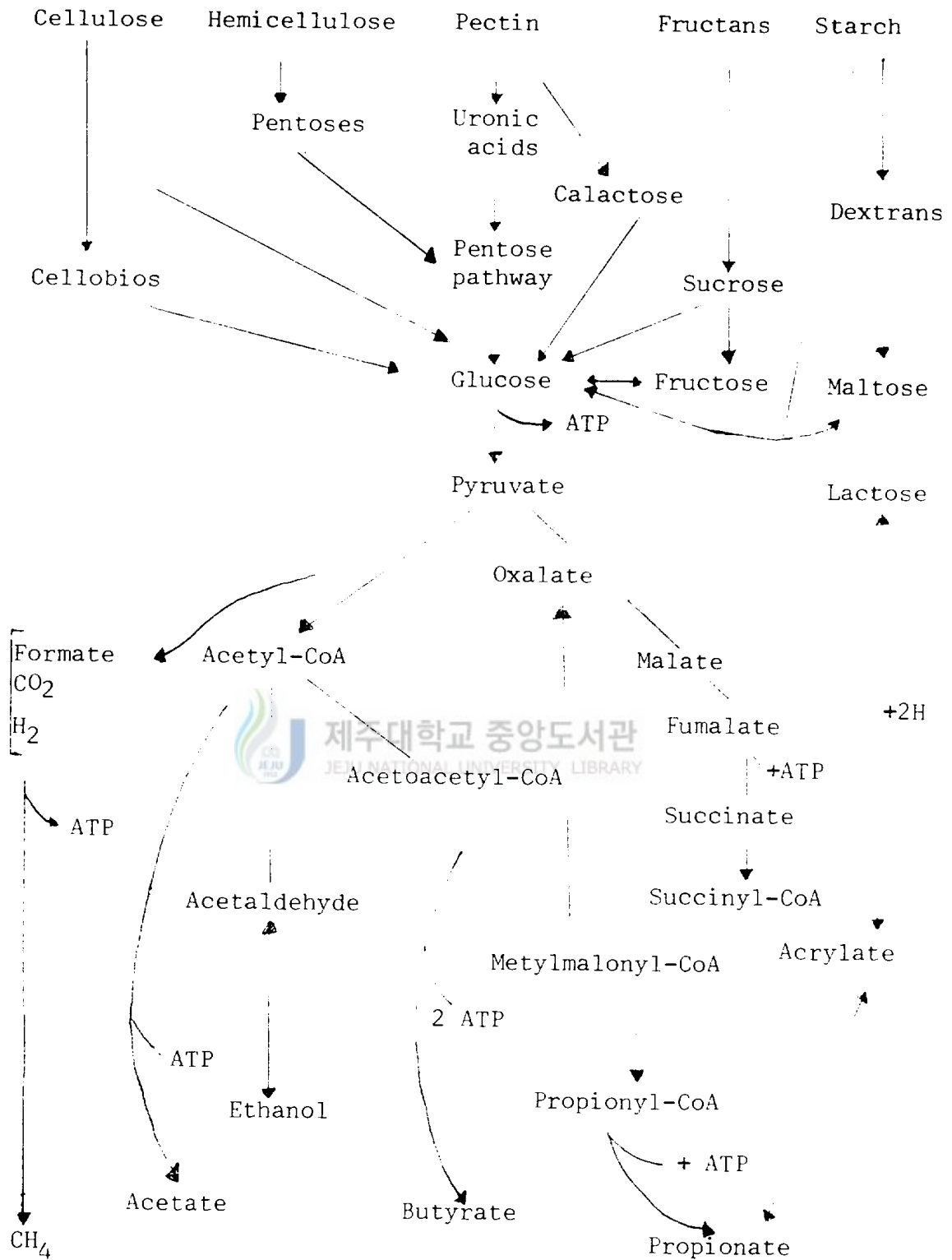


Figure 2.1 Pathways of carbohydrate metabolism in the rumen

Source : Van Soest, 1982

hydrolysis of other polysaccharides, resulting in the production of glucose and cellobiose ;

c) the hydrolysis of cellobiose to glucose by cellobiase.

The primary cellulolytic microbes in the rumen are Bacteroides succinogenes, Ruminococcus albus and Ruminococcus flavefaciens.

Pettipher and Lantham (1979) reported that the cellulases of the R. albus are active in the hydrolysis of denatured or amorphous cellulose, but are relatively inactive against crystalline cellulose. R. flavefaciens can hydrolyze crystalline cellulose. The ruminococci are able to degrade all the components of the cell wall, but where mature forages are concerned, tend to degrade quantitatively less than B. succinogenes (Dehority and Scott, 1967). Gorleau and Forsberg (1981) reported that B. succinogenes is the most prominent cellulolytic organism in the rumen contents of animals fed wheat straw, high in crystalline cellulose; suggesting that the cellulase of B. succinogenes is more active on crystalline cellulose than the enzymes of the ruminococci. Gorleau and Forsberg (1981), Dehority and Scott (1967) and Coen and Dehority (1970) are in unaimouse agreement that B. succinogenes was considered to be most active in digesting cell wall cellulose, but was unusual in being unable to utilize the hemicellulose which it degraded.

Hemicelluloses are a group of polysaccharides consisting of largely linear xylose chains with varying amounts of arabinose, uronic acid and galactose. Prins (1977) reported that the bran-

ching and solubility in water increase as proportions of uronic acids and galactose increase. As with cellulose, hemicellulose hydrolysis proceeds primarily through the extracellular release of the disaccharide, xylobiose, which is hydrolyzed by an intracellular enzyme-xylosidase-to form xylose. It appears that the cellulolytic bacteria are the most important species involved in hemicellulose hydrolysis. Dehority(1973) suggested this activity represents a nonspecific hydrolysis of the β -1, 4-xylosidic linkages by cellulase. Dehority and Scott(1967) found Butyrivibrio fibrisolvens, which may be numerically abundant in ruminants fed poor quality forage tend to digest more hemicellulose than cellulose. They also reported that hemicellulose hydrolysis can be quite gratuitous for closely associated noncellulolytic bacteria, which can use xylobiose, because several cellulolytic microbes, most notably B. succinogenes, do not themselves ferment the released pentoses(figure 2.1).

Pectin is a complex group of polysaccharides the basic building block for which is galacturonic Acid. Prins(1977) noted that varying amounts of the methyl ester of galacturonate and other sugars are present within the linear portion of the polymer and in side chains of pectin. At least two enzymes are required for pectin hydrolysis, methylesterase and polygalacturonidase. Pectin utilizers include B. succinogenes, B. ruminicola, B. fibrisolvens, S. bovis, Sachnospira multiparus, and ruminal spirochetes.

2.1.2. Volatile fatty acid production in the rumen

The V.F.A. which serve as a major source of energy to the ruminant, arises largely from the fermentation of dietary carbohydrates. Manta(who quoted from Rasmussen, 1965) commented that energy metabolism in ruminant is essentially one of fatty acids rather than carbohydrates. As much as 70% of the net energy requirements of ruminant is provided through V.F.A. Blaxter(1962) found that animal performance was highly related to the proportions of acetate to propionate, observed in rumen contents. It soon became common practice to measure rumen fluid V.F.A. in connection with performance trials and relate the acetate/propionate proportion to diet composition.

The amounts and proportions of individual V.F.A. produced during the fermentation of feed in the rumen vary according to the diet and the way in which it is prepared before feeding. In addition the observed ratios for V.F.A. vary from animal to animal and within animals, from time to time. Bath and Rook(1963) reported that with diets consisting entirely of hay it is usual to find 68-76% acetic, 14-19% propionic, 8-12% butyric acid. Manta(who quoted from Rasmussen, 1965) reported that the addition of concentrates to diets of hay or silage, fed to cattle has usually caused a decrease in the proportion of acetic acid and a rise in propionic acid.

The fine grinding of hay has been shown to decrease markedly the proportion of acetic acid and to increase the proportions of

propionic and butyric acids (Wright et al, 1963 ; Moore, 1964). But, Hogan and Weston(1967) found that changing sheep from chopped wheaten hay to ground wheaten hay, at the same level of intake, increases the proportion of acetate and reduces the proportion of propionate. Table 2.1 showed the effects of physical form of diet on V.F.A. production.

Recently, Murphy et al(1982) have studied a relationship between chemical composition of feeds and V.F.A. formation. Roughage diets are high in cellulose, intermediate in soluble sugar and low in starch. consequently, the numbers of cellulolytic and saccharolytic microbes are high and a large proportion of acetate is formed. This is due to cellulose fermentation, but also because the saccharolytic microbes compete favorably for soluble carbohydrates and the products of starch hydrolysis. Diets, high in starch, result in large numbers of amylolytic microbes which compete favorably for soluble carbohydrates and the products of starch and hemicellulose hydrolysis. Thus, propionate production is increased when cereals are fed, not only because of the high starch fermentation, but also because the fermentation products, formed from other carbohydrates, are altered to favor propionate.

2.2. Low quality roughage

No absolute definition of roughage quality has, so far, been given, and the term "quality" remains relative. The value of a

Table 2.1. Relation of diet to rumen volatile fatty acids in cattle

Ration	Rumen fluid V.F.A. as molar % of total V.F.A.					
	C2	C3	C4	C5	C6	
Long hay (38-42 lbs.)	70.5	19.5	7.5	2.6		
G and P hay (28-32 lbs.)	67.9	20.2	8.7	3.1		
G and hay (26 lbs.) + Ground corn (4 lbs.)	61.9	25.1	9.0	4.1		
G and P hay (28 lbs.) + Heated corn (4 lbs.)	53.9	31.1	9.4	5.5		
G and P hay (6 lbs.) + Heated corn (18 lbs.)	47.5	38.7	7.8	6.1		
G and P hay (16 lbs.) + Glucose (4 lbs.)	55.9	17.7	18.4	8.0		
Long hay (6 lbs.) + Heated corn (18 lbs.)	53.8	22.7	15.6	7.9		
Chopped hay, Ground corn + Pelleted linsed oil meal (1:1:0.2)	68.2	16.3	10.9	2.5	2.0	
G and P hay, Heated corn + Pelleted linsed oil meal (1:1:0.2)	47.2	41.1	9.2	1.9	0.8	

G and P : Ground and Pelleted

Source : Rasmussen, 1965.

roughage as a feed depends on the amount of dry matter consumed, its chemical composition, digestibility, balance of nutrients and economy of use.

Crop residues have potential as feedstuffs for ruminants. However, this practice has not been widely accepted because these low quality roughages are inefficiently utilized by ruminants compare to higher quality forages. This inefficiency is due to the low digestibility and poor nutritive value of crop residues.

Lignin is the chemical fraction of the cell wall most frequently associated with digestibility of forages by ruminants, and is a major factor associated with the poor digestibility of straw and other low quality forages (Van Soest, 1967, 1969; Waite et al, 1964).



It is now generally accepted that the decrease of digestibility accompanies with plant maturity. And this due to the deposition and extent of the lignin compounds, which form a protective covering against cellulose-splitting micro-organisms. Van Soest (1971) and Smith et al(1972) are unanimous agreement that lignin is a major endogenous plant factor, affecting digestibility of forage fiber. Lignin consists of polyphenols that are highly polymerized. It is intimately associated with other fiber components of the plant cell wall and apparently decreases the rate and extent of fiber digestibility. Digestibility of fiber or dry matter has been negatively related to the lignin content of forage (Van

Soest, 1971; Smith et al, 1972).

Kirk and Chang(1981) summarized the nature of lignin:

- a) lignin is the most abundant biopolymer next to cellulose and contains 1.5 times the carbon content of cellulose
- b) it is a complex macromolecule synthesized by the dehydrogenative radical polymerization of p-hydroxycinnamyl alcohols
- c) it contains several different interunit linkages, many of which are non-hydrolysable
- d) it physically protects the polysaccharides in lignocellulosics and must be disrupted for enzyme accessibility to the polysaccharides.

Baker(1973) and Cogswell et al(1976) reported that lignification has been considered to be one of the primary barriers to ruminant microbial digestion of fiber. Various mechanisms have been suggested by which lignin inhibits cell wall digestion, e.g. encrustation, toxicity to digesting microbes and lignin-polysaccharide complexes. Bolker(1963) found plant polysaccharides are apparently not simply encrusted by lignin but are probably covalently bonded to it. Hartley(1972), Barton and Akin(1977) suggested the type and extent of lignin-polysaccharide bonding could have more effect on digestion than the amount of lignin per se. Dehority and Johnson (1961) reported ball milling of lignified tissue greatly increased in vitro digestion of cellulose in intact forages, suggesting that lignin inhibited cellulose digestion by acting as a physical barrier.

between cellulose and cellulolytic rumen bacteria.

There are, however, several other factors which may account for anomalies in the relationship between lignin content and digestibility. Wilkins (1969) reported the digestibility of forage is influenced, in some cases, by rate of digestion in the rumen and by the rate of passage of the forage through the alimentary tract.

Van Soest(1964) observed that lignin appears to influence only the digestibility of other cell wall components. Johnston and Waite (1965) suggested that the effect of a particular quantity of lignin on the digestibility of other cell wall components is, therefore, likely to vary according to the pattern of distribution of lignin. Wilkins(1972) reported that the potential cellulose digestibility had significant negative correlations with both lignin content($r = -0.862$) and lignified tissue ($r = -0.905$).

In recent years, however, increasing evidence has been found of the existence of a complex formation between lignin and other cell wall constituents, particularly the hemicellulose. It is believed that lignin is amorously deposited along with the hemicellulose, between microfibrils, which appear to exist as an aggregated separate phase. Hartley and Jones(1976) found ester crosslinks between hemicellulose and lignin in monocotyledonous plants. In extracting the lignin carbohydrate complex of ryegrass, Morrison (1974) also discovered ester crosslinks between phenolic groups on lignin, and polymer of xylan and cellulose. These crosslinks hinder

swelling and digestion of fiber, but can be broken by alkaline agents during the process of saponification (Tarkow and Feist, 1969). Higuchi et al (1967) established the presence of ester linkages in the bridging units between lignin and cell wall carbohydrates.

However, there is evidence that lignin is not a totally inert compound. Philips (1934) concluded that some lignin could be degraded during digestion. Gaillard and Richards (1975) found that the cell-free rumen liquor of steer on a diet of spear grass, contained macromolecular substances in which carbohydrates and lignin-derived compounds were covalently bound to each other. The possibility then exists that the formation of soluble lignin-carbohydrate complexes, by the action of rumen microorganisms on a forage, might account for the dissolution and thus the apparent digestion of about half of the total lignin ingested (Gaillard and Richards, 1975).

Minson (1971) and Grant et al (1974) indicated that lignin was partly digested. They recovered only the lignin that remained in the fibrous residues of the feces. Therefore, apparent digestion of lignin may be obtained partially by the formation of soluble lignin-carbohydrate complexes that pass from the rumen. This digestion is due to the dissolution of lignin-carbohydrate complexes. Soluble lignin-carbohydrate complexes from forages which had been degraded by bacterial enzymes were isolated from rumen liquor of grass fed ruminants.

Morrison (1974) found that when lignin-carbohydrate complexes

were subjected to mild alkaline conditions, lignin was degraded to a lower molecular weight phenolic compound. He postulated that ferulic acid and p-coumaric acid, degradation products of lignin, acted as crosslinking agents between lignin and carbohydrate because they possess two important functional groups : the hydroxyl group and the carboxylic acid group. Hartley (1972) also found that two of these phenolic degradation products, ferulic acid and para coumaric acid-act as cross-linking agents between lignin and cell wall carbohydrates.

Cinnamic acid derivatives serve as building blocks of lignin, and other universal components of vascular plants (Harkin, 1973). Para coumaric and ferulic acids crosslink lignin to structural carbohydrates of plant cell walls (Hartley, 1973). These phenolic degradation products could be of considerable importance in the evaluation of carbohydrate digestibility.

Chaves et al (1982) obtained two high correlations between NDF digestibility and p-coumaric acid content, and lignin and p-coumaric acid content. Fahey et al (1980) report the disappearance of phenolic material from various sections of the gastrointestinal tract. All phenolic monomers of roughages decreased in concentration with passage through the tract.

Zemek et al (1979) who studied the inhibitory effects of 11 different lignin degradation products on bacterial growth. All compounds showed some inhibitory activity. Isoeugenol, a compound

containing double bond in the α , β positions of the side chain and a methyl group in the positions, was found to be the most inhibitory. Compounds with a carbonyl group in the α or β position or those with carboxyl and hydroxyl groups in the side chain were substantially less inhibitory.

2.3. Some treatments for improving the nutritive value of low quality roughage

The low digestibility and the poor nutritive value of crop residues are related to the extent of lignification of the cell wall component. Processes which delignify the fibrous fraction or otherwise solubilize cellulose and hemicellulose should improve digestibility of residues.



Numerous methods have been developed for improving the nutritive value of low quality roughages. Different methods : the chemical methods use of alkali and ammonia, grinding, gamma irradiation and enzyme addition.

2.3.1. Alkali treatment

NaOH is the most widely used alkali reagent. Most workers appear to consider that benefits attributable to NaOH treatment arise from the delignification of cell walls.

Tarkow and Feist (1969) reported that NaOH attribute beneficial effects to removal of lignin by saponification of acetates and

cleavage of ester bonds on xylan chains of timber. Crystalline cellulose is not soluble in strong alkalis, but is greatly swollen and undergoes modification to the crystalline structure (Warwicker and weight, 1967). Considerable variations exists in the method of alkali treatment used ; levels and type of alkali used ; type of forage materials and physical conditions during the treatment process.

Bechmann (1921) developed an alkali treatment process. In the Beckmann process for improving the nutritive value of poor quality roughages, the feed is soaked in a dilute solution of sodium hydroxide for 1-3 days, allowed to drain, and washed repeatedly with water to remove excess alkali. The process improves the digestibility of the straw from 40 to 60-70% and makes the feed more acceptable to the animal. Its major disadvantages are a high requirement of labour, the loss of soluble nutrients, and the need to preserve the product by drying or ensiling. In arid areas the requirement for large volumes of water (c.40 l/kg) would also be a disadvantage.

Recently a new process has been devised by Wilson and Pigden (1964) and Donefer et al (1969). Which involves spraying with a relatively small volume of a concentrated solution of NaOH and neutralization of excess alkali with an organic acid.

Many hydroxides are used : sodium hydroxids, ammonium hydroxide, calcium hydroxide and potassium hydroxide.

Waller (1976) compared the effect of chemical treatment of corn cobs with either sodium, calcium or ammonium hydroxide. He reported that alkali treatment, especially with sodium hydroxide, increased the rate of both in vitro cellulose and hemicellulose digestion. He also stated that chemical treatment solubilized some of the hemicellulose while not changing the cellulose content. Meang et al (1979) reported the in vitro dry matter digestibility of barley straw improved with increasing concentration of chemicals, however treatment with $\text{Ca}(\text{OH})_2$ and $\text{NH}_4(\text{OH})$ only improved a small extent. The solubility of barley straw increased and at the same time cell wall constituent of barley straw decreased with increasing concentration of NaOH , but acid detergent fiber, cellulose and lignin content of barley straw were not changed.

Klopfenstein (1978) concluded that the effect of chemical treatment, especially treatment with sodium hydroxide included :

- a) solubilization of hemicellulose, b) increasing the extent of cellulose and hemicellulose digestion and c) increasing the rate of cellulose and hemicellulose digestion, probably by swelling.

Klopfenstein et al (1972), and Rexen and Thomsen (1976) reported that lignin contents are generally not reduced by chemical treatment. This indicates that the increase in extent of digestion is probably due to breaking of the bonds between hemicellulose and cellulose without actually removing the lignin (Klopfenstein, 1978). Lesoing et al (1981) found that a significant amount of hemicellulose

was solubilized by chemical treatment with NaOH and Ca(OH)₂, but little cellulose was solubilized.

However, there is a different response to chemical treatment where measured by in vitro and in vivo results. Rexen and Thomsen (1976) reported that increases in digestibility of barley straws were consistent with in vitro and in vivo results when up to 4% of sodium hydroxide was added. With higher levels of NaOH, in vivo digestibility did not increase, but in vitro digestibility appeared to increase. Jackson (1977) observed a marked and consistent increase in dry matter digestibility of NaOH treated straw with in vitro fermentations ; however, in vivo digestibility increases were not so consistent. Mowat and Ololade (1970) found that in vivo digestibility of barley straw increased up to 4% NaOH treatment ; however, treatment at 6 and 8% did not produced a further increase. Undigested cell walls in the the feces decreased at higher levels of NaOH treatment, indicating that cellulose and hemicellulose digestibilities continued to respond to chemical treatment, however, increased excretion of neutral detergent solubles was apparent. Berger et al (1979) reported in vitro dry matter digestibility was greater than in vivo digestibility at the 4, 6 and 8 % NaOH levels. He also reported that NaOH decreased NDF content but only slightly decreased the percentage of ADF in corn cobs.

The low quality roughages which have been treated chemically are corn cobs (Waller and Klopfenstein, 1975), Wheat straw(Chandra

and Jackson, 1971) Barley straw (Rexen and Thomsen, 1976) oat straw (Sexena et al, 1971 ; Rexen and Thomsen, 1976), and rice straw (Chandra and Jackson, 1971).

Physical condition of roughages is also the factor affecting alkali treatment. Coombe et al (1979) reported that alkali treatment of either the chopped or pelleted straw increased digestibility of these components by 10% units compared to nonalkali treated straw. Potential digestibility of straw dry matter in the rumen, was increased by alkali treatment from 49-84% in chopped straw and from 59 to 73% in pelleted straw.

NaOH treatment of straw for feeding to animals has not always resulted in increased intakes and weight gains that might be expected with improved digestibility.

Jackson (1977) cited several possible reasons for such differences including an inhibitory effect on rumen microbial activity, a high pH and osmotic pressure of rumen fluid and a faster rate of passage of feed through the rumen.

2.3.2. Ammonia treatment

Attempts to improve the nutritive value of straw and other low quality roughages by chemical treatment have been made from about the end of the last century (Lehmann, 1895).

Today there is a world wide search for new practical methods to treat low quality roughages to increase their feeding value.

One of the most promising is the ammonia treatment. Both anhydrous ammonia and solution of ammonia in water have shown a positive effect in improving the nutritive value of low quality roughages.

Herrera-Saldana et al (1983) concluded that the ammonia treatment has some advantages over other alkali it increases both digestibility and crude protein content, does not require dehydration of the straw after treatment and can be carried out under farm conditions. Rounds et al (1976) observed that ammonia treatment appears to give slightly lower improvement in feeding value, than the methods involving "dry" treatment with sodium hydroxide, or ensiling with mixtures of sodium and calcium hydroxide. With damp materials, it may difficult to remove excess free ammonia, resulting in poor intakes.

Terashima et al (1980) and Tohrai et al (1978) commented that ammonia treatment increased the nitrogen content and the dry matter digestibility. Furthermore, the ammonia treatment decreased the content of neutral detergent fiber and increased the in vitro dry matter disappearances but did not affect the content of acid detergent fiber, composed of cellulose and lignin. Sundstol et al (1978) found that ammonia treatment increased nitrogen content by 0.8 to 1.0 percentage units equal to an increase of 5-6 percentage units in crude protein content ; in other words, the nitrogen content of the feed is roughly doubled. Horton and Steacy (1979) reported that ammonia treatment increased the average crude protein content of the straws, almost threefold, though improvement ranged from 50

to 276% when barley, oat and wheat straw were treated with 3.5% anhydrous ammonia.

Itoh et al (1975) found that most of the increased nitrogen was occupied with non-protein nitrogen. It was assumed that the increased N content, especially bound fibrous materials, existed as a lignified nitrogen compound.

Soundstol et al (1978) mentioned that there is no reason to doubt that N bound to the feed, through ammonia treatment can be utilized for protein synthesis in the rumen in a manner similar to other NPN. However, more research is required to determine if the nitrogen in ammonia treated materials is more slowly released from the rumen than that from ; the urea.

Coxworth et al (1976) reported an improvement of 14%, 8% and 12% units of digestibility when wheat, oat and barley straw were treated with 5% anhydrous ammonia. Arnason and Mo (1977) also reported an improvement of digestibility in barley straw, treated with 3.5% anhydrous ammonia.

In some fibrous materials, only the cell wall constituent was decreased, that is the non cell wall material was increased by ammonia treatment. Contents of acid detergent fiber and cellulose were not affected by the ammonia treatment.

Terashima et al (1980) found that ammonia treatment the decreased hemicellulose content, but did not affect the other fibrous materials such as cellulose and lignin. Lee (1981) reported that

NDF content decreased, but ADF and lignin content were not changed by ammonia treatment, indicating that considerable amounts of nutrients in these low quality roughages remained undigestible even after ammonia treatment.

There are many factors influencing ammonia treatment of low quality roughage : type and level of ammonia; moisture content; temperature and time of treatment; initial quality and type of material.

Kiangi and Kategile (1981) reported that anhydrous ammonia was most effective in improving in vitro dry matter and organic matter digestibility of maize stover; aqueous ammonia had a similar effect in increasing in vitro dry matter and organic matter digestibility of rice and wheat straws. Herrera-Saldana et al (1983) compared anhydrous ammonia and aqueous ammonia treatments. Crude protein content and in vitro dry matter digestibility increased from 3.6 to 9.8% and from 13.8 to 31.8%, with anhydrous ammonia treatment, and from 3.6 to 11.9% and from 13.8 to 32.9% with aqueous ammonia treatment. Borhami and Sundstol (1982) found that treatment with aqueous ammonia caused significantly higher improvement of in vitro dry matter and organic matter digestibility than with anhydrous ammonia. Solaiman et al (1979) concluded that NH_4OH had the potential to reduce the chemical cost of treatment and increase the crude protein content of the treated wheat straw.

Sundstol et al (1979) showed that the dry matter digestibility

increased as the level of ammonia increased up to 4% dry matter. No benefit in increasing the level about 5.5% was observed. Jackson (1977) found an increase of about 15% units in in vitro dry matter digestibility of straw when the level of ammonia was increased from 3% to 5.5%.

Borhami and Sundstol (1982) reported that the 4% level of ammonia was more effective than the 2% level; treatment with aqueous ammonia was more effective than with anhydrous ammonia.

Moisture content of the straw influenced the effect of ammonia treatment; moisture contents ranging from 10 to 50% have been cited as optimal (Waiss et al, 1972; Solaiman et al, 1979; Sundstol et al, 1979). Sundstol et al (1979), however, found a positive effect of increasing moisture content up to 50%, these workers stressed that high moisture content, in the material may cause some distribution problems when ammonia is injected.

The optimal time of treatment varies with the temperature of treatment. Kernan et al (1977) concluded that treatment time should be longer, at low temperatures. Solaiman et al (1979) reported that prolong treatment period had a significant linear effect on both in vitro dry matter digestibility and nitrogen content.

Horton and Steacy (1979) found that cereal straws do not respond uniformly to treatment with anhydrous ammonia in terms of increased crude protein content, intake and digestibility. Horton (1978) reported that barley, wheat and oat straw may differ in their

response to treatment with anhydrous ammonia.

2.3.3. Gamma irradiation

Considerable evidence indicates that the feeding value of many low quality roughages is limited, not by the absence of potential nutrients, but rather by the encrustation of carbohydrates within a lignin structure which is impenetrable to rumen bacteria.

Gamma irradiation can be expected to increase the amount of test-fed material in a finely particulate form and to increase, by cell rupture, soluble material in cells (McManus, 1972a). Gamma irradiation have been successfully to liberate these nutrients from the encrustation.

Timpa (1983) summarized the effect of gamma irradiation on the lignocellulosic materials. High levels of gamma irradiation causes degradation of cellulose. The manifestations of this degradation include increased the availability to rumen bacteria in the case of wood and grasses and alterations in physical properties, such as viscosity, swelling, and mechanical strength in the case of polysaccharides. Cleavage of 1,4-glucosidic bonds in cellulose results in reduction in chain length. Oxidative degradation related to the production of carbonyl and carboxyl groups at the chain ends.

Teszler and Rutherford (1956) commented that the general effects of radiation on polymers are at least twofold : polymers may be improved with respect to certain properties, through the medium

of cross-linking or they may be degraded because of scission of their long chain molecules. Thus, in one case, the effect is to increase the average molecular weight while in the other it is to decrease the molecular weight.

Various radiation sources were used : high energy cathod-ray (Charlesbay, 1955), thermal neutron (Teszler et al, 1958), gamma ray (Gilfillan and Linden, 1955 ; Teszler et al, 1958 ; Dilli et al, 1967 ; Ammerman et al, 1959 ; Pigden et al, 1966 ; Pritchard et al, 1962 ; McManus et al, 1972ab, and Timpa, 1983).

The effect of irradiation on cellulose has been reported by several worker. Lawton et al (1951) reported high energy irradiation can produce delignification, depolymerization and destruction of the crystalline structure of cellulose. Charlesbay (1955) showed that high energy cathod-ray bombardment of wood pulp and cotton linters resulted in chain-scission ; evidence of this is a decreased in viscosity and an increased in solubility. Gilfillan and Linden (1955) found that the strength of cotton was reduced by gamma irradiation. Teszler et al (1958) reported extensive depolymerzation of cotton fibers at low levels of irradiation.

Ammerman et al (1959) showed that the crude fibre and cellulose content of cotton linters, peanut hulls, corncobs and sugar cane bagasse irradiated at doses of 0, 10, 20, 40 megaroentgens, decreased with each increased in level of irradiation. Yu yu and Emery (1975) reported that electron irradiation markedly reduced fibrous

components at dose levels of 7.66 log rads and above. About 40% of the fibrous constituents in the original straw was solubilized at the highest dose level (9 log rads).

Gamma irradiation effects the in vitro and in vivo experiments. pigden et al (1966) studied effect of gamma irradiation on forages at different maturity stage and the faeces from animals fed these forages. They found that irradiation decreased in vitro dry matter digestibility of immature forages, but markedly increased in vitro dry matter digestibility at intermediated and mature stages ; the in vitro dry matter digestibility of faeces was increased at all growth stages, with maximum effects at mature stages. Total VFA production was generally decreased in the forages but markedly increased in faeces.

Pritchard et al (1962) studying the effects of gamma irradiation of ^{60}Co upon the feeding value of wheat straw, determined by in vitro fermentations, reported an increase of digestibility. However, increases the VFA production from the fermentations were found only up to doses at 2.5×10^8 rads, suggesting that above this level of radiation the carbohydrates are disintegrated to such a degree that they are no longer suitable substrates for rumen microorganisms.

Millett et al (1970) reported that electron irradiation increased the in vitro digestibility of spruce timber from 0 to 14% and digestibility of aspen from 32% to more than 75%.

McManus et al (1972^a) reported that gamma-irradiated rice straw, nassella and cotton lint in terylene bags in the rumen of sheep were degraded at substantially enhance rate compared to non-irradiated feed samples. However, evidence of reduced intra-ruminal dissimilation was also presented for the irradiation dose range 5-25 Mrad, especially of feeds irradiated in the wet state. This depression was not alleviated by supplying nitrogen as urea, indicating that nitrogen lack was not the only contributing factor. He suggested that factors generated by irradiation per se (active hydroxyl groups, peroxide or p^H effects) could have suppressed dissimilation. It was recognized that these factors could also exert effects directly upon the host ruminant animal as well as upon the microbiota. He also commented that the optimum dose of irradiation is 25 Mrad.

McManus et al (1972^b) reported that mean retention time and apparent digestibility were reduced when sheep fed irradiated wheat and rice straw. But voluntary feed intake was not significantly affected. He also reported that distinct changes occurred in the ratio of acetic to propionic acid in the rumen liquor of sheep, which suggest an alteration in the species composition in the foregut microbial population. This is the evidence presented for the presence in irradiated diets of a factor or factors toxic to the rumen microbiota. Yu yu and Emery (1975) reported that high level of electron irradiation (8.66 log rads) increased the total in vitro

cell wall digestion mainly by solubilization; rumen microbial cell wall digestion was dramatically reduced at high levels of irradiation.

Formation of free radicles and unknown toxic compounds by irradiation have been reported. Gilfillan and Linden (1955) suggested that the major cause of cellulose degradation was oxidation, resulting in the formation of cellulose peroxide, formed from the hydroxyl groups present in cellulose. Teszler et al (1958) found that acid groups were formed in cotton when the fiber arose by oxidation. Dilli et al (1967) reported that radiation induced the formation of trapped radicals in cellulose.

Some researchers have tried chemical, grinding and gamma irradiation. McManus and Choung (1976) studied the effects of prior grinding, and irradiation with gamma rays (0-50 Mrads) on NaOH treatment. Moderate increases in solubility and digestibility of straw followed irradiation but not for hulls. Major increases were found in irradiated straw further treated with alkali but hulls were little affected by 5g NaOH/100g D.M. excepted at the 50 Mrads level. Grinding had little effect on degradation of alkali-treated hulls. Alkali treatment removed encrusting silica and lignin from rice hulls and increased in vitro and in vivo digestibility.

Timpa (1983) reported effects of gamma irradiation and chemical treatments at low levels (HCl, H₂SO₄ and NaOH) on crystalline cellulose and amorphous cellulose. Irradiation treatment reduced the

fiber dry weight, but the greatest loss of weight was in the bagasse sample, treated with 1% sodium hydroxid and gamma irradiation. He suggested that the fiber weight loss reflects the amount of material that has been solubilized during the irradiation treatment. The p^H changes toward the acid range, with irradiation treatment compared with the corresponding control indicate the generation of acidic groups. The aliquots of samples from the sodium hydroxide treatment and gamma irradiation were extremely viscous and difficult to filter. By HPLC separation, irradiation and chemical treatments solubilize more material of an acidic nature as would be expected from a hemicellulose component. The amorphous cellulose powder was generally degraded into a greater number of components, detected by HPLC, than the more crystalline cellulose cotton. The most dramatic effect was found with combination of sodium hydroxide and irradiation in bagasse, when much larger amounts of materials were solubilized into a liquid phases.

2.3.4. Grinding

Some researchers have shown that finely ground and pelleted forages are less digestible than chopped forages, while others have observed little or no effect of these treatments on digestibility.

Blaxter et al (1956) showed that increasing the fineness of grinding led to a decrease the mean time spent in the rumen, and to a depression in the apparent digestion of dry matter and organic

matter. The digestion of crude fiber, nitrogen free extract and crude protein fractions were also depressed. The investigation of the effects of grinding on single feed, suggests that the depression in digestibility could be accounted for largely by increased rates of passage.

Alwash and Thomas (1971) observed that grinding and pelleting depressed cellulolytic activity in the rumen. Gharib et al (1975) reported that dry matter digestibility of poplar bark was not enhanced when the bark was ground through finer screens. Milne and Campling (1972) found only small differences between the physical forms of forage and digestibility of organic matter.

The digestibility of crude fiber and the mean retention time in the gut decreased with decreasing particle size.

Osbourn et al (1981) reported that the digestion of organic matter and cell wall was depressed by decreasing fineness of grinding. Osbourn et al (1976) found that the fiber or cell wall fraction is invariable fraction which is most affected by grinding. It seems logical therefore to assume that grinding, has less effect on the digestion of legume forages which contain a lower proportion of potentially digestible cell wall than grasses of similar organic matter digestibility.

2.3.5. Enzyme addition

The use of enzymes to increase the feeding value of low quality roughage has been investigated.

Yu yu and Emery (1975) reported that the addition of cellulase and pectinase did not reduce fiber content of oat straw nor improved in in vitro cell wall digestibility, but increased in vitro cell wall digestibility of alfalfa residue. Daniels and Hashim (1977) found increases of dry matter digestibility when ground rice hulls were added with fungal enzymes. Willis et al (1980) observed that the addition of enzymes (hemicellulase, pectinase and β - glucosidase) alone, caused a reduction in vitro dry matter digestibility of rice straw, but the highest in vitro dry matter digestibility was obtained when pretreated with 5% NaOH and an enzyme addition (20mg/100g DM).

2.4. Some techniques for measuring nutritive value of roughage

The value of roughages depend upon the number of animal they will support. Because feeding trials are expensive in terms of animal, labor, equipment, and feeds, investigations generally have adopted or are developing laboratory tests to screen potentially valuable ruminant feeds. These tests were correlated with animal production performance.

The detergent system of feed analysis, proposed by Van Soest (1967) has been satisfactory for temperate forages. Various equa-

tions using neutral detergent fiber, acid detergent fiber and acid detergent lignin, in various combinations, have been developed which accurately predict in vivo digestibility.

The two stage in vitro rumen fermentation method, developed by Tilly and Terry (1963) and used by many other investigators, has proven reliable in prediction in vivo digestibility. Enzymatic incubation values using pepsin, proteinase, cellulase or combination of these were shown high correlations with in vivo digestibility, total digestible nutrients and intake.

Because of the cost and labor involved in conducting in vivo experiments, alternative methods of determining degradability of feedstuffs are being investigated. The dacron bag technique is one of these procedures which has the potential to aid dry matter digestibility in the rumen.

2.4.1. Chemical Method

The Weende system of proximate analysis has been generally used for all the foregoing objectives in human nutrition, nonruminant and ruminant nutrition for more than 100 years. However, there has been much dissatisfaction with this system, particularly with the crude fiber determination and the calculation of nitrogen free extract (NFE).

From time to time efforts have been made to find a suitable replacement. The detergent partitioning of dry matter suggested by

Van Soest (1963) is now widely used for evaluation of forages.

Goering and Van Soest (1970) described a detergent system which included neutral detergent fiber (cell wall constituents), acid detergent fiber, acid detergent lignin, acid detergent cutin and acid detergent nitrogen. They also described assays for permanganate lignin, cellulose, insoluble ash and silica. They concluded that neutral detergent fiber appears to separate the nearly completely digestible nutritive available (98%) and the soluble forage constituents (cellular contents) from those that are not completely available (cell walls). However, they depend upon microbial fermentation in the rumen becoming partially available to the animal. Neutral detergent fiber is considered to be essentially hemicellulose, lignocellulose, and insoluble ash. Acid detergent fiber is considered to be lignocellulose and silica, so that the difference between neutral and acid detergent fiber residues is estimate of hemicellulose. But, this difference also included some cell wall protein.

Summative equations have been proposed for calculating digestibility of a mixed forage population, based on consideration of the cell walls and the cellular contents as separate digestive entities (Goering and Van Soest, 1970 ; Van Soest, 1967 ; Seoane, 1982).

There are :

Dry matter digestibility (%) : $0.98 \times (100 - \text{NDF}) + \text{NDF} \times \text{Digestion coefficient of the cell wall metabolic fecal losses}$; Goering

and Van Soest (1970).

Dry matter digestibility (%) : $0.98 \times (100 - \text{NDF}) - 12.9 + \text{NDF} (1.473 - 0.789 \log \text{lignin}) \times 100 / \text{ADF}$; Van Soest (1967).

Dry matter digestibility (%) : $0.98 \times (100 - \text{NDF}) - 12.7 + \text{NDF} (1.285 - 0.638 \log \text{lignin}) \times 100 / \text{ADF}$; Seoane (1982).

Many correlations between either detergent fiber and fibrous material, or between digestibility and intake level have been reported. Barnes (1973) obtained that there were high correlations between either NDF and ADF, or between ADL and in vivo digestibility or forages intake. Marten et al (1975) found that ADF was the best of numerous chemical assays for predicting in vivo digestibility of corn and sorghum silages. Seoane (1982) found high correlations between lignin and apparent cell wall digestibility, between logarithm (lignin x 100/ADF) and apparent cell wall digestibility.

Numerous modifications of the Van Soest detergent system have been reported. Clancy and Wilson (1966) proposed a modified acid detergent fiber. They found that increasing the acid strength and prolonging the boiling time improved the relationship between ADF and digestibility. But this treatment prevented the acid detergent fiber being used as a means of assaying for heat damage and unavailable protein. McLeod and Minson (1969) reported that the residual standard deviation for ADF as an index of digestibility, was higher for tropical than for temperate species. Consequently they suggested that modifications to the ADF procedure would be necessary to obtain

ADF values which could be used to predict digestibility for tropical grasses with the same residual standard deviation as for temperate grasses.

Bailey and Ulyatt (1970), Das et al (1975) and Van Soest and Robertson (1979) found some interference in the estimation of hemicellulose differences between NDF and ADF. These stem from the fact that neutral detergent dissolves pectin, tannin, and sometimes silica ; whereas acid detergent does not dissolve all pectin, or tannin-pectin complexes or silica. Thus, to obtain a purified ADF, neutral detergent extraction should precede acid detergent extraction.

These researchers outlined fiber system modifications that :

- a) omit decalin, because it increases fiber yield and contributes to difficult filtering ;
- b) add treatment with acetone or ethanol for high lipid feeds ;
- c) additional protease digestion for samples which contain an excess of 30% protein ;
- d) omit sodium sulfite from neutral detergent solution because it attacks lignin and causes significant lignin losses ;
- e) use amylases to overcome neutral detergent fiber filtering problems with high starch samples.

2.4.2. In vitro technique

Conventional digestion trials with large animals are frequently prohibitive, because of the time and expense involved. In some cases it may not be possible to obtain sufficient quantity of the

feed to conduct a digestibility trial. It may be of interest to evaluate, separately the contribution of the leaves and stems of herbage to the overall digestibility of the plant.

Considerable attention has been given to the development of in vitro techniques for estimating the forage feeding values, using small samples of herbage.

In his review, Johnson (1963) summarized the potential uses of the in vitro techniques, as follows :

- a) cellulose digestion and factors affecting it
- b) utilization of non protein nitrogen
- c) intermediate metabolism in both mixed and pure cultures
- d) studies of symbiosis
- e) studies of rate phenomena requiring a non steady state situation
- f) forage evaluation studies
- g) studies of bioenergetics of the rumen fermentation.

Since the two stage rumen fermentation method introduced by Tilley and Terry (1963), there has been extensive use of their procedure for the prediction the in vivo apparent dry matter digestibility of many different forages. An initial 48hr fermentation stage simulates the digestive process in the rumen and is followed by a second stage involving the solubilization of the dry matter residus from the first stage using acid-pepsin. The acid-pepsin digestion stage stimulates the in vivo breakdown of feed and microbial protein by the digestive enzymes in the abomasum of the ruminant.

Tilly and Terry (1963) proved that in vitro technique is the most accurate predictor of digestibility for both tropical and temperate grasses and legumes. They used 146 samples of grass, clover and alfalfa of known in vivo digestibility (Y), the regression equation $Y=0.99 X - 1.01$ (S.E. + 2.31) was calculated ; X= in vitro dry matter digestibility.

The in vitro techniques depend upon complete removal of microorganisms from the control or influence of the host animal. The principal objective of the in vitro technique is to duplicate, as closely as possible, the fermentation within the rumen of the animal, occurring as the result of microbial activity.

To minimize variation of in vitro techniques, Hungate (1966) have indicated the need for obtaining the rumen liquor from the class of stock from which the evaluations are being made and from animals fed diets similar to those being evaluated. Moore et al (1962) reported, sheep fed the same ration, showed considerable day-to-day variation in the amount of starch fermented. In addition they observed some differences between sheep in the relative numbers of predominant rumen bacteria. For these reasons they found it convenient to use a composite of the rumen liquor. Drawn from several animals to minimize variations.

The temperature in vitro techniques is controlled at 39°C by means of a water bath. Variations as little as 0.5°C may invalidate comparisons between individual fermentations. In addition special

care is necessary to prevent temperatures over 40°C which, if maintained for significant periods, may affect rumen bacteria activity (Johnson 1969). Anaerobic conditions are achieved by passing a steady stream of CO₂ through the test solution, or simply by saturating the medium with CO₂ and closing the fermentation vessels with a Bunsen valve. The p^H is adjusted by the addition of McDougall's solution, which is an artificial saliva first used by McDougall (1948). Most of the rumen microbial activity occurs between p^H 6.7 and 7.0 (Johnson 1969).

Instead of an acid pepsin digestion stage, Van Soest et al (1966) used neutral detergent solution. Neutral detergent solution is very effective in removing the bacterial cell wall and other endogenous products. They proposed the two stage in vitro procedure with neutral detergent, for the estimation of the true digestibility of forages, rather than the apparent digestibility.

2.4.3. Nylon (Dacron) bag technique

The nylon bag technique is a microdigestion method in which small samples are secured in bags made of indigestible materials (nylon, dacron, terylene or silk) and suspended in the rumen for microbial digestion. This technique is useful for the measurement of the digestion rate of forages or for measuring the effect of various treatment on the rate and extent of digestion. The rate and extent of digestion are measured by the loss of dry matter or

nutrient content from the sample after a specified period of incubation.

Factors influencing measurement of dacron bag digestibility in the rumen have been extensively studied : bag pore size and sample weight ; time ; basal diet ; rinsing method effects.

Samples from 1g (Van Dyne, 1962) to 2.85 kg (Lambert and Jacobson, 1958) have been reported. Erwin and Elliston (1959), Van Dyne (1962) and Van Keuren and Heinemann (1962) are in unanimous agreement that digestibility was linearly decreased when amount of sample was increased.

Fermentation times varying from a few hours to 96 hours have been adopted. Lusk et al (1962) compared dry matter disappearance values obtained by the nylon bag technique with those obtained from conventional trials of several roughages. They found that the 72 hours dry matter disappearance values were more closely associated with those obtained in conventional digestion trials than with any of the other time periods studied. Van Dyne (1962) and Hopson et al (1963) reported that digestibility is directly influenced by the duration of fermentation.

The influence of the basal diet on nylon bag digestibility has been reported. Hopson et al (1963) found that alfalfa fed to fistulated wethers significantly increased the cellulose digestion in the nylon bag containing alfalfa, bromegrass and timothy, compared with a basal ration of grass hays. Van Keuren and Heinemann (1963)

reported higher, but non-significant values when a steer was fed alfalfa hay than when the steer grazed alfalfa-orchard grass pasture. Neathery (1969) found that dry matter disappearance of samples of bermuda grass was higher in a steer was fed a ration of alfalfa-orchard grass mixed hay. He suggests that the reason bermuda grass gave significantly higher values might be that when the steer was on the bermuda grass diet, the number and species of microorganisms were more specific for cellulose digestion than the steer was on the alfalfa diet. Ayers et al (1976) reported that obtained 9 percentage units lower value when fed a wheat straw diet than when fed other diets. Ali and Stobbs (1980) suggested that the rate and final extent of loss of both protein and dry matter was related to the diet fed. He reported that the highest values were found when the fistulated cattle consumed a grass/legume diet and lowest values when eating a grass diet supplemented with grain sorghum.

The rinsing method on nylon bag digestibility were studied by Van Dyne (1962), Hopson et al (1963) and Chonest et al (1970). Van Dyne (1962) comparing two rinsing methods, in a first experiment the bags were rinsed as a group under running top water to minimize variations due to rinse procedures. In a second experiment they were processed in two different ways ; they were rinsed as in the first experiment and half of them received additional soaking in 75% ethyl alcohol solution. The latter group were thrice alternately

rinsed in running tap water and soaked in 75% ethyl alcohol solution and agitated in a large beaker of water prior to drying. They found differences between average percent cellulose digested for the two rinse procedures were highly significant although relatively small. There was greater than 10% difference (highly significant) in estimates of dry matter digestion by the two rinse procedures. Cellulose and dry matter digestion values both include losses of small undigested parages particles which might pass through the weave of the bag. Dry matter digestion values also include losses of soluble components from the bag during rinsing that may not have been digested. But, Hopson et al (1963) found that samples of ground forage placed on dacron bags and suspended in running water for a 24 hour period, have lost approximately 1% of the forage.

Chonest et al (1970) reported that the addition of pepsin treatment of the residue remaining in the nylon bags after removal from the rumen may improve the reliability of the nylon bag method. The pepsin treatment permits a more effective washing and reduces variation among triplicates, which is attributed to the elimination of microorganisms.

The nylon bag technique is subject to considerable variability and is difficult to standardize. Pigden (1969) and Lowrey (1970) concluded sources of variation are size and type of bags, cloth mesh size, sample size and fineness of grind, number or sample per

trial, diet of host animal, method of suspension, and method of cleaning and rinsing the bags after removal from the rumen. Orskov (1976) suggested standardized procedure of nylon bag technique. The standardized procedure include : a) the sheep should be fitted with a large rumen cannula, 30-40 mm in diameter and should be fed on a high quality hay or artificially dried grass, defined as hay with a digestibility of not less than 700g/kg dry matter ; b) the recommended bag size is 200 mm long and 90 mm wide ; c) the mesh size should be about 100 ; d) the length of nylon strings should be approximately 25 cm ; e) 4-6 bags can be incubated at the same time.

Ayers et al (1976) suggested that nylon bag technique will only closely reflect in vivo dry matter digestibility values provided that : a) the procedure of nylon bag technique is properly standardized in terms of sample size, forage consistant, incubation time, rinsing and pouch fabric, b) a nylon bag technique is used which permits ready ruminal insertion and withdrawal and flexible movement of pouches in the rumen, and c) adequate account is taken of the effects of different animal's basal diets.

III. MATERIALS AND METHODS

Barley straw, barley hull and rape husk were used in Experiment I, and barley straw, barley hull, rape stem and rape husk were used in Experiment II. These barley and rape byproducts were obtained from farms around Cheju city.

3. 1. Chemical analysis

Proximate analysis was performed by A.O.A.C. (1980) procedure. Neutral detergent fiber (NDF), cellulose, permanganate lignin and acid detergent fiber nitrogen (ADF-N) analysis were carried out according to the method proposed by Goering and Van Soest (1970). Acid detergent fiber (ADF) content was measured by the method outlined by Clancy and Wilson (1966), after pre-extracted with a neutral detergent solution. Hemicellulose content was calculated by difference between NDF and ADF.

The chemical composition of the barley straw, barley hull, rape stem and rape husk used in these studies, is shown in table 3.1 and table 3.2.

Total volatile fatty acid concentration was measured by the steam distillation method as described by Fenner and Elliot (1963), and individual volatile fatty acid production was determined by gas liquid chromatography, using hydrogen flame detector (Varian 3700), as proposed by Cottyn and Boucque (1968).

Table 3.1 Chemical composition of barley straw, barley hull and rape husk in

Experiment I

(Unit : DM basis %)

Item	Barley straw	Barley hull	Rape husk
Dry matter	87.47	87.42	86.20
Crude protein	4.27	4.56	4.17
Crude fat	0.51	1.86	0.82
Crude fiber	29.32	21.83	34.01
Ash	7.88	11.76	9.17
NFE	46.00	49.27	38.85
NDF	80.35	75.23	64.8
ADF	59.31	40.45	59.90
Hemicellulose	21.04	34.77	4.90
Cellulose	50.40	33.11	53.55
Lignin	7.34	4.70	6.76

Table 3.2. Chemical composition of barley straw, barley hull, rape stem and rape husk in Experiment II

Sample	(Unit : DM basis %)			
Item	Barley straw	Barley hull	Rape stem	Rape husk
Dry matter	90.82	90.74	87.42	88.84
Crude protein	5.89	4.34	4.38	4.38
Crude fat	3.12	1.10	0.96	1.25
Crude fiber	29.32	21.83	35.25	34.01
Ash	7.88	11.76	6.74	9.17
NFE	47.73	52.81	41.05	41.28
NDF	66.19	70.48	64.38	65.89
Hemicellulose	22.47	32.48	12.35	13.00

Reducing sugar formation was evaluated by the DNS method with glucose as a standard describe by miller (1959).

X-ray diffraction was carried out by the powder method described by Fan et al (1980), using X-ray diffraction analyzer. The sample was ground with cyclon mill using 200 mesh sieve.

3.2. Animals

Three adult wether sheep weighing about 40kg and fitted with rumen fistula, were used. The sheeps were individually maintained and housed in metabolism cases. They were fed high quality italyan ryegrass hay ad libitum, and fed twice daily.

These sheep were used as a donor source of pooled, strained rumen liquor in the in vitro study, and as test animals in the determination of dacron bag dry matter digestibility.

3.3. Treatment of samples and experimental design

3.3.1. Experiment I. Effect of gamma irradiation on low quality roughages

Barley straw, barley hull and rape husk were used in this experiment. Gamma irradiation was carried out using the ^{60}Co source of the Korea Atomic Energy Research Institute, Seoul, Korea. About 700g of barley straw, barley hull and rape husk were placed individually in plastic bags and then packed in appropriate cardboard

cylinders. Four levels of irradiation were applied : 2.5, 5, 10 and 25 Mrad.

Following gamma irradiation, about 100g of irradiated samples were ground through 1 mm or 2 mm screen.

3.3.1.1. Experiment I-1

This experiment was conducted to assess the effect of gamma irradiation on the fibrous material degradation, heat damage, reducing sugar formation, cellulose crystallinity and enzyme susceptibility of barley straw, barley hull and rape husk.

All samples were tested to determine the contents of NDF, ADF, cellulose, hemicellulose and ADF-N.

Following extraction with 0.05 M citrate buffer (pH 4.8) for 12 hours at 10 °C, reducing sugar content was measured using extraction solution.

The crystallinity of cellulose was measured by means of X-ray diffraction analyzer. The sample was mounted horizontally, while the geiger counter moved in a vertical arc. A Cuka target with filter was used. The sample was scanned through a range of 2θ from 5° to 30° . Relative crystallinity was calculated by following equation.

$$\text{Crystallinity index (I)} = \frac{\text{relative intensity of } 2\theta = 22.5^\circ}{\text{relative intensity of } 2\theta = 18^\circ}$$

Enzyme susceptibility was measured using cellulase, obtained from *Trichoderma viride* (ONOZUKA RS, Yakult Pharmaceutical Industry Co. LTD), as production of reducing sugar. 1g enzyme powder was dissolved in 100 ml of 0.05 M citrate buffer (pH 4.8), and then enzyme activity was checked. Including C.M.C. activity : 0.3953 IU, Filter paper activity : 0.4893 IU and Cotton fiber activity : 4.1098 IU per ml enzyme solution.

The standard mixture contained 50 mg ground sample, 0.5 ml enzyme solution and 1 ml of 0.05 M citrate buffer. The mixture was incubated at 50 °C for 30 minutes, 1 hour and 12 hour intervals in a shaking incubator. After incubation, 3 ml of DNS solution were added to each sample to stop enzymatic reaction. Following 5 minutes boiling, the reducing sugar production was determined as a glucose using a spectrophotometer at 550 nm.

3.3.1.2. Experiment 1-2

This experiment was designed to test the effect of different levels of gamma irradiation on the **dry matter disappearance and cell wall digestion of barley straw, barley hull and rape husk** in the rumen.

Following gamma irradiation, ground (2 mm screen) samples weighing about 2g were placed in 6 x 10 cm dacron bags. After insertion of the samples, each bag was tied with nylon string. Bags were secured at about 0.5 cm intervals near the end of a

weighted 69 cm nylon line. The line was tached to the rumen fistula and placed into the vental sac of the rumen for 12, 24, 36, 48 and 72 hours. Every sheep recieved one dacron bag from each of the 15 treatment groups. After removal from the rumen, the bags were washed throughly in running water, and then dried at 105°C for 12 hours. The dried residue was weighed and calculated dacron bag dry matter disappearance.

NDF content determination was performed on the dried residue in dacron bag to measure the cell wall digestion.

3.3.1.3. Experiment 1-3

In the in vitro system there was no opportunity for certain toxic compounds being diluted as happens in the dacron bag technique. Therefore, this experiment was designed to measure the effect of gamma irradiation on the microbial activity. In vitro dry matter disappearance, total and individual VFA production was determined using ground (1 mm screen) barley straw, barley hull and rape husk, after gamma irradiation.

In the determination of in vitro dry matter disappearance, gamma irradiated samples were incubated for 12, 24, 36 and 48 hours using the first stage of the in vitro fermentation described by Tilley and terry (1963). 0.25 g ground samples were incubated with 30 ml of inocula in water bath at $39 \pm 1^{\circ}\text{C}$. The inocula was composed of artificial saliva (McDougall, 1948) : 25 ml and strained fresh

rumen liquor from italyan ryegrass hay fed sheeps : 5 ml. Each tube had a blank control tube containing rumen liquor but no test substrate thus allowing calculation of the dry matter loss of samples following fermentation. After incubation, mixture of inocula and substrate in the tube filtered through filter paper (No. 5A, TOYO). After that, residue dried at 105°C for 12 hours. The dried residue was weighed and calculated in vitro dry matter disappearance. Filtered solution was refrigerated for determining the VFA concentration.

Total VFA concentration was determined on control and treatment liquor at the end of each fermentation. Determination of total VFA concentration was performed by steam distillation method (Fenner and Elliot, 1963). Determinations were carried out triplicate.

For the determination of in vitro individual VFA production, 0.25 g samples of all test materials were incubated for 1, 2, 4 and 6 hours, in the same manner as the in vitro dry matter disappearance determination. After fermentation, incubated liquor filtered through No. 5 filter paper (TOYO), and 5 ml of filtrate added to 1 ml of 25% metaphosphoric acid in a centrifuge cell. following centrifuging at 3000 rpm for 30 minutes, the supernatent was used to determine the total and individual VFA production. Individual VFA production was measured using gas liquid chromatography.

3.3.2. Experiment II. Effects of chemical treatment and mixed treatment by grinding, chemical treatment, enzyme addition with gamma irradiation on low quality roughages

Barley straw, barley hull, rape stem and rape husk were used in this experiment.

3.3.2.1. Experiment II-1

This experiment was designed to assess the effect of chemical treatment on improving the nutritive value of barley and rape by-products, and to determine the optimal treatment level of chemical treatment.

Two kinds of chemical reagent were used : sodium hydroxide (NaOH) and ammonium hydroxide (NH₄OH). Sample was ground through 100 mesh or 2 mm screen using laboratory mill.

About 100 g of ground samples were treated with 1.5, 3, 4.5 and 6 g NaOH/100 g DM, using 50 ml of distilled water/100 g DM. The NaOH solution was sprayed on the samples while contained in a plastic bag and allowed to react for 24 hours.

Another 100 g ground samples of each materials were treated with 1.5, 3, 4.5 and 6% NH₄OH (NH₃ basis). The samples contained 50% moisture. NH₄OH solution was sprayed on the samples while contained in plastic bags and allow to react for 7 days.

After reaction, NaOH and NH₄OH treated samples were dried for

overnight at 80°C.

NDF, ADF and hemicellulose and total nitrogen content were determined in all test materials.

Two grams samples of treated materials were inserted in dacron bag, and were suspended for 24 hours in the rumen of three rumen fistulated, italyan ryegrass hay fed sheeps.

3.3.2.2. Experiment II-2

This experiment was conducted to evaluate the effect of mixed treatment by grinding, chemical treatment with gamma irradiation on improving the nutritive value of barley and rape byproducts, and to determination of optimal combination level of gamma irradiation and chemical treatment.

Barley straw, barley hull, rape stem and rape husk were exposed to gamma ray at a level of 2.5 Mrad using the Korea Atomic Energy Research Institute's ^{60}Co source at Seoul, Korea.

Following gamma irradiation, the samples were ground through 100 mesh or 2 mm screen using the laboratory mill. After grinding, samples were treated with 1.5, 3, 4.5 and 6% NaOH or NH_4OH as the same manner in Experiment II-1.

NDF, ADF, hemicellulose contents and dacron bag dry matter digestibility were measured as the same manner in Experiment II-1.

3.3.2.3. Experiment II-3

Effect of enzyme addition on the digestibility of gamma irradiated barley straw, barley hull and rape husk was measured.

The enzyme used in this experiment was harvested from *Trichoderma viride* QM 9414 using dialysis with ammonium sulfate. The harvested enzyme solution was freeze dried and preserved. This enzyme has a relatively lower enzyme activity than a commercial enzyme (C.M.C. activity : 0.3695, Filter paper activity : 0.2692 and Cotton fiber activity : 2.6225 IU per ml enzyme solution).

Four levels of gamma irradiation were applied : 2.5, 5, 10 and 25 Mrad. About 100 g irradiated and ground (2 mm screen) samples of each material were treated with 25 mg enzyme/100 g dry matter, using 80 ml of 0.05 M citrate buffer (pH 4.8) to dissolve the enzyme. The enzyme solution was sprayed on the samples while contained in a plastic bag and allowed to react for 24 hours.

Two gram of enzyme treated samples were suspended in a dacron bag for 48 hours in the rumen of three rumen fistulated italyan ryegrass hay fed sheep.

3.4. Statistical Analysis

Differences between means of treatment were tested using Duncan's New Multiple Range Test (Duncan, 1955).

IV. RESULTS AND DISCUSSION

RESULTS

4.1 Experiment I. Effect of gamma irradiation

4.1.1 Experiment I-1. Effect of gamma irradiation on fibrous material degradation, ADF-N content, reducing sugar formation, cellulose crystallinity and susceptibility to microbial cellulase

Table 4.1.1. 1 shows the effect of gamma irradiation on the degradation of fibrous material. The NDF and hemicellulose contents of barley straw (BS), barley hull (BH) and rape husk (RH) markedly decreased by irradiation. A significant decrease was found at the 25 Mrad level in BS, and at 10 and 25 Mrad irradiation levels in BH and RH. Nearly 15% of NDF in RH and nearly 20% of hemicellulose in BH were solubilized at the highest irradiation level (25 Mrad).

Except for the ADF content of BH, gamma irradiation does not change the ADF, cellulose and lignin content of BS, BH and RH. The ADF content of BH was increased by gamma irradiation. The highest value of ADF was obtained at the 25 Mrad.

Table 4.1.1.1 Effect of gamma irradiation on the fibrous material degradation of low quality roughages

(Unit : DM basis %)

Sample	Item	Irradiation dose (Mrad)					
		0	2.5	5	10	25	
Barley straw	NDF	80.35 ^c	74.80 ^b	74.40 ^b	76.27 ^b	70.67 ^a	
	ADF	59.31	57.17	56.30	59.22	58.27	
	Cellulose	50.40	49.36	48.15	50.25	48.96	
	Lignin	7.34 ^b	7.56 ^b	7.67 ^b	8.51 ^b	7.92	
	Hemicellulose	21.04 ^b	17.62 ^b	18.11 ^b	17.05 ^b	12.40 ^a	
Barley hull	NDF	75.23 ^d	71.77 ^c	70.89 ^c	66.48 ^b	61.76 ^a	
	ADF	40.45 ^a	42.28 ^a	41.71 ^a	43.44 ^a	47.21 ^b	
	Cellulose	33.11	32.18	34.70	35.97	34.77	
	Lignin	4.70	4.27	4.58	4.27 ^b	5.56 ^a	
	Hemicellulose	34.77 ^d	29.48 ^c	29.18 ^c	23.04 ^b	14.55 ^a	
Rape husk	NDF	64.80 ^c	67.78 ^c	69.42 ^c	54.50 ^b	49.11 ^a	
	ADF	59.90	62.30	63.44	64.17	62.95	
	Cellulose	53.55	54.53	56.97	54.84	53.28	
	Lignin	6.76 ^{bc}	7.55 ^b	5.99	8.97 ^a	9.17	
	Hemicellulose	4.90 ^{bc}	6.23 ^b	5.77 ^c	0	0	

a-d Means with different superscripts within the same line are significantly different (P < 0.01).

It may be that gamma irradiation of the test materials has resulted in the production of sufficient cellulose-derived reducing sugar to after the nitrogen : substrate ratio available to micro-organisms, at the degradative sites. The content of ADF-N and reducing sugar were then determined. ADF-N and reducing sugar content of irradiated BS, BH and RH varied with test materials (Table 4.1.1.2 and Table 4.1.1.3).

The ADF-N content of BS was decreased by gamma irradiation. The lowest ADF-N content was obtained at the 25 Mrad level. The BH, ADF-N content was markedly increased at the irradiation level of 25 Mrad. However, the ADF-N content of RH was not affected by gamma irradiation, and the lowest ADF-N content was found at the 25 Mrad irradiation level.

The reducing sugar content of BS was increased at the 2.5 and 10 Mrad levels, but decreased at an irradiation level of 25 Mrad. All levels of gamma irradiation increased the reducing sugar content of BH. The highest value was obtained at 25 Mrad. In the case of BH, the reducing sugar content was affected by gamma irradiation. A 2.5 Mrad irradiation level decreased the reducing sugar content, and other levels increased the reducing sugar content of RH. The highest value of reducing sugar content was obtained at an irradiation level of 25 Mrad.

The x-ray diffractograms of the low quality roughages irradiated with gamma ray, were measured for estimation of cellulose crystallinity.

Table 4.1.1.1.2 Effect of gamma irradiation on the ADF-N content of low quality roughages

Sample	Irradiation dose (Mrad)				
	0	2.5	5	10	25
Barley straw	2.35	1.91	1.74	1.66	1.57
Barley hull	2.12 ^a	2.24 ^a	2.01 ^a	1.96 ^a	2.55 ^b
Rape husk	2.20	2.22	1.94	2.09	1.76

a-b Means with different superscripts within the same line are significantly different ($P < 0.01$).

Table 4.1.1.3 Effect of gamma irradiation on the reducing sugar content of low quality roughages

Sample	(Unit : mg glucose/g DM)				
	Irradiation dose (Mrad)				
	0	2.5	5	10	25
Barley straw	5.05	3.37	5.05	5.14	4.91
Barley hull	5.09 ^f	5.18 ^f	5.50 ^f	5.52 ^g	5.50 ^f
Rape husk	5.28 ^b	5.05 ^a	5.46 ^c	5.50 ^c	6.06 ^d

a-d Means with different superscripts within the same line are significantly different ($P < 0.01$)

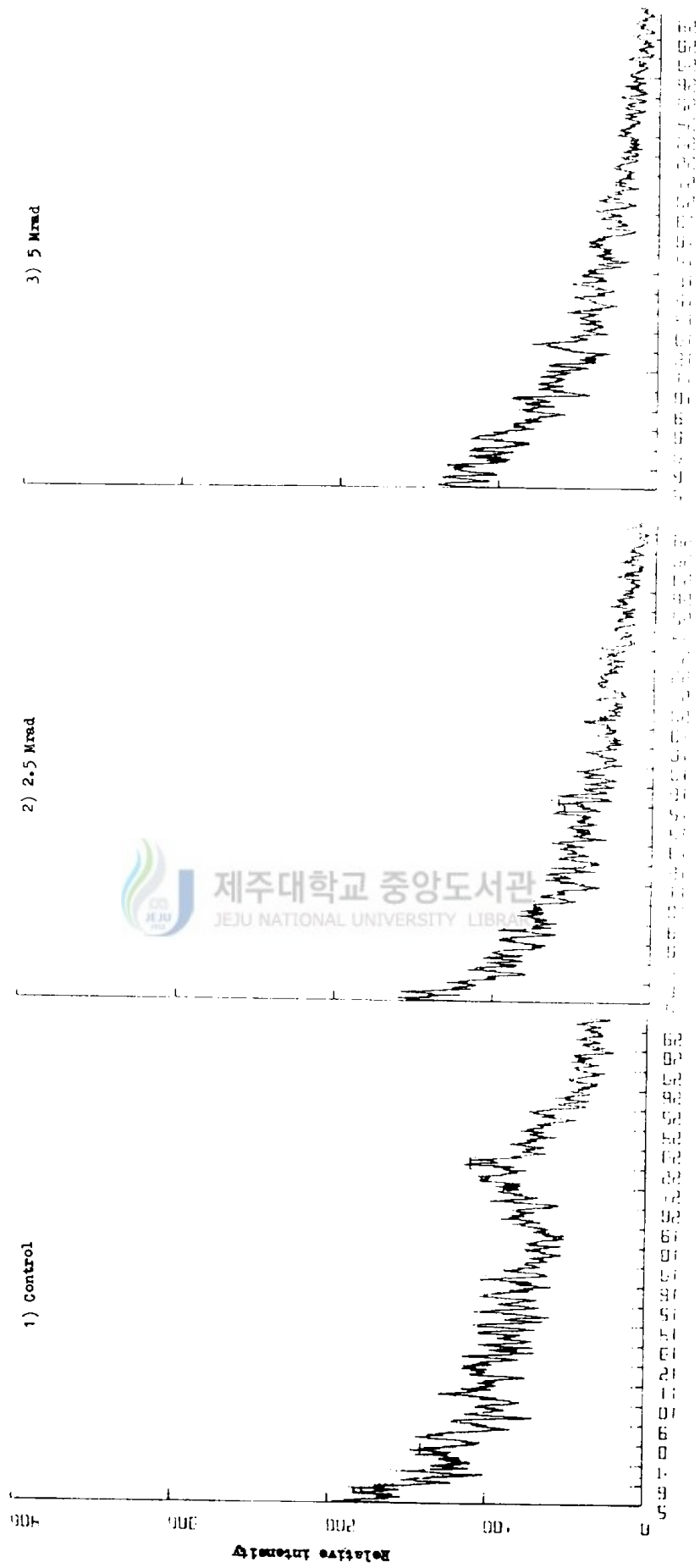
f-g Means with different superscripts within the same line are significantly different ($P < 0.05$).

Figure 4.1.1.1 shows the x-ray diffractogram of gamma irradiated barley straw. This diffractogram showed that unirradiated BS peaked between $2\theta=18^\circ$ and $2\theta=25^\circ$. The x-ray diffraction pattern of 2.5 and 5 Mrad irradiated BS showed no peaks. This result suggested that the crystalline structure of BS was disrupted at the 2.5 and 5 Mrad irradiation levels. 10 Mrad irradiated BS had a peak but, it was weaker than the control. However, 25 Mrad irradiation gave rise to a very strong peak higher than the control and other irradiation levels.

Gamma irradiation affected the crystalline structure of barley hull (figure 4.1.1.2). The x-ray diffraction pattern of unirradiated BH showed no peak. All levels of irradiation raised the peak, particularly the 5 Mrad level which had high peak.

The x-ray diffractogram of irradiated rape husk is shown in figure 4.1.1.3. Gamma irradiation disrupted the crystalline structure of RH. There was no peak with any levels of gamma irradiation, however, unirradiated RH gave rise to a peak. There were no differences among the irradiation levels.

The crystallinity index of cellulose, from x-ray diffractogram, is calculated in table 4.1.1.4. The cellulose crystallinity of BS and RH was decreased by gamma irradiation, but gamma irradiation increased the cellulose crystallinity of BH. The lowest value of cellulose crystallinity was obtained at 2.5 Mrad in BS. A 25 Mrad irradiated BS gave a higher crystallinity than other irradiation



2 (Degree)

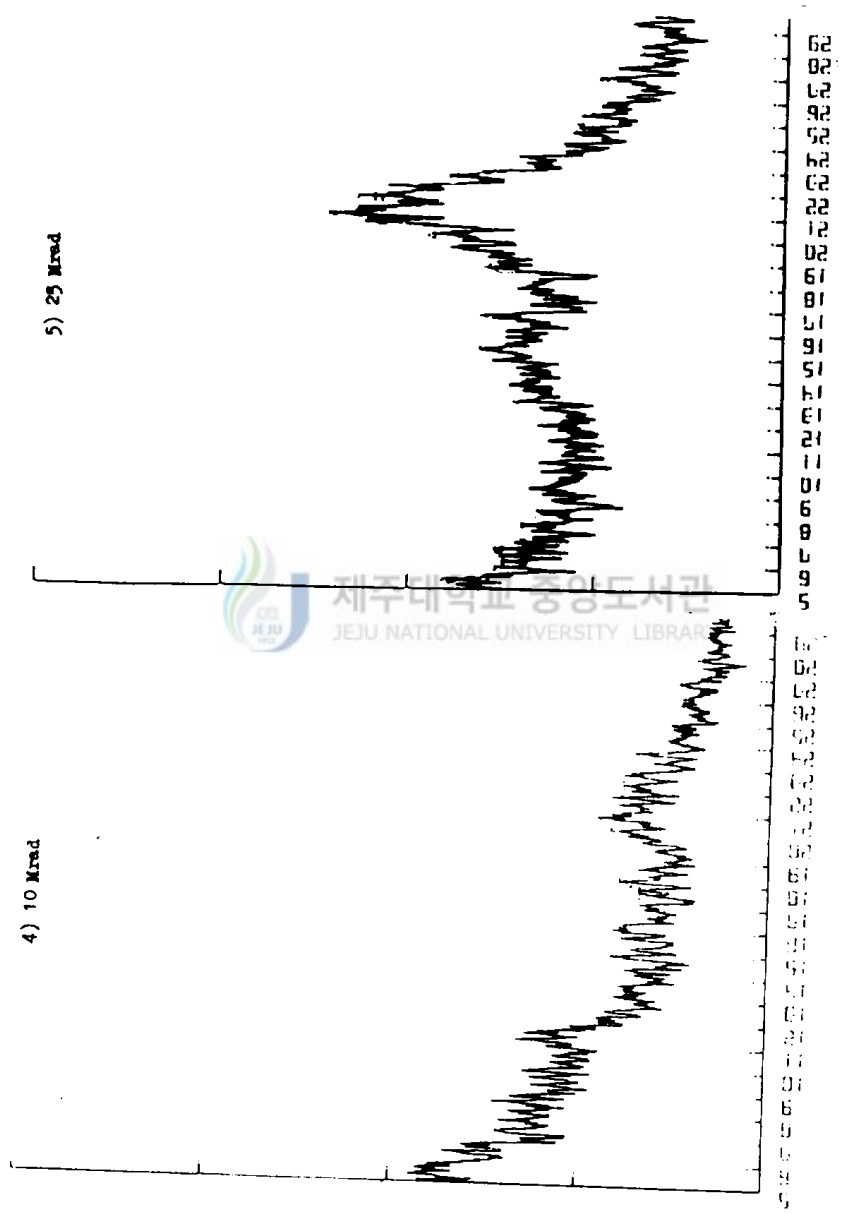
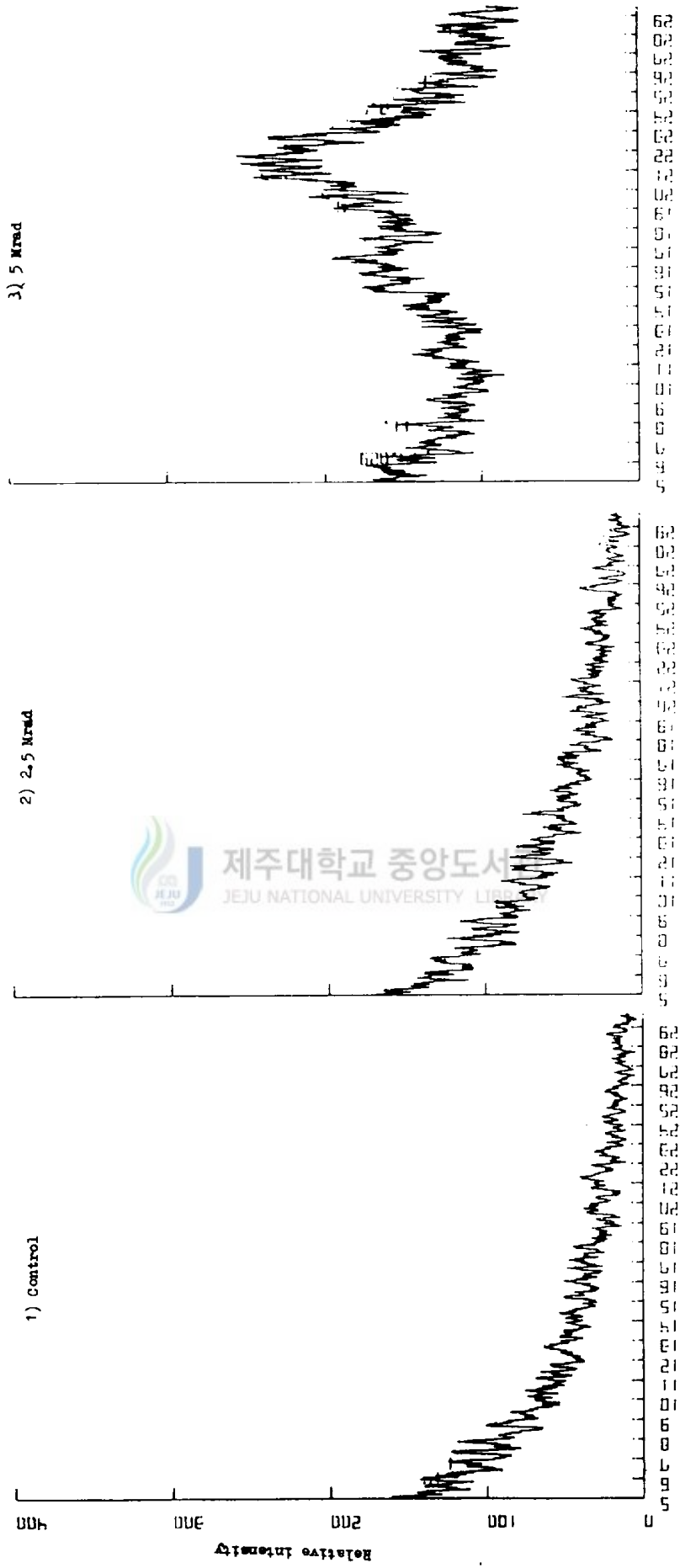


Figure 4.1.1.1 X-ray diffractogram of gamma irradiated barley straw
2 (Degree)




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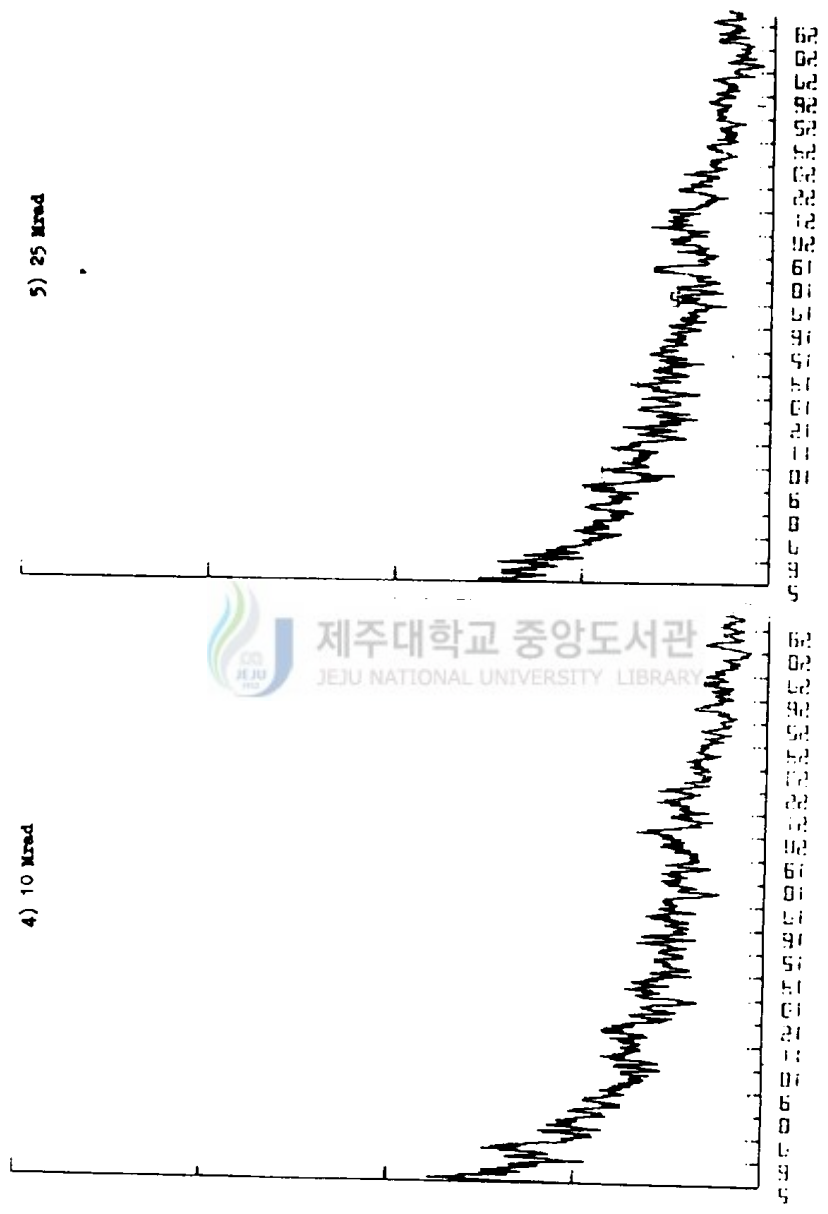
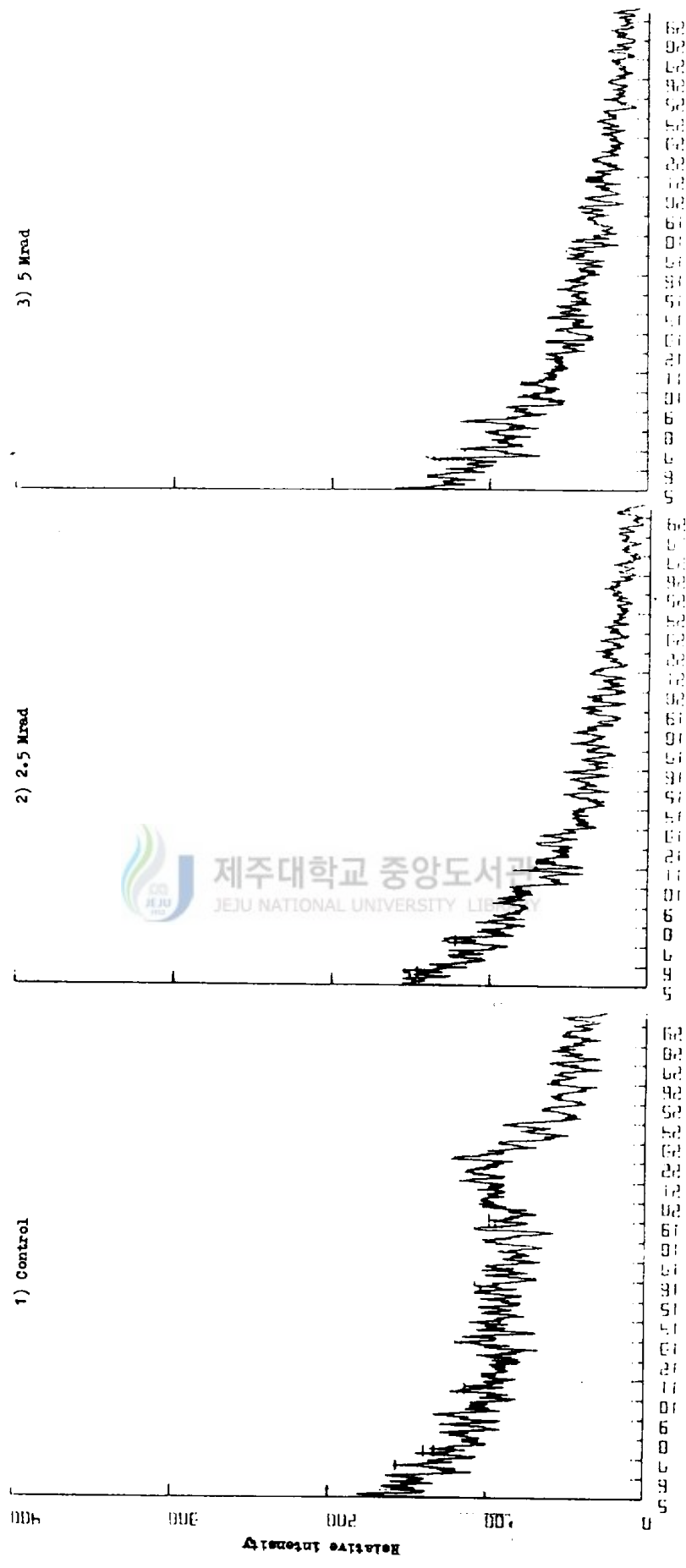


Figure 4.1.1.2 X-ray diffractogram of gamma irradiated barley hull




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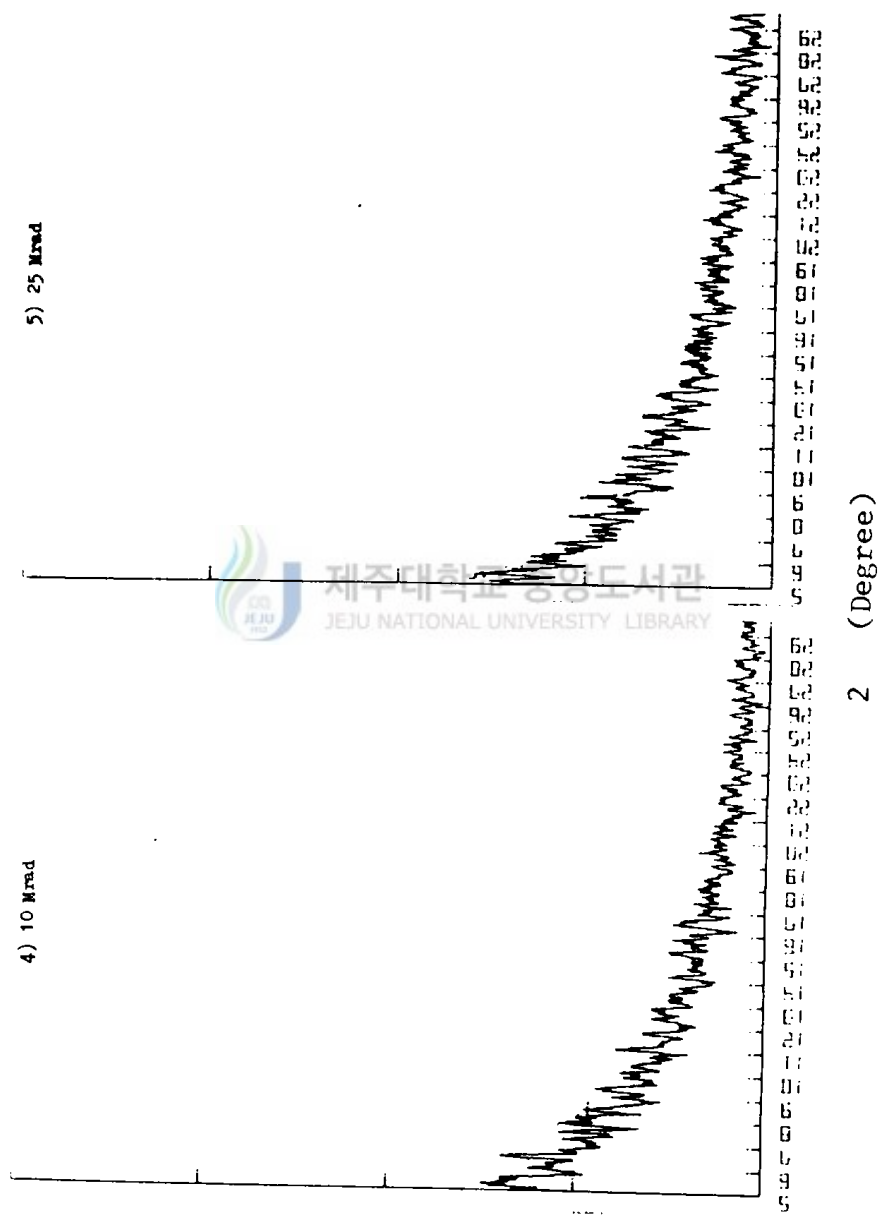


Figure 4.1.1.3 X-ray diffractogram of gamma irradiated rape husk

Table 4.1.1.4. Cellulose crystallinity of gamma irradiated low quality roughages

Sample	Irradiation dose (Mrad)				
	0	2.5	5	10	25
Barley straw	1.65	1.05	1.14	1.12	1.46
Barley hull	0.78	1.14	1.42	1.11	1.33
Rape husk	1.19	0.73	1.08	0.74	1.07

levels. With barley hull, the highest value of crystallinity was obtained at 5 Mrad level. The 2.5 and 10 Mrad levels gave similar crystallinity and there were no differences in crystallinity of RH between 5 and 25 Mrad.

The biological susceptibility of gamma irradiated low quality roughages was checked with cellulases, obtained from *Trichoderma viride*. Figure 4.1.1.4 shows the hydrolysis of gamma irradiated low quality roughages. BS, BH and RH irradiated with 10 and 25 Mrad were extensively hydrolyzed in a short time (30 mins), however, these irradiation levels decreased or did not increase the rate of hydrolysis at 1 hour incubation. There were no significant differences in hydrolysis rates between the control and 5 Mrad level in BS. The hydrolysis rate of 2.5 Mrad irradiated BS, BH and RH was linearly increased by prolonging the incubation time. At 12 hours incubation, 2.5 Mrad irradiation increased hydrolysis more than the 5 and 10 Mrad levels in BS. However, the hydrolysis rate was higher than that of 5 Mrad in BH. Irradiation of 2.5 Mrad increased hydrolysis more than any other irradiation levels, at 12 hours incubation.

4.1.2 Experiment I-2. Effect of gamma irradiation on dry matter disappearance and cell wall digestion

The dacron bag dry matter disappearance of gamma irradiated

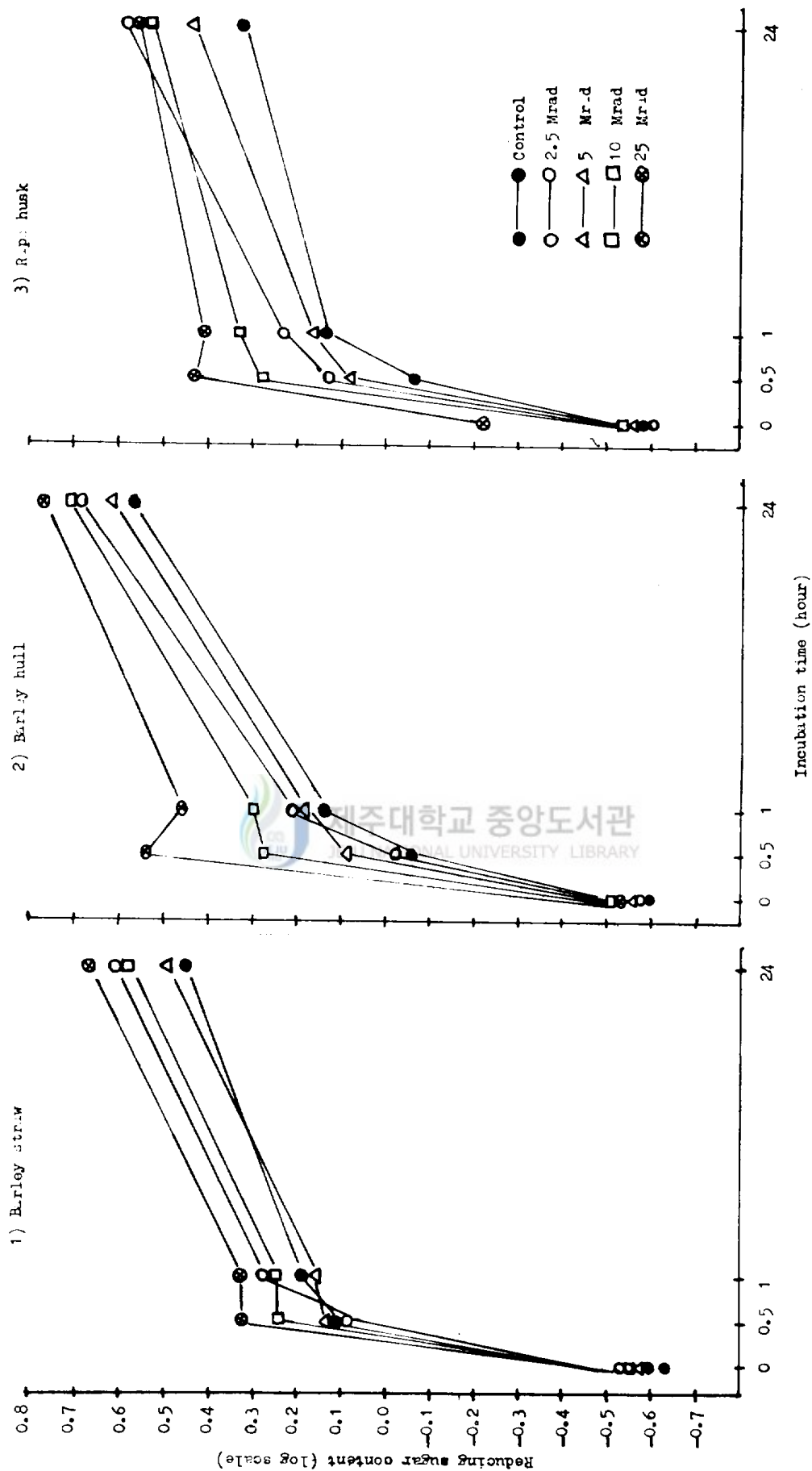


Figure 4.1.1.4 Hydrolysis of gamma irradiated low quality roughages by cellulase

low quality roughages is shown in figure 4.1.2.1. The dry matter disappearance (DMD) of unirradiated BS, BH and RH was improved by prolonging the suspension time up to 48 hours, after which DMD was not improved, although the suspension time was increased to 72 hours. The DMD of gamma irradiated BS was markedly increased up to 36 hours, after which time the DMD differed according to level of irradiation. The DMD of gamma irradiated BS was lower than the control at a suspension period of 48 hours. After 72 hours suspension, the DMD at the 2.5 and 10 Mrad irradiation levels were lower than the control but, at the 5 and 25 Mrad was higher than the control.

With barley hull, the disappearance pattern, when irradiated with levels of 2.5 and 25 Mrad, was similar to the control. Irradiation levels of 5 and 10 Mrad markedly increased the DMD by prolonging the suspension time up to 36 hours. There was, however, no increase at 48 hours suspension, but an increase was obtained at 72 hours. The DMD at the treatment level of 5 Mrad was lower than that of the 2.5 Mrad. The highest DMD was obtained at an irradiation level of 25 Mrad.

In rape husk, higher irradiation levels (10 and 25 Mrad) increased the DMD more than the control, but lower irradiation levels (2.5 and 5 Mrad) had the opposite effect. The greatest DMD was found at 25 Mrad.

Figure 4.1.2.2 shows the changes of residual cell wall

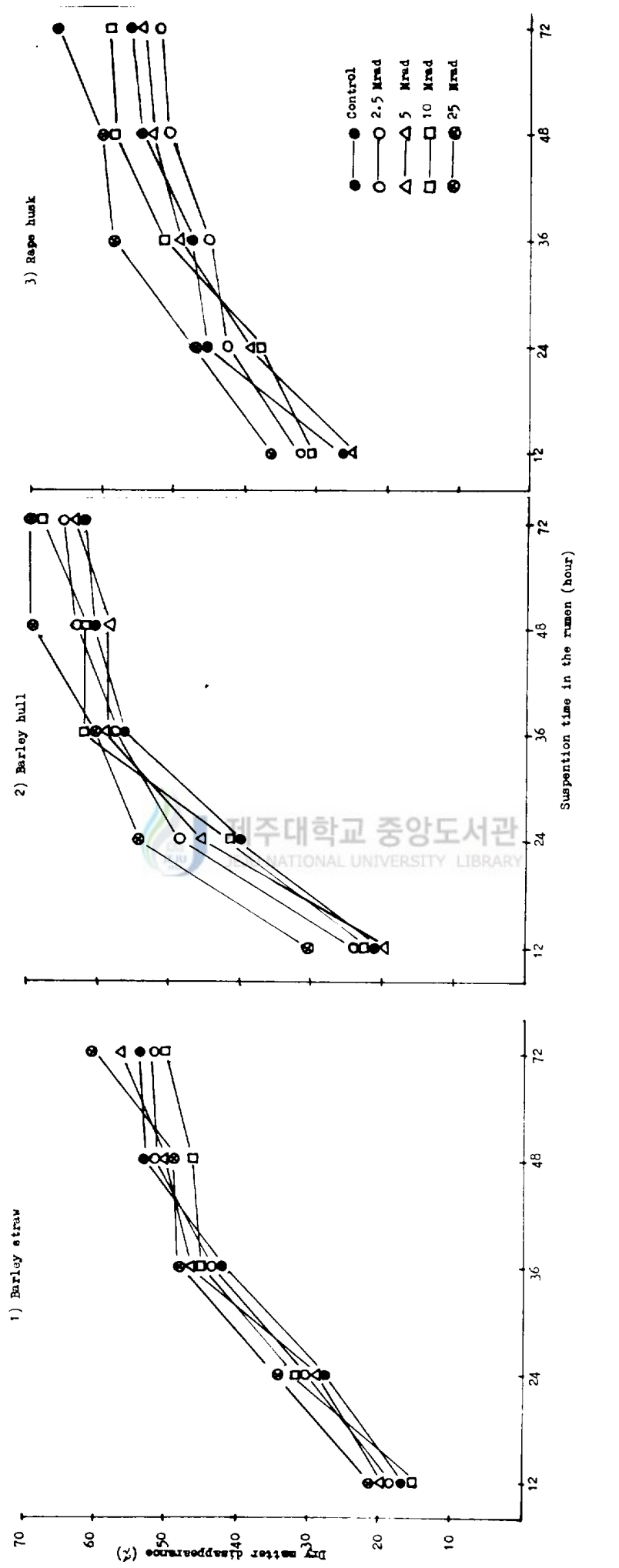


Figure 4.1.2.1 Dry matter disappearance from dacron bag of gamma irradiated low quality roughages

content after suspension in the rumen. Gamma irradiation improves cell wall digestion and lengthens cell wall digestion time, except 2.5 Mrad and 5 Mrad irradiation in RH. Most cell wall digestion of unirradiated BS and BH occurred within 12 hours but, cell wall digestion continued up to 24 hours in RH. Gamma irradiation increased cell wall digestion of BS and BH up to 24 or 48 hours suspension. The cell wall digestion of BS was reduced when irradiation level was increased to 12 hours suspension. The lowest cell wall digestion was found at 12 hours suspension when irradiated with 25 Mrad. However, 25 Mrad irradiation prolonged the cell wall digestion of BS up to 48 hours.

However, opposite effect was found in BH, as cell wall digestion increased by raising the irradiation level. During the 12 hours suspension period, the lowest cell wall digestion value was obtained at the 5 Mrad level.

These results suggested that lower cell wall digestion of 25 Mrad irradiated BS and 5 Mrad irradiated BH were due to an increase of cellulose crystallinity of cell wall (see figures 4.1.1.1 and 4.1.1.2).

Low irradiation levels (2.5 and 5 Mrad) decreased the cell wall digestion of RH, but high levels (10 and 25 Mrad) increased it more than the control. All irradiation levels decreased cell wall digestion more than unirradiated RH at 24 hours suspension.

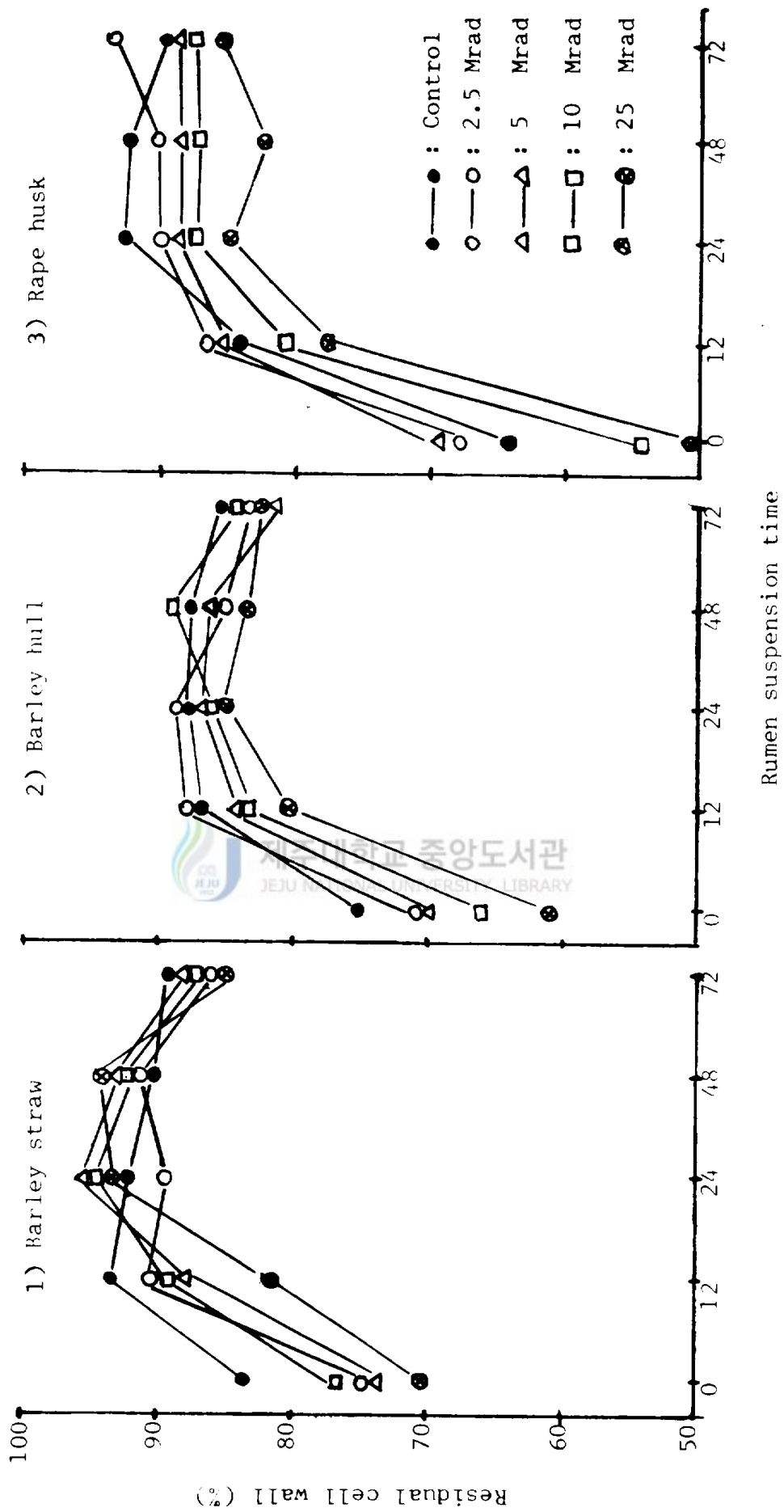


Figure 4.1.2.2 Cell wall digestion of gamma irradiated low quality roughages.

4.1.3. Experiment I-3. Effect of gamma irradiation on in vitro fermentation

In vitro dry matter disappearance of gamma irradiated low quality roughages is shown in figure 4.1.3.1. In vitro dry matter disappearance (DMD) of all test materials increased by prolonging the incubation time. Irradiation at 5 and 25 Mrad decreased the DMD of BS, whereas irradiate at 2.5 and 10 Mrad increased the DMD at 48 hours incubation. The lowest value of DMD was obtained at 25 Mrad. At 36 hours incubation, all levels of irradiation decreased the DMD of BS than the control.

Gamma irradiation greatly affected the DMD of BH. The DMD of low levels (2.5 and 5 Mrad) irradiated BH was decreased at 12 hours incubation. Higher irradiation levels (10 and 25 Mrad) significantly increased the DMD and the highest DMD was obtained at the 25 Mrad level.

In rape husk, the DMD was improved by raising the gamma irradiation level, but a 5 Mrad level decreased the DMD of RH more than the 2.5 Mrad level.

In vitro total VFA production of gamma irradiated low quality roughages is shown in table 4.1.3.1. Total VFA production of all test materials was increased by lengthening the incubation period. Total VFA production of BH was higher than BS or RH.

The 5 and 10 Mrad irradiation levels decreased the total VAF

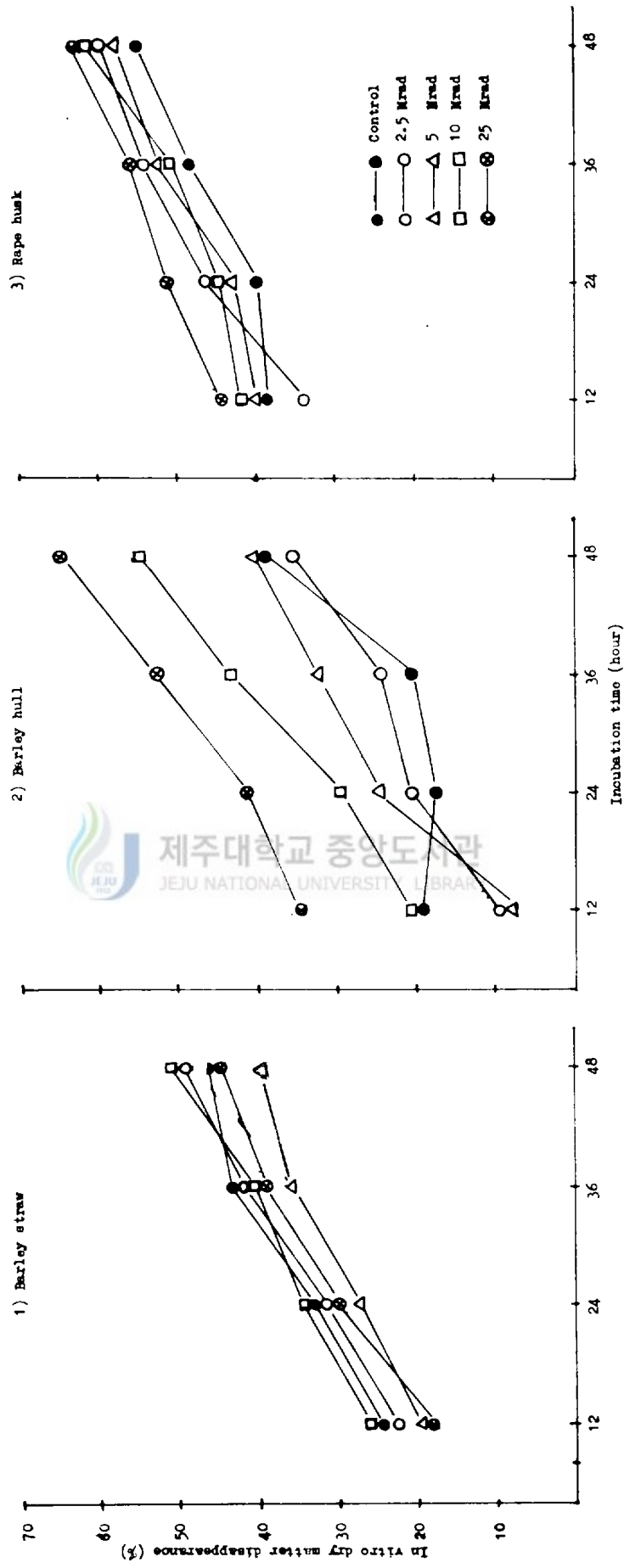


Figure 4.1.3.1 In vitro dry matter disappearance of gamma irradiated low quality roughages

Table 4.1.3.1. Total in vitro VFA production of gamma irradiated low quality roughages
(Unit ; m M/l)

Sample	Irradiation dose(Mrad)	Incubation time (hour)		
		12	24	36
Barley straw	0	16.86 ^a	22.13 ^b	27.7 ^c
	2.5	6.56 ^a	22.92 ^{ab}	29.61 ^b
	5	16.55 ^f	21.01 ^{fh}	26.43 ^{gh}
	10	15.28 ^a	19.74 ^a	26.01 ^b
	25	18.46 ^a	21.65 ^b	28.33 ^c
Barley hull	0	19.1 ^{a.l}	23.56 ^{b.l}	29.29 ^c
	2.5	17.19 ^{a.l}	23.56 ^{b.l}	27.38 ^c
	5	17.83 ^{a.l}	24.83 ^{b.l}	29.29 ^c
	10	19.1 ^{a.l}	25.47 ^{b.l}	29.61 ^c
	25	22.28 ^{f.m}	28.34 ^{f.m}	31.86 ^{fg}
Rape husk	0	20.01 ^a	26.43 ^{b.j}	29.93 ^c
	2.5	18.47 ^a	26.74 ^{b.j}	32.79 ^c
	5	19.74 ^a	23.14 ^{a.j}	28.33 ^b
	10	21.97 ^a	24.51 ^{b.j}	31.23 ^c
	25	23.88 ^a	29.96 ^{b.k}	34.52 ^c

a-d : Means in the same line with different superscripts differ (P < 0.01)

f-h : Means in the same line with different superscripts differ (P < 0.05)

i-k : Means in the same column with different superscripts differ (P < 0.01)

l-m : Means in the same column with different superscripts differ (P < 0.05).

production of BS at all incubation periods. At 36 hours incubation, the 5 and 10 Mrad irradiation lowered the total VFA production, while only the 25 Mrad level increased the total VFA production.

Similar results were obtained for barley hull. At 48 hours incubation, total VFA production of BH, irradiated at 2.5, 5 and 10 Mrad levels, was decreased. Only exposure to 25 Mrad increased the total VFA production.

Raising the gamma irradiation level increased the total VFA production of rape husk irradiation at 5 Mrad decreased total VFA production in all incubation periods. The highest total VFA production was obtained at the 25 Mrad.

Tables 4.1.3.2 ; 4.1.3.4 and 4.1.3.5 shows in vitro individual VFA production of gamma irradiated low quality roughages according to fermentation intervals.

The total VFA and acetic acid (C₂) production of barley straw was decreased at 5 and 10 Mrad irradiation levels, however, irradiation levels of 2.5 and 25 Mrad increased the total VFA and C₂ production of BS. The lowest total VFA and C₂ production were obtained at 10 Mrad level and treatment level of 25 Mrad lowered than the 2.5 Mrad in total VFA and C₂ production of BS.

All levels of irradiation increased the propionic acid (C₃) and butyric acid (C₄) production of BS. The highest production of C₃ and C₄ were obtained at 2.5 Mrad level. However, gamma irradiation reduced the acetic acid/propionic acid (C₂/C₃) ratio of BS.

Table 4.1.3.2 Effect of gamma irradiation on in vitro individual VFA of barley straw

(Unit : m M/l)

Incubation time (hr)	Irradiation dose (Mrad)														
	0			2.5			5			10			25		
	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4
1	8.47	6.59	1.00	15.25	8.85	1.78	10.12	5.70	1.16	10.41	8.98	1.31	7.23	5.40	0.84
2	12.13	4.68	1.17	10.73	7.19	1.34	11.22	5.52	1.31	5.67	5.92	0.81	12.02	5.92	1.38
4	10.91	4.11	0.83	11.15	6.91	1.32	8.91	6.61	1.01	5.15	3.61	0.74	12.65	6.06	1.50
6	10.26	7.84	1.32	11.07	5.04	1.32	8.50	5.83	1.05	12.53	6.29	1.48	13.95	8.07	1.78
Mean	10.45	5.81	1.08	12.05	7.00	1.44	9.69	5.92	1.13	8.44	6.20	1.09	11.46	6.32	1.38
Total	70.28			82.95			67.67			63.45			77.55		
Mean C2/C3 ratio	1.96			1.76			1.65			1.38			1.82		

C2 : Acetic acid C3 : Propionic acid C4 : Butyric acid.

Except 10 Mrad irradiation, other irradiation levels increased the total VFA production of barley hull (figure 4.1.3.3). But, irradiation level of 25 Mrad lowered the total VFA production of BH than the 2.5 and 5 Mrad. All levels of gamma irradiation increased the C₂ production of BH. The C₂ production of BH irradiated at 5 and 10 Mrad lowered than the 2.5 Mrad level and the highest C₂ production was obtained at 25 Mrad. High irradiation levels (10 and 25 Mrad) decreased the C₃ production, whereas low irradiation levels (2.5 and 5 Mrad) increased the C₃ production of BH. Gamma irradiation decreased the C₂/C₃ ratio of BH than the control, but 5 Mrad level increased the C₂/C₃ ratio.

The total VFA production of rape husk was increased by raising irradiation level. Gamma irradiation increased the C₂ production of RH. Irradiation of 5 Mrad lower C₂ production of RH than other treatment level, and there were no differences among the 2.5, 10 and 25 Mrad irradiation. The C₃ production of RH was increased by gamma irradiation, however, there were small differences among treatment levels. The C₄ production was increased by raising the irradiation level. Except for the 5 Mrad irradiation, other treatment level increased the C₂/C₃ ratio of RH and the highest C₂/C₃ ratio was obtained at 10 Mrad.

Table 4.1.3.3 Effect of gamma irradiation on in vitro individual VFA of barley hull

(Unit : m M/l)

Incubation time (hr)	Irradiation dose (Mrad)														
	0			2.5			5			10			25		
	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4			
1	7.38	2.95	0.77	11.23	6.99	1.19	12.39	4.29	1.28	6.55	8.10	0.02	8.01	5.59	T
2	5.60	3.61	0.61	9.24	7.59	1.04	12.88	5.81	1.29	7.91	8.25	T	8.55	8.07	T
4	13.73	5.37	1.41	11.39	8.26	1.31	11.75	6.23	1.21	11.77	5.06	0.43	10.31	8.96	0.26
6	8.93	4.69	0.92	10.50	6.63	1.14	13.86	8.17	1.60	5.89	3.21	0.5	11.44	6.44	0.69
Mean	8.91	4.16	0.93	10.59	7.37	1.17	12.72	6.13	1.34	8.03	6.16	0.24	9.58	9.69	0.24
Total	57.58			77.24			81.56			58.16			68.81		
Mean C2/C3 ratio	2.08			1.45			2.18			1.48			1.36		

T : Trace

C2 : Acetic acid C3 : Propionic acid C4 : Butyric acid.

Table 4.1.3.4 Effect of gamma irradiation on in vitro individual VFA of rape husk

Incubation time (hr)	Irradiation dose (Mrad)														
	0			2.5			5			10			25		
	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4
1	3.97	2.12	T	-	-	-	7.97	7.86	0.47	15.31	5.56	0.64	10.75	8.30	0.88
2	7.54	5.20	0.06	18.43	10.83	T	11.82	8.71	0.64	14.99	9.94	1.17	14.70	8.04	1.13
4	6.73	4.02	0.05	9.91	5.74	0.1	6.61	6.86	0.47	13.76	7.64	1.00	17.40	7.43	0.61
6	8.75	10.03	0.20	14.51	7.75	0.65	11.91	11.79	0.89	14.12	9.33	1.04	16.50	10.05	1.32
Mean	6.75	5.35	0.08	14.28	8.11	0.25	9.58	8.81	0.62	14.55	8.12	0.96	14.84	8.46	0.99
Total	49.0			68.15			77.04			96.38			98.0		
Mean C2/C3 ratio	1.47			1.76			1.08			1.89			1.78		

T : Trace - : Not measured.

C2 : Acetic acid C3 : Propionic acid C4 : Butyric acid.

4.2 Experiment II. Effect of chemical treatment and mixed treatment by grinding, chemical treatment, enzyme addition with gamma irradiation on low quality roughages

4.2.1 Experiment II-1. Effect of chemical treatment

Effect of NaOH treatment on the dry matter digestibility (DMD) of low quality roughages is shown in table 4.2.1.1. Dry matter digestibility of barley straw (BS), barley hull (BH), rape stem (RS) and rape husk (RH) was improved by increasing the NaOH treatment level. NaOH treatment was more effective to improve the DMD of BS than that of BH, RS and RH. Particle size affected the NaOH treatment. The 2 mm screen grinding was effective in improving the DMD of BS, RS and RH than fine grinding. In BH, fine grinding was more effective to improve the DMD than the 2 mm screen grinding.

Figure 4.2.1.1 shows the effect of NaOH treatment on the nutritive value of low quality roughages. The NDF and hemicellulose contents of BS, BH and RH were decreased by increasing NaOH treatment levels. But, NaOH treatment did not change the ADF content of BS and BH, however, the ADF content of RS and RH was decreased by NaOH treatment.

Table 4.2.1.2 shows the effect of NH_4OH treatment on the DMD of low quality roughages. The effect of NH_4OH treatment varied

Table 4.2.1.1 Effect of NaOH treatment on the DMD of low quality roughages

Sample Level of NaOH (%)	(Unit : DM basis %)									
	Barley straw		Barley hull		Rape stem		Rape husk			
	2G	FG	2G	FG	2G	FG	2G	FG	2G	FG
0	36.41 ^a	25.59 ^a	27.63 ^a	35.43 ^a	30.30 ^a	27.08 ^b	28.72 ^a	29.97 ^{ed}		
1.5	37.67 ^a	38.06 ^b	37.99 ^b	41.22 ^b	47.98 ^b	32.21 ^b	35.52 ^b	34.85 ^{bd}		
3	49.12 ^b	46.66 ^c	47.37 ^c	53.93 ^c	41.86 ^b	37.05 ^b	43.39 ^c	36.09 ^b		
4.5	53.13 ^b	56.69 ^d	51.02 ^c	54.55 ^c	52.35 ^b	39.15 ^{ab}	46.58 ^c	43.10 ^b		
6	64.62 ^c	61.43 ^e	56.82 ^c	62.22 ^d	53.08 ^b	39.31 ^a	56.01 ^d	46.94 ^a		

a-e Mean with different superscripts within the same column are significantly different (P < 0.01).

2G : 2mm screen grinding FG : Fine grinding.

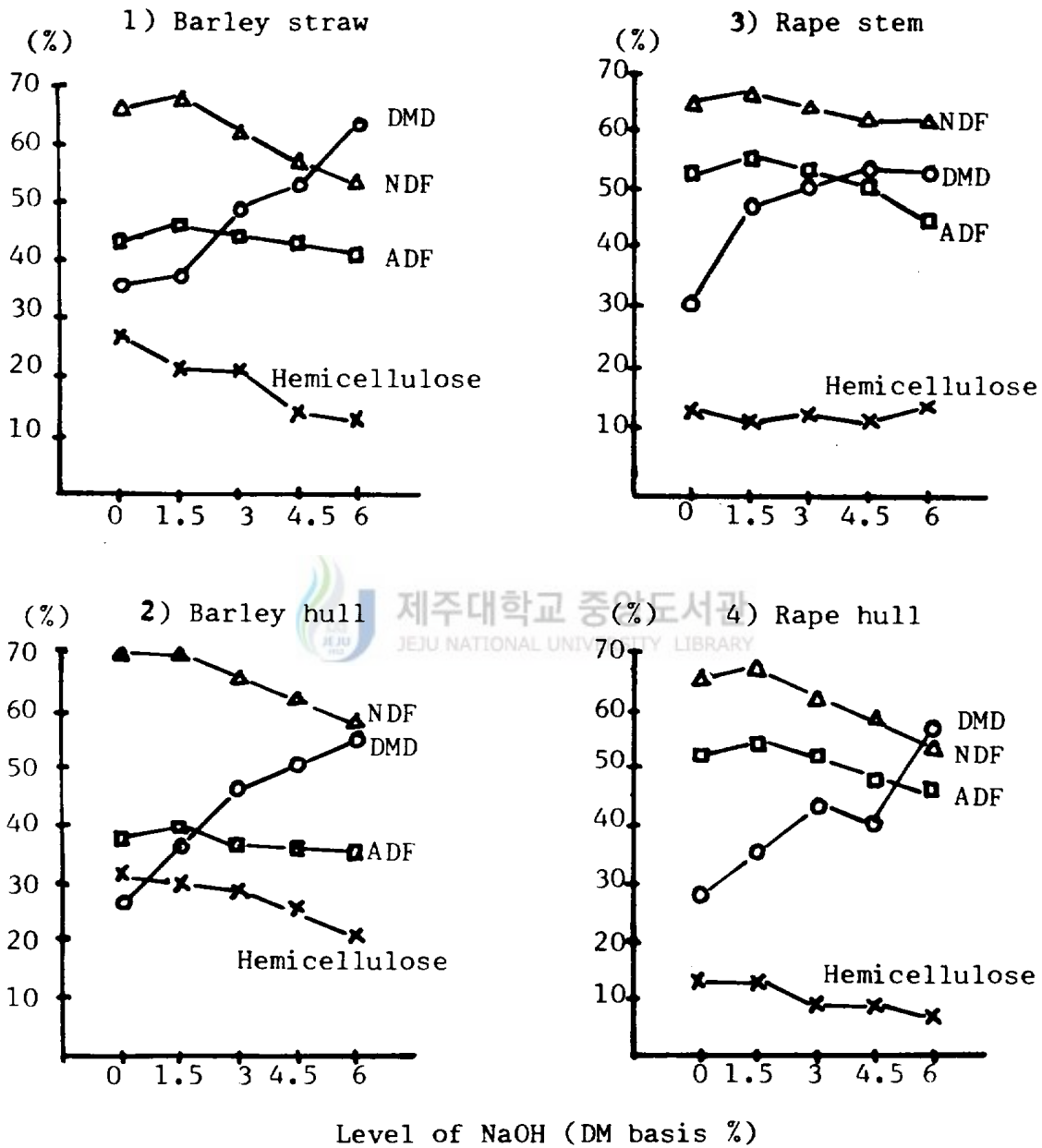


Figure 4.2.1.1 Effect of NaOH treatment on the nutritive value of low quality roughages.

with particle size. The DMD of NH_4OH treated barley straw was markedly improved by increasing the NH_4OH treatment level up to 3%. There was no further increase of DMD in the case of treated with 4.5 and 6% of NH_4OH . The fine grinding was effective on improving the DMD of BS than the 2 mm screen grinding. The DMD of BH was markedly increased by raising the NH_4OH treatment level up to 1.5% in 2 mm screen grinding. Above 3% of NH_4OH treatment, there was no economical increase of DMD. However, the DMD of fine ground BH was significantly improved by increasing the NH_4OH up to 3%. The DMD of NH_4OH treated rape stem and rape husk were increased up to 3% and 1.5% of NH_4OH level in 2 mm screen grinding. However, in the fine grinding, the DMD of RS and RH were increased up to 4.5% NH_4OH level. The fine grinding was effective to improve the DMD of BS and BH. The DMD of 2 mm screen grinding was higher than the fine grinding in RS and RH.

Effect of NH_4OH treatment on nutritive value of low quality roughages is shown in figure 4.2.1.2. NH_4OH treatment decreased the NDF content of BS and BH, while the NDF content of RS and RH were not changed by NH_4OH treatment. NH_4OH treatment did not decrease the ADF content of BS, BH, RS and RH. Total N content of all test materials increased up to 3 or 4% of NH_4OH level. The RH showed a lower total N content than that of BS, BH and RS.

Table 4.2.1.1.2 Effect of NH₄OH treatment on the DMD of low quality roughages

Sample Level of NH ₄ OH(%)	(Unit : DM basis %)							
	Barley straw		Barley hull		Rape stem		Rape husk	
	2G	FG	2G	FG	2G	FG	2G	FG
0	36.41 ^h	25.59 ^a	27.63 ^a	35.43 ^a	30.39 ^a	27.08 ^a	28.72 ^a	29.97
1.5	46.65 ^{h1}	54.60 ^b	45.33 ^{bc}	55.79 ^b	39.48 ^b	29.35 ^{bfg}	46.11	39.55
3	50.01	57.19	49.31	62.69	43.77	31.91 ^{cg}	45.87	42.29
4.5	40.76 ^{h1}	58.19 ^b	52.48 ^b	40.94 ^b	33.41 ^b	43.87 ^d	43.87 ^b	44.74
6	49.10 ^{ik1}	58.43 ^b	54.87 ^b	48.93 ^c	44.91 ^b	34.12 ^{ce}	47.54 ^b	40.56

a-g Means with different superscripts within the same column are significantly different ($P < 0.01$)

h-1 Means with different superscripts within the same column are significantly different ($P < 0.05$).

2G : 2mm screen grinding FG : Fine grinding.

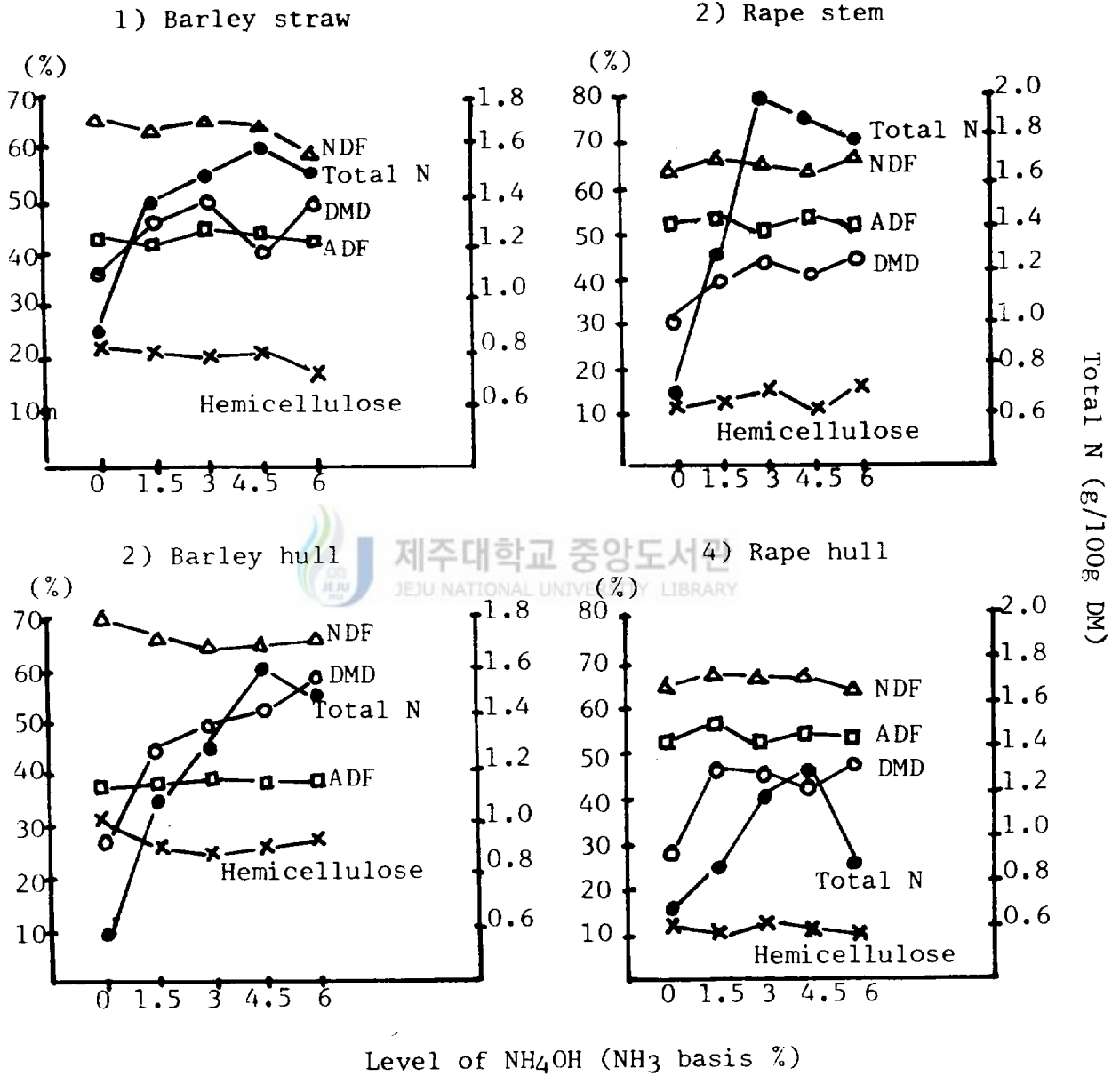


Figure 4.2.1.2 Effect of NH_4OH treatment on the nutritive value of low quality roughages.

4.2.2 Experiment II-2. Effect of mixed treatment by grinding,
chemical treatment with gamma irradiation

Table 4.2.2.1 shows the effect of grinding and gamma irradiation on the DMD of low quality roughages. The DMD of barley straw and rape husk was increased by 2 mm screen grinding. This grinding was the most effective in improving the DMD of BS. The DMD of BH and RH was increased by fine grinding. The fine grinding was the most effective in improving the DMD of BH. Mixed treatment with grinding and gamma irradiation showed compensation effect. The DMD of 2 mm screen ground BS was higher than the fine grinding, however, mixed treatment with fine grinding and gamma irradiation gave a higher DMD of BS than the mixed treatment with 2 mm screen grinding and gamma irradiation. The opposite phenomena was found in BH. The DMD of RS and RH was increased by mixed treatment; grinding and gamma irradiation. The highest DMD of RS was obtained in mixed treatment with fine grinding and gamma irradiation. The highest DMD of RH was found by mixed treatment with 2 mm screen grinding and gamma irradiation.

Effect of mixed treatment with grinding, NaOH and gamma irradiation on the DMD of low quality roughages is shown in table 4.2.2.2. The DMD of all materials treated with grinding, NaOH and gamma irradiation was improved by increasing NaOH treatment level. Mixed treatment effect was varied with particle size. Mixed

Table 4.2.3.1.1 Effects of grinding and gamma irradiation on the dry matter digestibility of low quality roughages

(Unit : DM basis %)

Treatment Sample	Control	2G	FG	IR-2G	IR-FG
Barley straw	22.22 ^a	36.41 ^c	25.59 ^{abd}	29.78 ^{bc}	32.28 ^{cd}
Barley hull	24.43 ^{fg}	27.67 ^{fi}	35.43 ^{hi}	30.43 ^{fi}	29.44 ^{fh}
Rape stem	20.09 ^a	30.39 ^{be}	27.08 ^{bc}	31.27 ^{bc}	32.29 ^{de}
Rape husk	22.32 ^a	28.72 ^{bc}	29.97 ^{be}	33.97 ^{de}	30.86 ^{be}

a-d Means with different superscripts within the same line are significantly different ($P < 0.01$)

f-g Means with different superscripts within the same line are significantly different ($P < 0.05$).

2G : 2mm screen grinding FG : Fine grinding

IR-2G : 2mm screen grinding after gamma irradiation

IR-FG : Fine grinding after gamma irradiation.

Table 4.2.2.2 Effect of mixed with grinding, NaOH and gamma irradiation on the DMD of low quality roughages

Sample Level of NaOH(%)	Barley straw		Barley hull		Rape stem		Rape husk	
	2G	FG	2G	FG	2G	FG	2G	FG
0	29.78	32.28	27.63	35.43	30.30	27.08	33.97	30.86
1.5	40.47	41.86	37.99	41.22	47.98	32.21	40.77	46.12
3	40.69	50.12	47.37	53.93	41.86	37.05	44.02	50.13
4.5	45.11	54.62	51.02	54.55	52.35	39.15	49.09	45.90
6	45.44	59.46	56.82	62.22	53.08	39.31	52.43	48.58

2G : 2mm screen grinding FG : Fine grinding.

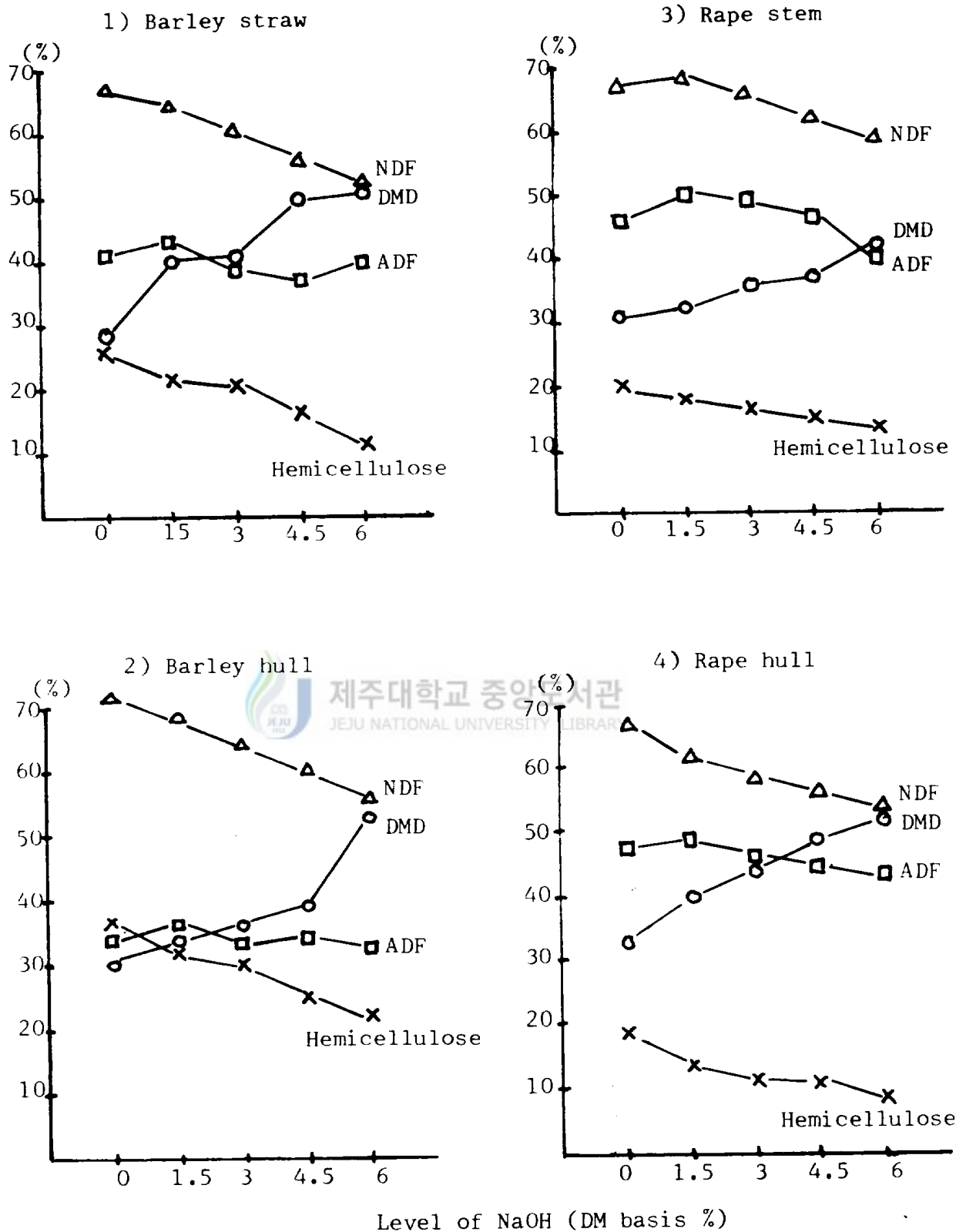


Figure 4.2.2.1 Changes in nutritive value when treated with NaOH and gamma irradiation.

treatment with fine grinding, NaOH and gamma irradiation was effective to improve the DMD of barley byproducts, whereas mixed treatment with 2 mm screen grinding, NaOH and gamma irradiation was effective in improving the DMD of rape byproducts.

Figure 4.2.2.1 shows the changes in nutritive value when treated with NaOH and gamma irradiation. Mixed treatment with NaOH and gamma irradiation markedly decreased the NDF and hemicellulose contents of BS, BH, RS and RH. The ADF content of BS was slightly decreased by mixed treatment with NaOH and gamma irradiation, but the ADF content of BH did not change. Mixed treatment with NaOH and gamma irradiation markedly decreased the ADF content of RS and RH.

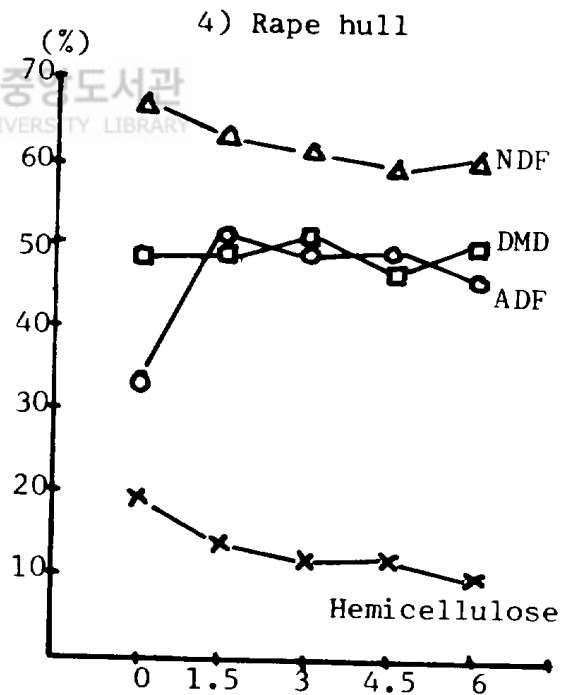
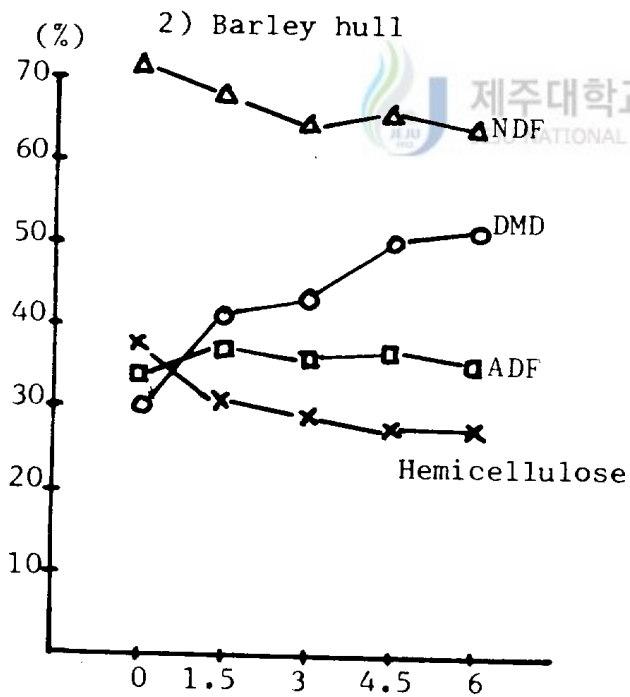
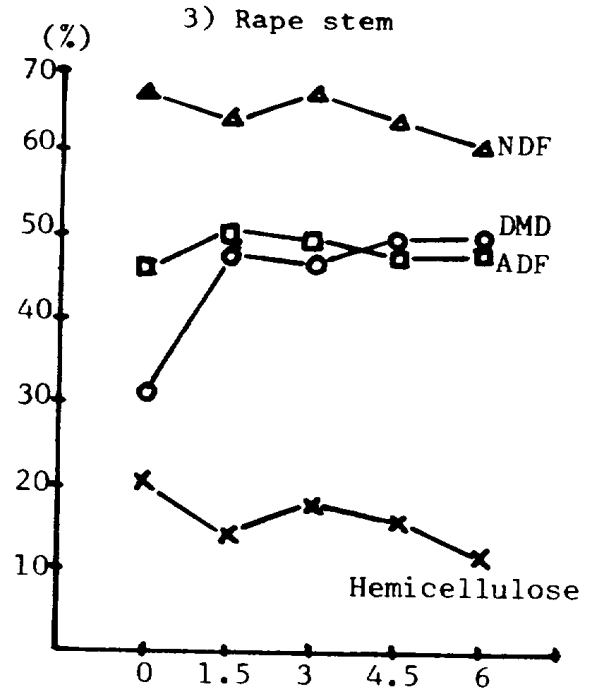
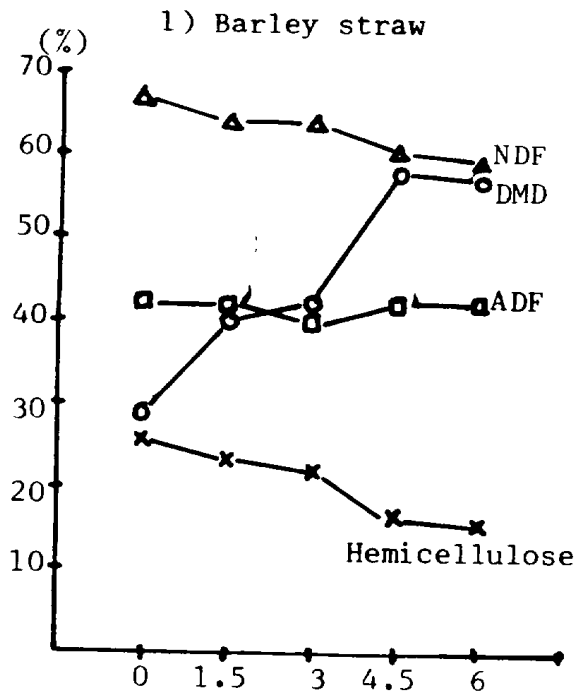
Effect of mixed treatment with grinding, NH_4OH and gamma irradiation on the DMD of low quality roughages is shown in table 4.2.2.3. Mixed treatment with grinding, NH_4OH and gamma irradiation improved the DMD of BS when treated with NH_4OH treatment level up to 3%, but further increase of DMD was obtained in 2 mm screen grinding when treated with 4.5% NH_4OH . In barley hull, mixed treatment with grinding, NH_4OH plus gamma irradiation was more effective in improving the DMD than only NH_4OH treatment. The DMD of RS and RH were markedly improved with increasing NH_4OH level up to 1.5%, and mixed treatment of grinding, NH_4OH and gamma irradiation more effective in improving the DMD of RS and RH than only grinding and NH_4OH treatment. The 2 mm screen grinding was effective in RS and the fine grinding was effective in RH.

Table 4.2.2.3 Effect of mixed treatment with grinding, NH₄OH and gamma irradiation on the DMD of low quality roughages

Sample Level of NH ₄ OH (%)	Barley straw		Barley hull		Rape stem		Rape husk	
	2G	FG	2G	FG	2G	FG	2G	FG
0	29.78 ^a	32.28 ^a	30.34 ^a	29.44 ^a	31.27 ^a	32.29 ^a	33.97 ^a	30.86 ^a
1.5	40.06 ^b	52.80 ^b	41.65 ^b	51.95 ^b	48.84 ^b	45.67 ^b	51.35 ^b	52.88 ^b
3	42.75 ^b	56.90 ^b	43.09 ^b	61.11 ^b	47.82 ^b	46.07 ^b	49.26 ^b	53.64 ^b
4.5	58.96 ^c	52.16 ^b	45.05 ^b	58.39 ^b	49.05 ^b	47.75 ^b	49.25 ^b	56.91 ^b
6	57.18 ^c	52.16 ^b	46.17 ^b	67.26 ^b	50.61 ^b	50.32 ^b	46.23 ^b	54.48 ^b

a-c Means with different superscripts within the same column are significantly different (P < 0.01).

2G : 2mm screen grinding FG : Fine grinding.



Level of NH₄OH (NH₃ basis %)

Figure 4.2.2.2 Changes in nutritive value when treated with NH₄OH and gamma irradiation.

Changes in nutritive value when treated with NH_4OH and gamma irradiation is shown in figure 4.2.2.2. Mixed treatment with NH_4OH and irradiation decreased NDF content of all test materials. The ADF content did not change by treatment with NH_4OH and irradiation.

4.2.3 Experiment II-3. Effect of enzyme addition on gamma irradiated low quality roughages

Table 4.2.3.1 shows the effect of enzyme addition on the dry matter digestibility of gamma irradiated low quality roughages. The dry matter digestibility of irradiated barley straw was increased by enzyme addition only at the 25-Mrad level. Enzyme addition increased the DMD of irradiated BH and RH. The highest DMD of BH was found at the 5 Mrad, and the 10 Mrad irradiation level reached the highest DMD in RH.

Discussion

4.3.1 Experiment I. Effect of gamma irradiation on low quality roughages

There is considerable evidence that the feeding value of many low quality roughages is limited not so much by the absence of

Table 4.2.2.4 Effect of enzyme addition on the digestibility of gamma irradiated low quality roughages

Sample	Irradiation dose (Mrad)				(Unit : DM basis %)
	0	2.5	5	10	
Barley straw	53.55	49.38	49.35	52.67	57.97
Barley hull	61.83	69.36	70.15	63.98	69.84
Rape husk	58.99	59.77	60.35	66.06	65.94

potential nutrients, but rather by encrustation of carbohydrates within the lignin structure of the cell wall which is considered to be impenetratable to rumen bacteria or their enzyme (McManus et al, 1972^a).

Gamma irradiation has been used to liberate the nutrient from the encrustation (Ammerman et al, 1959; Lawton et al, 1951; McManus et al, 1972^{a, b}; McManus and Choung, 1976; Millett et al, 1970; Pigden et al, 1966; Pritchard et al, 1962; Timpa, 1983; Yu yu and Emery, 1975).

Our study shows that low quality roughages do not respond uniformly to treatment with gamma irradiation in terms of fibrous material degradation, ADF-N content, reducing sugar formation cellulose crystallinity, enzyme susceptibility, dacron bag dry matter disappearance, in vitro dry matter disappearance and VFA production. These suggest that different response due to innate physical structural differences between low quality roughages. Similar results were reported by McManus et al (1972 a,b) and Yu yu and Emery (1975).

Gamma irradiation markedly decreased the NDF and hemicellulose contents but contents of ADF, cellulose and lignin were not changed by gamma irradiation. (Table 4.1.1.1.) This indicates that degradation of gamma irradiated cell wall is due to the solubilization of hemicellulose. It also may be that the 25 Mrad irradiation dose is not sufficient to solubilize the cellulose, ADF and lignin. Similar results were reported by Yu yu and Emery (1975). They reported that

electron irradiation markedly reduced fibrous components (NDF, ADF, cellulose and lignin) of wheat straw at a dose level of 7.66 log rads and above.

They also found that fibrous constituents were slightly increased at the low irradiation level. It is the reverse of the situation expected from the work of Ammerman et al (1959), who showed that the crude fiber and cellulose content, samples of cotton linters, peanut hulls, corn cobs and sugar cane bagasse irradiated at doses of 0, 10, 20 and 40 megaroentgen, decrease with each increase in level of irradiation. These decrease may be due to innate physical differences between test materials.

The x-ray diffraction pattern of 25 or 5 Mrad irradiated barley straw or barley hull showed a very strong peaks higher than the control. Exposure to 2.5 and 5 Mrad in BS and gamma irradiated RH showed no peak. These results suggested that crystalline structure of BS was changed to a noncrystalline form (amorphous type) at the 2.5 and 5 Mrad irradiation levels, and gamma irradiation disrupted the crystalline structure of RH. The x-ray diffractogram of the 25 or 5 Mrad irradiated BS or BH showed these irradiation levels stronged crystalline structure of BS and BH.

Gamma irradiation decreased cellulose crystallinity of BS and RH, whereas cellulose crystallinity of BH was increased by gamma irradiation. It may be agree with result commented by Teszler and Rutherford (1956). They commented that the general effect of

radiation on polymers are at least twofold : polymer may be improved with respect to certain properties, through the medium of cross-linking or they may be degraded because of scission of their long chain molecules. Thus, in one case, the effect is to increase the average molecular weight while in the other it is to decrease the molecular weight.

Similar results were reported by Sasaki et al (1979). They reported that x-ray diffractogram of cotton cellulose treated with H_2SO_4 showed no sharp peaks, but only weak and broad peaks. However, x-ray diffractogram of the cryo-milled cellulose to 250 mesh showed that it had still crystalline structure of cotton cellulose powder, and was disrupted by acid dissolving treatment and changed to a noncrystalline form. Yoon et al (1983) found that NaOH treatment decreased crystallinity of rice straw, whereas ammonia treatment increased crystallinity of rice straw cellulose, when compared with NaOH and ammonia treatments.

Gamma irradiation increased hydrolysis of low quality roughages. Enzyme hydrolysis of low quality roughages was rapidly increased in a short time at higher irradiation levels (10 and 25 Mrad), suggesting that gamma irradiated cellulose markedly changed to amorphous form at higher irradiation levels. These results suggest that gamma irradiation can affect both the amorphous and the crystalline regions of the cellulose molecule. In this respect, our study agrees with McManus et al (1972).

The relationship between degrees of crystallinity of cellulose and its susceptibility to cellulose was found only at a dose level of 2.5 Mrad. These results differ from those reported by Sasaki et al (1979). They found that in pure cellulose there are good correlations between cellulose crystallinity and susceptibility to microbial cellulose when cellulose is treated with various solvents. Further studies are required to solve the disagreement between crystallinity and its susceptibility.

Dacron bag dry matter disappearance of gamma irradiated low quality roughages varied with samples types and irradiation doses (4.1.2.1). Barley hull showed a more positive to gamma irradiation than barley straw or rape husk. In 72 hours suspension, dry matter disappearance of barley straw decreased at 2.5 and 10 Mrad levels, and lower irradiation levels (2.5 and Mrad) decreased the dry matter disappearance of barley hull. All levels of gamma irradiation increased the dry matter disappearance of rape husk while DMD irradiated with 5 Mrad level was lower than that of 2.5 Mrad. Similar result was reported by McManus et al (1972^a). They reported a marked depressive action of irradiation on apparent loss of test material dry matter, over the dose range of 5-25 Mrad. This loss least for nassella, on either base ration, and greatest for cotton on a rice straw base diet. They also suggested that the lose is due to the innate structural differences between these materials or to formation of toxic factors.

Gamma irradiation increased the cell wall digestion and lengthen the cell wall digestion time up to 48 hours. The lowest cell wall digestion was found at the 25 Mrad irradiated BS and 5 Mrad irradiated barley hull in 12 hours suspension period. It may be these results due to increase of cellulose crystallinity in cell wall so inhibiting the cell wall digestion by the microorganism and its enzyme.

In the in vitro system there is no opportunity for certain toxic compounds being diluted, as exists in the dacron bag technique. Postive effect of gamma irradiation on the in vitro trial was also found in barley hull.

In the in vitro trial, 5 and 25 Mrad irradiation levels reduced dry matter disappearance of barley straw and low irradiation levels (2.5 and 5 Mrad) decreased the DMD of barley hull at 12 hours incubation. Gamma irradiation increased dry matter disappearance of rape husk, whereas 5 Mrad level was lower than that of 2.5 Mrad level (figure 4.1.3.1).

In vitro total VFA production of all test samples was increased by increasing incubation time. Total VFA production of barley straw was decreased at 5 and 10 Mrad levels and irradiation of 2.5 and 5 Mrad decreased VFA production of barley hull while VFA production of rape husk were decreased at 5 Mrad irradiation level.

There were good correlations between dry matter disappearance and total VFA production in barley hull and rape husk; however, dry

matter disappearance and total VFA production was poorly correlated in BH.

The reduction of dry matter disappearance and total VFA production may be due to formation of toxic compounds when exposed to gamma irradiation. McManus et al (1972 a,b) suggested that toxic factors generated by irradiation per se (active hydroxyl groups, peroxide or p^H effect) could have suppressed dissimilation. Gilfillan and Linden (1955) suggested that the major cause of degradation by gamma ray was oxidation resulting in the formation of a cellulose peroxide, which formed from the hydroxyl groups present in cellulose. Dilli et al (1967) reported that radiation induced formation of trapped radicals in cellulose and maximum concentration of trapped free radicals were found at the 10 Mrad irradiation dose. Timpa (1983) found the p^H changes toward the acid range with irradiation treatment when compared to that of the corresponding controls, indicating the generation of acidic groups.

In view of in vitro dry matter disappearance, Millet et al (1970) reported that electron radiation increased the in vitro digestibility of wood. Pigden et al (1966) found that irradiation decreased in vitro dry matter digestibility of immature forages, but markedly increased in vitro dry matter digestibility at intermediate and mature stages. Pritchard et al (1962) reported in vitro dry matter digestibility of wheat straw irradiated with 5-9 log rad was increased the DMD. Yu yu and Emery (1975) found

that in vitro cell wall digestion was improved by increasing the irradiation level up to a maximum of 7.66 log rad.

Similar results was reported by pritchard et al (1962). They found that total VFA production of wheat straw was increased only up to doses of 2.5×10^8 rads, suggesting that above this level of radiation the carbohydrates are disintegrated to such a degree that they are no longer suitable stbstrates for rumen microorganisms.

Gamma irradiation increased the production of propionic acid in all test materials. However, acetic acid production varied with irradiation levels and samples. In C2/C3 ratio, irradiation decreased C2/C3 ratio of BS, only the irradiation level at 5 Mrad increased the C2/C3 ratio of BH. Except for 5 Mrad level, other level increased C2/C3 ratio of RH. This suggesting concomitant alteration in species composition of the ruminal microorganism. McManus et al (1972 c) found that C2/C3 acid ratio was decreased with increasing irradiation levels when wheat straw was treated at 25, 50 and 75 Mrad levels. They suggested that changes in the C2/C3 ratio could be due to particle size effects or to chemical factors consequent upon irradiation.

Experiment II. Effects of chemical treatment and mixed treatment with grinding, chemical treatment and gamma irradiation

NaOH treatment increased DMD of BS, BH, RS and RH with increasing NaOH level. Digestibility results of this experiment are in general agreement with those of other research by Rexen and Thomsen (1976), Jackson (1977) and Berger et al (1979).

Particle size affected alkali treatment. Fine grinding was more effective in improving DMD of NaOH treated BH, and 2 mm grinding was more effective in improving the DMD of NaOH treated RS and RH.

NaOH treatment markedly decreased NDF content and hemicellulose content of BS, BH, RS and RH, ADF content of BS and BH was not changed by NaOH treatment, however, the ADF content of RS and RH was markedly decreased by increasing NaOH treatment levels. The optimal treatment level of NaOH was 6% in BS, BH and RH but 4.5% in RS. Similar results were reported by many researchers. Waller (1976) and Lesoing et al (1981) reported that NaOH treatment markedly solubilized hemicellulose content while not or slightly changed the cellulose content. Berger et al (1979) found NaOH treatment decreased NDF content while only a slight decreased in the percentage ADF of corn cobs. Klopfenstein et al (1972), and Rexen and Thomsen (1976) reported that lignin contents are

generally not reduced by NaOH treatment. Klopfenstein (1978) suggested that the increase in extent of digestion is probably due to breaking of bonds between lignin and hemicellulose, of cellulose without actual removal of lignin.

NH₄OH treatment improved DMD of BS, BH, RS and RH by increasing the NH₄OH levels up to 3%. Similar results were reported by Sundstol et al (1979) and Borhami and Sundstol (1982). Sundstol et al (1979) reported that dry matter digestibility increased as the level of ammonia increased up to 4%. No benefit in increase the level to 5.5% observed. Borhami and Sundstol (1982) found that the 4% level of ammonia was more effective than the 2% level; this trend was more clear with aqueous than with anhydrous ammonia.

NDF and hemicellulose contents of BS and BH were decreased by NH₄OH treatment whereas NDF and hemicellulose contents of RS and RH were not changed by NH₄OH treatment. (Figure 4.1.1.2). The optimal treatment level of NH₄OH was 3% in BS, RS and RH but 4.5% in BH. Similar results were reported by many researcher. Terashima et al (1980), and Tohrai et al (1987) found that ammonia treatment decreased the content of ADF. Itoh et al (1975) reported that ammonia treatment decreased NDF content, however, contents of ADF and cellulose were not affected by the ammonia treatment. Lee(1981) reported that NDF content decreased, but ADF and lignin content were not changed by ammonia treatment. He suggested that unchange of ADF and lignin, indicating considerable amounts of

nutrients in low quality roughages were remained undigestible even after ammonia treatment.

Total N content of BS, BH, RS and RH markedly increased by ammonia treatment, however, the lowest total N content was found in RH. (Figure 4.2.1.2). The highest total N content of BS, RS and RH was obtained at 4.5% level of NH_4OH treatment, but the highest total N content of BH was obtained 3% level of NH_4OH . Similar results were obtained by Sundstol et al (1978) and Horton and Steacy (1979). Sundstol et al (1978) found the ammonia treatment increased nitrogen content by 0.8 to 1.0 percentage units. Horton and Steady (1979) reported that ammonia treatment increased the average crude protein of the straw almost threefold though improvement ranged from 50 to 276% when barley, oat and wheat straw treated with 3.5% anhydrous ammonia.

BS, BH, RS and RH do not respond uniformly to treatment with NH_4OH in terms of DMD, total N content and fibrous material contents. Horton and Steacy (1979) and Horton (1978) found barley, wheat and oat straw were ammonia treated indicated that cereal straws may differ in their response to treatment with anhydrous ammonia.

Grinding increased dry matter digestibility (DMD) of varley straw (BS), barley hull (BH), rape stem (RS) and rape husk (RH). (Table 4.2.2.1) 2mm grinding was effective to improve DMD of BS and RS, and fine grinding was more effective in BH and RH.

This results indicate that there are physecal structural differences

between these materials. Similar results by Gharib et al (1975). They reported that DMD of the poplar bark was not enhanced when the bark was ground through the finer screens. Blaxter et al (1956) suggested that increasing the fineness of grinding led to a decrease the mean time spent in the rumen, and to a depression in the dry matter digestibility.

Combination of grinding and gamma irradiation showed compensation effect on improving the DMD of low quality roughages.

The effects of mixed treatment with grinding chemicals and gamma irradiation varied with types of samples. Mixed treatment with grinding, NaOH and irradiation on the BS and BH were less effective than only grinding and NaOH treatment. The positive effect of mixed treatment with grinding, NaOH and irradiation was found in barley and rape by product at 6% NaOH level. Mixed treatment with NaOH and gamma irradiation linearly decreased NDF and hemicellulose contents of BS, BH, RS and RH than only NaOH treatment. But, ADF content was not changed by mixed treatment with NaOH and irradiation. These results suggest the presence of different physical structure between BS, BH, RS and RH, and that gamma irradiation aid to solubilize the NDF and hemicellulose of NaOH treated low quality roughages. The optimal combination level of NaOH and irradiation was 6% NaOH in all test materials.

Similar results were obtained in mixed treatment with grinding, NH_4OH and gamma irradiation. Mixed treatment with grinding

NH₄OH and irradiation on BS and BH was less effective than NH₄OH treatment. The positive effect was obtained only treated with 4.5 and 6% NH₄OH in BS and BH. Mixed treatment with grinding, NH₄OH and irradiation was effective in improving the DMD of RS and RH than only NH₄OH treatment. NDF and hemicellulose contents were decreased by mixed treatment with NH₄OH and irradiation than only NH₄OH treatment. But, ADF content was not changed by mixed treatment with NH₄OH and gamma irradiation than only NH₄OH treatment. But, ADF content was not changed by mixed treatment with NH₄OH and gamma irradiation. The optimal combination level of NH₄OH and irradiation was 4.5% in BS, 6% in BH, 1.5% in RS and 3% in RH.

Enzyme addition increased the DMD of gamma irradiated BS only at the 25 Mrad irradiation level, however, decreased the DMD at other irradiation levels. (Table 4.2.3.1). Enzyme addition increased the DMD of gamma irradiated BH and RH at all levels. Similar results were obtained by Willis et al (1980). They observed that addition of enzymes (hemicellulose, pectinase and glucosidase) alone caused a reduction in in vitro digestibility of rice straw, but that the highest in vitro dry matter digestibility when pretreat with 5% NaOH and enzyme addition. These results suggest certain level of gamma irradiation cause a formation of toxic compounds to enzyme or polymer may be improved with respect to certain properties through the medium of cross-linking by gamma irradiation.

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