An-Najah National University

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In Situ Sheep Ruminal Degradation Kinetics of Crude Protein and Fiber for two Agricultural By-Products in Palestine

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Dedication

This thesis is lovingly dedicated to my respective parents and to my brothers and sisters who have been my constant source of inspiration. They have given me the drive and discipline to tackle any task with enthusiasm and determination. Without their love and support this project would not have been made possible.

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إقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

In Situ Sheep Ruminal Degradation Kinetics of Crude Protein and Fiber for two Agricultural By-Products in Palestine

اقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name:	**اسم الطالب:**
Signature:	**التوقيع:**
Date:	**التاريخ:**

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List of Abbreviations

CP	Crude Protein
NDF	Neutral Detergent Fiber
ADF	Acid Detergent Fiber
OC	Olive Cake
AOAC	Association of Official Analytical Chemists
GHW	Green house wastes products
TW	Tomato waste
CW	Cucumber waste
ED	Effective degradability
DM	Dry matter
RDP	Rumen Degradable Protein
RUP	Rumen Undegradable Protein
N	Nitrogen
OM	Organic Matter
EE	Ether Extract
NPN	Non Protein Nitrogen
CMS	Condensed Molasses of Fermentation Soluble
MSG	Monosodium Glutamate
PROC NLIN	Nonlinear Procedure of SAS
SAS	Statistical Analysis Software
CF	Crude Fiber

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In Situ Sheep Ruminal Degradation Kinetics of Crude Protein and Fiber for two Agricultural By-Products in Palestine

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Abstract

Two agricultural by-products (crude olive cake, OC and a mix of green house wastes of tomato and cucumber, GHW) used for sheep nutrition in rural communities in Palestine were evaluated for ruminal dry matter (DM), crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) degradation kinetics. Two bags each containing 5 g ground forage were incubated in the rumen of two ruminally cannulated Assaf rams for 4, 8, 16, 24, 48 and 72 h. Rate and extent of ruminal digestion were estimated. Significant differences between by-product types were observed in rapidly soluble and potentially degradable fractions, and degradation rates of DM, CP, ADF and NDF. The rapidly soluble DM, CP, ADF and NDF fractions were 16.19, 15.21, 10.64 and 14.06% for GHW, and 7.2, 6.6, 6.0 and 5.62% for OC. The potentially degradable DM, CP, ADF and NDF fractions, respectively, were 55.82, 62.3, 49.4 and 55.09% for GHW, and 47.6, 44.26, 46.34 and 48.53% for OC. The DM, CP, ADF and NDF disappearance in GHW were 47.3, 55.7, 40.39 and 40.7 at 24 h, 53.58, 67.45, 47.84 and 45.26 at 48 h, 65.38, 72.7, 54.96 and 57.95 at 72 h. for olive cake 34.99, 25.66, 32.80, and 32.90 at 24 h, 46.44, 33.87 42.44, 41.98 at 48 h, 49.21, 40.31, 47.39 and 48.19 at 72 h. The disappearance of all tested nutrients was higher ($P<0.05$) in GHW compared to OC. The

effective degradability (ED) of DM and CP were also higher ($P < 0.05$) for GHW than OC. Taking these findings into consideration, one can propose using both by-products, together or separately as part of ruminant rations. According to their degradability, they can be introduced in these rations as part of roughage (wheat and barley straw). However, the GHW can be incorporated in ruminant rations as part of good quality roughage (legume hay).

Chapter One

Introduction

1. Introduction:

Livestock production is growing rapidly as a result of the increasing demand on animal products. Projections of the FAO (2001) suggest that global meat production and consumption will rise from 233 million tonnes in 2000 to 300 million tonnes in 2020, and milk from 568 to 700 million tonnes over the same period. Egg production will also increase by 30%. This forecast shows a massive increase in animal protein demand, needed to satisfy the growth in the human population. Asia is experiencing the world's highest growth rates in production and consumption of livestock products (meat, milk and eggs) (Delgado *et al.*, 1999),. The issues to be addressed are the environmental and feed supply problems arising from the intensification of livestock production.

The population in Palestine is increasing at high rate. The population growth rate was estimated at 2.9% (Palestinian Central Bureau of Statistics, 2012). This growth is accompanied by harsh economic conditions and general change in climate. Growth of human population is also accompanied by a simultaneous increase in the demand for feed ingredients. This has already resulted in the deterioration of natural grazing areas, in addition to rising the prices of raw materials used in the manufacture of feed, which is controlled by the Israeli side, and leading to a marked decrease in animal performance. It is therefore important to study the utilization of agricultural by-products as feed ingredients for farm animals especially ruminants.

The majority of the sheep population in Palestine is of the Awassi breed. Awassi sheep graze on the rangelands. In order to cope with the lack of feed resources and the high costs, sheep can be fed on agro-industrial wastes. Large amounts of agro industrial wastes are available locally where olive cake (OC) and green house wastes (GHW) are the most abundant (50 and 500 thousand tonnes/year, respectively; (PCBS, 2010).

Dumping or burning wastes of agro-industrial by-products presents potential air and water pollution problems. Also high-moisture wastes are difficult to burn. Consequently, it is very important for both economic and ecological reasons that such a high quantity of by-products of the local plant production and processing be included in animal feed. Many by-products have a substantial potential value as animal feedstuffs. Ruminants have the unique capacity to utilize fiber, because of their rumen microbes. This means that cereals can be largely replaced by these by-products in ruminant rations. Consequently the competition between human and animal nutrition can be decreased.

Local research proved the positive potential of these wastes as feed ingredients (Abo Omar and Gavoret, 1995; Abo Omar *et al*., 1997; Abo Omar, 2001; Abo Omar, et al.,2003; Abo Omar, *et al.,* 2011; Abo Omar and Naser, 2011, 2011; Hejazi and Abu Omar, 2009; Zaza and Abo Omar, 2008).

Local research proved the potential of these wastes as feed ingredients such as olive cake which was fed to fattening lambs (Abo Omar and Gavoret, 1995; Abo Omar *et al.*, 2012), to broilers (Rabayaa., et al 2001) and to layers (Shanti *et al.*, 2012). Olive cake was fed at levels ranging from 5 to 30% of rations on dry matter. However, several other by- products were utilized in livestock rations. Corrugated cardboard was fed to fattening Awassi lambs at 20% of DM (Abo Omar., et al 2001). Citrus pulps were fed to broilers at levels up to 10% of rations dry matter (Khawaja, 2003). Silage made of various agro industrial wastes was fed to fattening lambs with positive effects on lamb general performance (Zaza and Abo Omar, 2008). Silage made of tomatoes, poultry litter and wheat straw was fed to lambs with promising advantages (Azmuti, 2003). Citrus pulp silage when fed to lambs had similar effects on lamb performance (Zaza and Abo Omar, 2008). Similarly, lambs performance and carcass merits were improved when fed olive cake poultry litter tomatoe silage (Abo Omar *et al.*, un published data). Other by-products were also fed to livestock with advantages. Sesame cake was successfully fed to lactating goats at levels reaching 20% of rations DM (Hejazi and Abo Omar, 2009). Abu Baker (2008) fed broilers with almond hulls at levels of 10% with no negative effects on growth.

There is no information about degradability of nutrients, effective degradability (ED) values and digestion kinetics of wastes used for sheep feeding in Palestine. Accurate values are important for the feed manufacturing industry. The feed manufacturing industry and farmers performing feed mixing practices, therefore, rely on nutritive values, such as those published by research institutions. Unfortunately, the accuracy of ration formulation depends on the assumption that all wastes are represented by these limited published values describing rumen degradability.

The nylon bag technique (*In Situ*) is usually used for estimation of rumen degradability (Ørskov and McDonald, 1979). Ruminally undegradable nutrients (i.e. Dry matter (DM), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF)) of these raw materials are important as are used for ration formulation. Estimation of ruminally degradable and undegradable fractions of the raw ingredients is complicated. The technique has become increasingly popular means to estimate the rumen degradable and undegredable fractions (Ørskov and McDonald, 1979; Wilkerson *et al.*, 1995).

This technique allows a number of feed ingredients to be evaluated at the same time, and is accepted as one of the basic techniques required by the new evaluation systems (i.e. protein) proposed by different organizations (Shand *et al.*, 1988). Many factors, such as varieties of the vegetable, growing conditions, nitrogen fertilization and stage of maturity at harvest time and extraction method (i.e. OC) affect ruminal degradability of DM, CP, ADF and NDF contents of OC and GHW (Van Straalen and Tamminga, 1990; Hoffman *et al.*, 1993).

It is important to understand the concept of dietary nutrient degradability (i.e. CP) since the extent and rate of its degradation determines the nitrogen available for rumen micro-organisms and undegradable CP available for digestion in the small intestine (Ørskov, 1992).

Ruminal CP degradability of a feedstuff is an important characteristic to determining the CP value of a feed (Madsens and Hvelplund, 1994; Michalet-Doreau and Ould-Bah, 1992). Dietary CP requirements are best expressed in terms of the rumen degraded protein (RDP) and the rumen undegraded protein (RUP).

Degradable protein is used by ruminal microbes, to convert feed nutrients to microbial protein, rather than by the animal itself. Ruminally degraded protein can come in the form of NPN (non-protein nitrogen) and true protein, which supplies the rumen microbes with amino acids and peptides. These nutrients are used by the microbes to support microbial fermentation. A deficiency in RDP would result in reduced carbohydrate digestion, VFA and microbial protein production. This would decrease animal performance (Cooper et al., 2002).

The RUP fraction is relatively more expensive because rumen micro-organisms can utilize products of relatively poor quality CP (i.e., NH3, peptides, amino acids) to synthesize better quality microbial protein. Apart from ruminal CP degradability, factors such as the amino acid profile and digestibility of RUP should be taken into account when estimating the CP requirements of ruminants (NRC, 2001).

Neutral Detergent Fiber is a good indicator of "bulk" and consequently feed intake. Acid detergent fiber is a good indicator of digestibility and energy intake.

Considerable quantities of olive cake and vegetable by-products (i.e. green house wastes: fruits, leaves, stems) that are suitable for feeding to livestock are generated every year in the Mediterranean countries. However, they are lost or not utilized due to lack of technical knowledge and lack of information about their nutritive value. The nutritive value of this by product varies greatly with the processing system. The main factor that influenced nutritive value in ruminant is the ruminal digestible fraction of feed (i.e. olive cake).

Due to lack of information available on ruminal degradability of DM, CP, NDF and ADF of olive cake and waste products of green houses, this study was undertaken to determine *in situ* ruminal degradability, fractional rates of digestion and effective degradability of DM, CP, ADF and NDF of olive cake and a mixture of tomato and cucumber wastes of green houses.

Chapter Tow

Literature review

2. Literature Review:

2.1. Cultivated Areas:

Palestine is located between 29° and 33° north latitude and 35° and 39° longitude, with a total area of 6245 Km^2 (area of West Bank include east Jerusalem and Gaza Strip). Palestine has a Mediterranean climate with a cold rainy winter and hot dry summer. The precipitation is ranges from 150 mm in the south east to 700 mm in the northern western part of the West Bank. The West Bank consists of four agro-ecological zones, semi coastal, central highlands, eastern slopes, and Jordan valley (MoA, 2000).

In Palestine the the total area of cultivated land is 2,150,800 dunums. The rain-fed area is 1.9 million dunums (PCBS, 2012). Recent statistics showed that the cultivated or arable land represents 42.5%, while the area of open land with or without significant vegetation cover represents 29.3%. Grazing area represents 12.5%, the area of Palestinian built-up land represents 6.6%, and the area of built-up land in Israeli occupied sites including the expansion and annexation wall represents 4.1% during the year 2006.

Land and water are fundamental agricultural resources. Roughly, 62.9% of Palestine's arable land is located in C designated areas with just 18.3% in A areas. Although grazing lands comprise 2.02 million dunums, Israeli settlement activity and Separation Wall construction has reduced grazing lands to only 700, 000 dunums. The western section of the Separation area is 900, 000 dunums of agricultural land.

The olive tree is considered one of the main fruit tree in Palestine. More than 10 million olive trees are planted in an area of more than 3600 thousands dunums which constitute 50% of the cultivated area, mostly in mountains. Compared to other fruit trees, the olive tree is the most widespread constituting 80% of the area planted with fruit trees.

2.2. Livestock Production in Palestine:

Livestock in Palestine includes: poultry; sheep and goats; and small numbers of beef and dairy cattle. Most rural Palestinian families have some form of livestock, which they use to provide dairy products, eggs and occasionally meat. However, livestock in Palestine has increased more slowly compared to population growth, resulting in a production shortage, especially in red meat. The livestock sector makes up about 40% of the total Palestinian agricultural revenues (UNCTAD, 1993). Restriction of the grazing area, mostly in the Palestinian eastern slopes, has been the major reason for this production shortfall. Prior to 1967, grazing areas consisted of 400, 000 to 500, 000 dunums. This figure has declined sharply since the Israeli occupation, as 85% of the land has been confiscated or closed to provide for Israeli security and settlement needs. With such limited grazing areas available to shepherds, there is both an unsustainable ratio of livestock per dunum, and thus overgrazing, this leads to higher production costs for livestock raising due to the use of purchased supplemental feed.

More recently there has been a decline altogether in the number of livestock in Palestine because of reduced profits (PCBS, 2012). .

Livestock production in Palestine varied according to economical and political situation. There are 39, 625 heads of cattle, 732, 399, 240, 136 heads of sheep and goats, 39, 419 million of broilers and 3.045 million layers. In Palestine, sheep is the major small ruminant. In 2010, the total number of sheep in the West Bank was about 732.399 heads (Awassi, 54.5%; Assaf, 34% and Awassi x Assaf, 11%; (PCBS 2012)).

The majority of sheep raised locally is of the Awassi breed (PCBS, 2012). It is a fat-tailed breed. This breed contributes about 50% in the animal production sector (PCBS, 2010).

The Assaf (Awassi and East Friesian breeds) is gaining increased importance in Palestine, the Assaf breed is managed under an intensive production system involving weaning lambs at birth, rearing them on milk replacement, and milking ewes after parturition.

2.3. Fattening projects in Palestine:

The fattening operations are among the important activities within animal livestock sector. Recent statistics showed that about 400 thousand heads of lambs were fattened in 2000 (MOA, 2000). The income from fattening operations was estimated to be more than 50% of total income resulted from the animal sector (MOA, 2000; Naser, 2009). However, the cost of feed for fattening lambs is estimated to be as much as 80% (Abo

Omar, 2001; Abo Omar *et al*., 2012). Therefore, any attempts to reduce the high input cost will be of considerable importance as most of the ingredients are imported from foreign sources and at high cost (Azmouti, 2003; Neirat, 2006). The execution of fattening operations varies widely in Palestine. The variations are due to several reasons: among these are the sizes of investment, location of the fattening farm, and the experience of farmers. Locally, there are two types of lamb fattening systems: the commercial (intensive) and the extensive systems. In the first, commercial fattening feeds are used in these operations while a barley-based diet is used in the second system (Abo Omar, 1992). The extensive fattening system is mainly practecd in eastern slopes of the West Bank (Barghuti and Abo Omar, 2001). Fattening animals in this system are mainly fed with roughage either from pastures or market. Adoption of the extensive fattening system has negative impact on local pastures. Lambs fed under this system are considered as potential destroyers of the pastures especially in the eastern slopes, which can lead to environmental hazards.

The intensive systems of livestock production might be friendlier to local environment. The majority of lambs under fattening operations in either system is of Awassi breed. Cereal grains such as barley are common feed ingredients in local fattening operations. It makes a major part of manufactured feed.

Barley is incorporated in mixed feed at a rate of about 25% (Abo Omar, 1992; Abo Omar *et al*., 2012). However, it is used as a sole grain feed ingredient in the extensive fattening system. The amount of barley used in local fattening operations is estimated to be 100 thousand tons/year (MOA, 2000).

The use of barley in feeding ruminants for the extensive fattening operations used to give satisfactory results. The estimated average daily gain of lambs under such feeding program is about 120-200 g (MOA, 1999); while in the intensive fattening system is estimated to be more than 240 g/day (MOA, 1999). The outcome of such fattening operations is variable especially when the extensive fattening system is considered.

2.4. Feed consumption in Palestine:

The total amount of concentrate feeds consumed by local livestock is about 767 thousand tons (PCBS, 2010). The annual consumption by class of animals is shown in Table (1).

Table(1). Annual concentrated feed consumption by class of livestock

Animal	Feed consumed (ton)	%
Poultry	319061	41
Sheep and goats	330000	43
Cattle	117636	16
Total	766697	100

Source: PCBS, 2010.

2.5. Problems of Livestock Sector:

Many obstacles are facing the livestock. The most important one is the limited feed resources. It is well documented that feed costs make more than 80% of total production costs (Abo Omar *et al.*, 2011). To overcome this situation, attempts were made to use unconventional feed ingredients in feeding sheep. Large amounts of agro-industrial wastes are available and can partially reduce dependence on concentration.

2.6. Agro-industrial By-products:

Agro-industrial by-products are subdivided into energy feeds and protein feeds. However, by-product feeds can have the characteristics of both a concentrate and roughage. Several by-product feeds are available both fresh (high moisture) or dried. The most commonly available by-products in our region include green house wastes: (tomatoes, cucumber, squash, beans, cauliflower and other vegetables). Others like olive cake, citrus, banana, almond, grape, and field crops (mainly wheat). Some of the agriculture crops are available all year long, like green house by-products and some are seasonal like olive cake. The amounts of available by-products are shown in Table (2).

Table (2). Amounts of local by-products according to season

Type of by-product	Annual amount (thousand ton)	Season
Cucumber	Not available	All seasons
Tomato	Not available	All seasons
Squash	Not available	All seasons
Beans	Not available	Fall
Olive cake	48	Fall
Citrus	18	Spring
Banana	18	All seasons
Almond	Not available	Summer
Grapes	1	Summer – Fall
Field crops	100	Summer
Cauliflower	Not available	Spring – Fall

Source: Abo Omar et al., 2012.

Certain wastes are used in feeding sheep. However, no information is available on the degradable and un-degradable fractions of their contents. In ruminant nutrition, degradation and digestion characteristics are useful information to evaluate feedstuffs.

Olive cake (OC) is the by-product obtained by pressing olive fruits for olive oil. It is composed of the pulp and the crushed stone. The Olive cake is composed of 50% pulp and 50% stones on air-dry basis. Production of OC has been rapidly increasing in recent years. Approximately, 48 thousand tons are available as a byproduct each year (Abo Omar and Gavoret, 1995).

Unlike many other by-products, OC is available as a dry by-product. Low moisture content makes this by-product attractive to livestock feed by reducing transportation costs and allowing for long-term storage.

Similarly, large amounts of green house wastes are available each year. The estimated amount was about 500 thousand tons in 2010 (PCBS, 2010). Also, the low moisture content and the high leave to stem ratio of these wastes makes it an important feedstuff for ruminants.

The chemical composition and feeding values of OC and green house wastes (GHW) were examined by various researchers; however, important information about degradability in the rumen is missing.

2.7. Upgrading of the Agro-industrial By-products:

Many types of feed ingredients or feedstuffs that meet the nutritional needs of livestock are available. These feedstuffs are the raw materials that can be converted into animal cells, tissues, organs, and products. Familiarity with the chemical and nutritional composition of the various classes of feedstuffs is essential in order to formulate most economical and profitable rations.

A feedstuff is loosely defined as any component of a ration that serves some useful function. Feedstuffs generally are included in the ration to help meet the requirement for one or more nutrients. However, they may also be included in the ration to provide bulk, reduce oxidation, emulsify fats, provide flavor, improve animal health, or improve characteristics of the livestock products.

2.8. Use of Agro-industrial By-products in Livestock Rations:

The yield of by-products from food industry which are suitable as feed for livestock is determined by two main factors: the dietary habits of human beings and the production capacity of alternative feed resources. The latter is often determined by the land area available for feed production. Palestine is a small country with a high population density, and lacks natural feed resources. Unfortunately, most by-products at present are being discarded as wastes, which cause environmental pollution. Therefore, changes in government policy might be necessary to facilitate the utilization of by-products and more research on their use is also needed.

Brewers' Grain:

Is a by-product from brewing barley to make beer, and contains non-soluble materials such as barley husk. Kim *et al*. (1992) indicated that adding 10% dry brewers' grain to the diet of fattening pigs reduces their daily weight gain, but improves their carcass quality.

Distillers' Grain:

Raw materials are used for distillation include rice, tapioca and sweet potato. The nutritional characteristics depend on the raw materials; Distillers' grain has relatively high crude protein content, but low energy content. The palatability is rather poor, so less than 5% should be included in the rations of most animals *(Kim et al.* 1992).

Condensed Molasses of Fermentation Soluble (CMS):

Is the organic residue of microbial fermentation, produced in making monosodium glutamate (MSG) from raw sugar and molasses. CMS supplement may decrease the viscosity of molasses, making it a more efficient feed resource. In addition, since CMS has a freezing point of -40°C, it can be preserved during the winter.

Since CMS contains a high level of crude protein (45.0%), it is a good potential feed resource. Particularly, high levels of non-protein nitrogen (32.0%) in CMS have attracted ruminant nutritionists to study whether it can replace the non protein nitrogen (NPN) in conventional feeds. Cha *et al.* (1990) and Maeng *et al*. (1990) suggested the utilization of CMS for feed.

By-products from Fruit and Vegetable Processing:

The main by-product of citrus processing is citrus pulp. Citrus pulp contains 10.8 - 12.7% crude fiber, 8.3 - 9.7% crude protein, 59.3 - 69.5%

nitrogen free extract, and 62.8 - 76.1% total digestible nutrients, which make the pulp a promising energy source for livestock. In addition, the relatively high level of crude fiber makes it a possible feed source for ruminants. Citrus pulp is discarded as waste, and is seldom used as ruminant feed.

Two experiments (Park et al., 1981, Oh et al., 1981) have been carried out to determine the appropriate level of citrus pulp silage in the diet of fattening cattle. Results indicated that cattle fed 20% citrus pulp silage had the same total and daily average weight gains as the control, while the group given 40% citrus pulp silage had a lower weight gain. Therefore, 20% citrus pulp silage is an appropriate level to use as a partial replacement for feed concentrates.

2.9. Evaluation of By-products:

Various methods available for feed evaluation and digestibility studies through feeding experiments are expensive and require sophisticated laboratory equipment facilities for keeping animals. The diversity in the nutritive value of different feedstuffs needs some easy and efficient method of evaluation; therefore, some alternative laboratory methods are required. *In vitro* methods are widely used to evaluate the nutritive value of different classes of feeds (Menke *et al*., 1979: Getachew *et al*., 1998).

2.10. The *In Situ* Technique:

Two different aspects are considered to evaluate feed contents for ruminants: microbial degradability of the feed contents and intestinal digestibility (Kopecny *et al*., 1989). The nylon bag technique (the *in Situ*) is usually used for estimation of rumen degradability (Ørskov and McDonald, 1979). Quin et al (1938) used the fiber bag technique to investigate the digestion of feeds in the rumen of cannulated sheep. They used cylindrical bags composed of a very fine natural silk. Subsequent workers have used bags made of artificial fibers (Erwin and Elliston 1959; Johnson 1966). The artificial fiber bag (dacron bag, nylon bag, rumen bag) technique, provides a powerful tool for the initial evaluation of feedstuffs and for improving our understanding of the processes of degradation which occur within the rumen. The *in Situ* procedure has become popular in recent years for the estimation of the rumen degradable and undegradable fractions of feeds (Wilkerson *et al*., 1995).

The *in Situ* technique allows a number of feeds to be evaluated at the same time, and is accepted as one of the basic techniques required by the new protein evaluation systems proposed by National Research Council (NRC) and other organizations (Von Keyserlingk *et al*., 1996; Turgut and Yanar, 2004). However, it must be noted that the technique has three important limitations. Firstly, since the sample is confined within the bag it is not exposed to any breakdown by chewing and rumination. Secondly food would normally be able to leave the rumen once broken down to a suitable particle size. Thirdly, what is actually measured is the breakdown of material to a size small enough to leave the bag and not necessarily a complete degradation to simple chemical compounds.

Fiber bags: Various materials have been used in the construction of the bags. Quin *et al* (1938), as already mentioned, utilized fine silk, while Mehrez and Ørskov (1979) used Dacron material obtained from an old parachute.

The optimum size of the bag has been investigated by a number of workers (Mehrez 1976). The optimum size is essentially a compromise between two opposing factors. On one hand, the necessity to have the bag large enough relative to the sample size used, so as to ensure that rumen fluid can easily enter the bag and mix with the sample. On the other hand, there is the necessity to have a bag small enough to be easily withdrawn through the rumen cannula.

Comminution of dry feeds is rather easier to define (by type of mill and screen size). Erwin and Elliston (1959) found that the fineness of grinding of the sample had less effect on the disappearance of dry matter as the period of incubation was increased. This is to be expected, since a decrease in the particle size will increase the surface area per unit weight of substrate. This increase may affect the initial rate of degradation, but not necessarily the final extent of degradation. Van Keuren and Heinemann (1962) found no differences between samples (dried grasses, clover and alfalfa) when ground through a 20, 40 or 60 mm mesh screen. Lawrey

(1969) found no differences in dry matter losses with forages ground through 4, 3, 2 or 1 mm screen sizes, although passive losses of materials through the pores of the bag occur with the use of 1 mm screen (Payne *et al*., 1972).

Sample size: A reduction in degradability was observed by many workers as the sample size, for a given bag size, was increased (Tomlin *et al*., 1967; Mehrez and Ørskov, 1976). The smallest amount of sample necessary may be defined as that which will provide adequate material for analysis after incubation (for example Nitrogen), or possibly by the precision of the balances available for weighing the bag and sample. The amount of sample incubated in the hag will also depend on the bulk density of the prepared sample. Ørskov and McDonald. (1979) found that about 2 g air dry ground straw, 3 g good hay or dried grass, 5 g of concentrate (e.g barley, protein supplements) and 10 - 15 g of fresh herbage are suitable for the size of bag.

Incubation time: Much of the published data relate to experiments in which the workers tended to incubate bags for only a few different times, and attempted to relate dry matter losses from the bags to the appearent digestibility of the feedstuff. The interest is in measuring the rate of degradation, which requires a number of measurements of degradation after different times. The total time for complete degradation will vary with the material being incubated, and hence the intermediate times chosen will also vary. As a rough guide: concentrates require 12-36 hours, good quality forages 24-60 hours, poor quality roughages 48-72 hours. These are the times required to reach, or nearly reach, the asymptote (potential degradation).

Replication: Mehrez and Ørskov (1977) found that the greatest source of variation in the disappearance of DM from the bags was the between sheep component (6.2% of the mean), followed by that of between days (4.9%). The least variation was found between the bags (3.3%) incubated together and withdrawn at the same time, that the use of one bag, two days (i.e. a repeated measurement) and three sheep was a reasonable combination.

The ruminal degradability of feed fractions is influenced by type of waste, growing conditions of the forage, N fertilization and stage of maturity at harvest time (Hoffman *et al*., 1993).

There is no research on olive cake and green house wastes of tomatoes and cucumbers degradability, but there is much research on degradability of other by- products.

Maheri-Sis, *et al*., (2011) evaluated the effects of microwave irradiation for 2.5 and 5 min on ruminal dry matter (DM) degradation parameters of tomato pomace. These authers reported that microwave irradiation reduced DM solubility in the rumen, but had no significant effect on the extent of total and effective degradability of tomato pomace. Gencoglu, *et al*., (2011) estimated the *In Situ* ruminal crude protein and starch degradability of some grains and by-product feeds in Turkey. Nahand, *et al*., (2011) determined the nutritional value of apple tree leaves for ruminants using the nylon bags.

This study provides estimates of ruminal degradation kinetics of feeds to improve the accuracy of formulation of sheep diets.

Chapter Three

Materials and Methods

3. Materials and Methods

3.1. Preparation of Animals and Experimental Design

This experiment was conducted at An Najah National University farm, Faculty of Agriculture, Tulkarm, Palestine. Initially, three 2-yr old castrated Assaf rams (50 ± 5 kg) were fitted with rumen cannulas with a 4 cm internal diameter to determine the *in situ* degradation characteristics of two agricultural by-products. One of the rams was later removed from the study due to health problems. After surgical operation, rams were housed in a pen (4m*4m) and were fed *ad libitum* on wheat straw and a concentrate for two weeks before the start of experiment (adaptation period). Hay contained 910, 90, 330, 450, 70 and 20 g/kg of DM, CP, ADF, NDF, ash and fat, respectively. Chemical composition of the concentrate was 880, 160, 120, 340, 60, 30, 18 and 6 g/kg of DM, CP, ADF, NDF, ash, fat, Ca and P, respectively. The concentrate contained vitamins A, D3 and E at levels of 50000, 700, and 30 IU/kg, respectively.

3.2. Preparation of By-products

Tomato wastes (TW) and cucumber wastes (CW) were obtained from nearby greenhouses (mixtures of leaves, stems and some fruits) during summer after finishing a green house cultivation period, while olive cake (OC) was collected in November during the olive fruit pressing season. Raw ingredients (*i,e.* OC, TW, and CW) were dried and ground to pass through a 2 mm screen. Five-gram samples were weighed into nylon bags

(8 cm × 16 cm) with 40–45 µ pore size. Equal amounts of TW and CW (2.5g TW, 2.5g CW) were used to form each sample of a green house waste mixture (GHW).

3.3. Chemical Analysis

Prior to *in situ* degradation procedure, proximate analyses were performed on double samples of OC and a mixture of TW and TC (3 g) according to the Association of Official Analytical Chemists. (1990).

Olive cake and green house waste mixture were analyzed for CP, DM, total ash (Ash), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al*., 1991). Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as N*6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by procedures outlined by Goering and Van Soest (1970) with modifications described by Van Soest *et al*. (1991).

Table (3) Chemical composition of olive cake (OC) and green house wastes (GHW):

Chemical composition %	OC	GHW
Dry matter	89	88.2
Crude protein	10.92	17.5
NDF	49	43
ADF	33	37
Ash	1.4	16

3.4. In Situ Procedure

Five grams samples of each wse type were weighed into Dacron bags (8 cm × 16 cm) with 40–45 µm pore size. The bags are made from nylon filter cloth. They were tied and closed by the use of polypropylene twines. The bags were inserted in the ventral rumen of the two remaning rams at 08:00 on d 1; 08:00 on d 2; and 08:00, 16:00, 00:00 on d 3, and 04:00 on d 4. These times of insertion correspond to incubation times of 72, 48, 24, 16, 8, and 4 h, respectively. Two samples were used per time and per animal, with a total of four independent observations per time. The soluble fraction (0h) measurement was obtained by soaking the two bags of sample in warm water for 1 h. After incubation, bags were removed from the rumen and were rinsed with 39^{o}C water to remove particles adhering to the outside of the bags and the 0h sample bags were rinsed immediately after soaking in tap water. All bags were then washed in a washing machine on the delicate setting for a 10-15 min rinse and a 2-min spin cycle and this sequence was repeated 10 times. Then they were dried at 65◦

C for 48 h in an oven and then weighed as described by Ørskov (1982) and Janicki and Stallings (1988).

3.5. Statistical analysis

Ruminal disappearances (p) at each incubation time were calculated as the difference between the residues and original samples. The amounts of CP and DM in the residues, expressed as percentages of original samples, were determined for each bag. Two observations from each ram were obtained for each raw material and occupation time. Ruminal kinetics parameters were estimated using PROC NLIN procedure of SAS (SAS, 2003) fitting the exponential model of Ørskov and McDonald (1979):

$$p = a + b(1 - e^{-ct}) \qquad (1)$$

Where *p* is rumen disappearance at time *t*, *a* is the rapidly soluble fraction, *b* is the potentially degradable (fermentable) fraction, and *c* the constant rate of degradation of *b* (h^{-1}).

Effective degradability (ED) of nutrient components was calculated applying the equation of Ørskov and Mc Donald (1979):

$$ED = a + \{(bc)/(c+k)\} \qquad (2)$$

Where *a*, *b*, *c* are the same as in (1), k = Rate constant of passage (h^{-1}). When calculating effective degradability, the rate constant of passage was assumed to be 0.02, 0.05 and 0.08 per hour (Bhargava and Ørskov, 1987).

The data obtained (degradation characteristics, effective degradability and disappearance rates) were subjected to statistical analyses using PROC MIXED procedure of SAS (SAS, 2003). Because of the inherent differences between rams, each ram was considered as a block (randomized complete block design with two replicates per treatment). Treatment (type of by- product) was fitted as a fixed factor while block (animal) and block*treatment were fitted as random factors according to the following model (Kaps and Lamberson, 2004):

$$y_{ijl} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \varepsilon_{ijl} \qquad (3)$$

Where y_{ijl} is observation l in treatment i and block j, μ is the overall mean, τ_i is the effect of treatment i, β_j is the effect of block j, $\tau\beta_{ij}$ is the interaction effect of treatment i and block j, and ε_{ijl} is random error.

Chapter Four
Results

4.1. Results:

Proximate analyses of OC and GHW are presented in Table 3. The CP was 109.2 and 175 g/kg DM, ADF 330 and 370, NDF 490 and 430, crude ash 10 and 160 g/kg DM, respectively.

4.1.1Degradation Kinetics of Dry Matter

The rapidly soluble fraction '*a*' of DM was higher ($P< 0.05$) in GHW (16.19%) compared to OC (7.21%), (Table 4). The insoluble but rumen degradable fraction of DM, 'b' was lower ($P< 0.05$) in OC (47.61%) compared to that in GHW (55.82%). GHW had higher ($P< 0.05$) amount of potentially degradable DM (a+b) than OC (72.01% and 54.82%).

The effective degradability (ED) of DM was calculated using rumen outflow rates of 2, 5 and 8 h^{-1}. There were significant differences ($P< 0.05$) between OC and GHW in effective degradability of DM (ED of GHW was higher than that of OC for all passage rates, Table 4). ED estimates of DM were 48.26, 35.77, 30.28 for GHW and 36.68, 25.96, 20.96 for OC at k values of 2, 5 and 8 h^{-1}, respectively. Disappearance rates (%) of DM for OC and GHW at different rumen incubation times are presented in Table 4. At 24 h of incubation, the disappearance of DM was higher ($P< 0.05$) for GHW (47.30%) compared to that for OC (34.99%). At 48 and 72 hours of incubation, disappearance value of DM was also higher ($P< 0.05$) for GHW (53.6%, 65.4%) compared to OC (46.4%, 49.2%).

4.1.2 Degradation Kinetics of Crude Protein:

The rapidly soluble fraction '*a*' of CP was higher (P< 0.05) for GHW (15.21%) compared to that of OC (6.64%) (Table 5). The insoluble

rumen degradable fraction of CP, 'b' was lower (P< 0.05) for OC (44.26%) compared to that in GHW (62.30%). GHW had higher (P< 0.05) amount of potentially degradable CP (a+b) than OC (77.50% and 50.81%). Effective degradability of CP was consistently higher (P < 0.05) for GHW than OC at all passage rates (55.74%, 41.80%, 35.01% for GHW and 28.75%, 19.28%, 15.49% for OC), Table 5. Disappearance rates of CP at 24, 48, and 72 hours were higher (P< 0.05) in GHW (55.70%, 67.45%, 72.70%) compared to OC (25.7%, 33.9%, 40.3%).

4.1.3 Degradation Kinetics of ADF:

The rapidly soluble fraction '*a*' of ADF was higher (P< 0.05) in GHW (10.46%) compared to OC (6.0%) (Table 6). The insoluble but rumen degradable fraction of ADF, 'b' was lower (P< 0.05) in OC (46.34%) compared to that for GHW (49.40%). GHW had higher (P<0.05) amount of potentially degradable ADF (a+b) than OC (60.1% and 52.36%).

The effective degradability of ADF were significant differences (P< 0.05) between OC and GHW in effective degradability of ADF (ED of GHW was higher than that of OC for all passage rates, Table 6). ED estimates of ADF were 40.63%, 29.59%, 24.41% for GHW and 34.55%, 24.08%, 19.24% for OC at k values of 2, 5 and 8 h^{-1}, respectively.

Disappearance rates (%) of ADF for OC and GHW at different rumen incubation times are presented in Table 6. At 24 h of incubation, the disappearance values of ADF was higher (P< 0.05) for GHW (44.34%) compared to that OC (32.80%). At 48 and 72 hours of incubation, disappearance value of ADF was also higher (P< 0.05) for GHW (47.84%, 54.96%) compared to that for OC (42.44%, 47.39%).

4.1.4 Degradation Kinetics of NDF:

The rapidly soluble fraction '*a*' of NDF was higher (P< 0.05) for GHW (14.06%) compared to that for OC (5.62%) (Table 7). The insoluble but rumen degradable fraction of NDF, 'b' was lower (P< 0.05) for OC (48.53%) compared to that for GHW (55.09%). GHW had higher (P<0.05) amount of potentially degradable NDF (a+b) than OC (69.15% and 54.35%). Effective degradability of NDF was consistently higher (P < 0.05) for GHW than that for OC at all passage rates (41.84%, 30.0%, 25.24% for GHW and 34.53%, 23.88%, 18.64% for OC) (Table 7). Disappearance rates of NDF at 24, 48, and 72 hours were higher (P< 0.05) for GHW (40.70%, 45.26%, 57.95%) compared to that for OC (32.90%, 41.98%, 48.19%).

Table (4). Degradation characteristics and disappearance of dry matter (DM) in olive cake (OC) and green house wastes (GHW)

	GHW	OC	SEM[5]	P value
Degradation characteristics				
A%[1]	16.19	7.21	0.1072	0.002
b%[2]	55.82	47.61	0.2753	0.031
c[3]	0.027	0.032	0.0006	0.089
a+b%[4]	72.01	54.82	0.3547	0.015
Effective degradability (%)				
k=0.02	48.26	36.68	0.456	0.019
k=0.05	35.77	25.96	0.411	0.020
k=0.08	30.28	20.96	0.356	0.017
Disappearance (%)				
24hrs	47.30	34.99	0.524	0.017
48hrs	53.58	46.44	0.659	0.018
72hrs	65.38	49.21	0.399	0.018

[1,2,3,4] constants in the equation $P = a + b (1-e^{-ct})$, where P is level of degradation at time "t", "a", rapidly soluble fraction; "b", insoluble fraction but degradable in rumen; "c", rate of degradation of "b" per hour; "a + b", potentially degradable fraction.
K: Rate constant of passage (h^{-1})
[5] Standard error of the mean.

Table (5). Degradation characteristics and disappearance of crude protein (CP) in olive cake (OC) and green house wastes (GHW)

	GHW	OC	SEM[5]	P value
Degradation characteristics				
a%[1]	15.21	6.64	0.1406	0.011
B%[2]	62.30	44.26	0.4114	0.021
c[3]	0.037	0.019	0.0006	0.033
a+b(**%**)[4]	77.50	50.81	0.4662	0.016
Effective degradability (%)				
k=0.02	55.74	28.75	0.3993	0.006
k=0.05	41.80	19.28	0.3279	0.006
k=0.08	35.01	15.49	0.2657	0.006
Disappearance (%)				
24hrs	55.70	25.66	0.6279	0.014
48hrs	67.45	33.87	0.7909	0.010
72hrs	72.70	40.31	0.4349	0.012

[1,2,3,4] constants in the equation $P = a + b\ (1\text{-}e^{-ct})$, where P is level of degradation at time "t", "a", rapidly soluble fraction; "b", insoluble fraction but degradable in rumen; "c", rate of degradation of "b" per hour; "a + b", potentially degradable fraction.
K: Rate constant of passage (h^{-1})
[5] Standard error of the mean.

Table (6). Degradation characteristics and disappearance of acid detergent fiber (ADF) for olive cake (OC) and green house wastes (GHW)

	GHW	OC	SEM[5]	P value
Degradation characteristics				
a%[1]	10.64	6.00	0.1704	0.019
b%[2]	49.40	46.34	0.4453	0.013
c[3]	0.030	0.032	0.0009	0.057
a+b%[4]	60.01	52.36	0.6062	0.007
Effective degradability (%)				
k=0.02	40.63	34.55	0.3251	0.037
k=0.05	29.59	24.08	0.2010	0.033
k=0.08	24.41	19.24	0.1749	0.030
Disappearance (%)				
24hrs	40.34	32.80	0.5554	0.048
48hrs	47.84	42.44	0.3640	0.047
72hrs	54.96	47.39	0.4097	0.037

[1,2,3,4] constants in the equation $P=a+ b (1-e^{-ct})$, where P is level of degradation at time "t", "a", rapidly soluble fraction; "b", insoluble fraction but degradable in rumen; "c", rate of degradation of "b" per hour; "a + b", potentially degradable fraction.
K: Rate constant of passage (h^{-1}).
[5] Standard error of the mean.

Table (7). Degradation characteristics and disappearance of neutral detergent fiber (NDF) for olive cake (OC) and green house wastes (GHW)

	GHW	OC	SEM[5]	P value
Degradation characteristics				
a%[1]	14.06	5.62	0.2083	0.022
b%[2]	55.09	48.53	0.6295	0.049
c[3]	0.020	0.029	0.0012	0.108
a+b**%**[4]	69.15	54.35	0.5003	0.027
Effective degradability (%)				
k=0.02	41.84	34.53	0.2983	0.037
k=0.05	30.00	23.88	0.4854	0.049
k=0.08	25.24	18.64	0.2301	0.032
Disappearance (%)				
24hrs	40.70	32.90	0.4178	0.048
48hrs	45.26	41.98	0.7413	0.017
72hrs	57.95	48.19	0.5661	0.030

[1,2,3,4] constants in the equation $P = a + b (1-e^{-ct})$, where P is level of degradation at time “t”, “a”, readily soluble fraction; “b”, insoluble fraction but degradable in rumen; “c”, rate of degradation of “b” per hour; “a + b”, potentially degradable fraction.
K: Rate constant of passage (h^{-1}).
[5] Standard error of the means.

4.2. Discussion:

A number of authors suggested that degradation characteristics of feeds in the rumen provide a useful basis for the evaluation of their nutritive value (Ørskov et al., 1988; Shem et al., 1995). The NDF degradability of roughages is an essential parameter in predicting their energetic value. The cell wall components in these feedstuffs are the main nutritive constituents and the extent of rumen degradation is the main factor that influences their energetic value (NRC, 2001). Moreover, NDF degradability has been used in models to estimate the physical fill of fibrous feeds in the rumen (Madsens and Hvelplund, 1994) and, therefore, the intake capacity of ruminants. As suggested by previous research, it is likely that digestibility of forage fiber measured *in vitro* or *in situ* is a better indicator of the potential of forages to enhance dry matter (DM) intake than NDF digestibility measured *in vivo* (Varga, 2006).

Many experiments have shown that nonstructural carbohydrate sources have a positive effect on fiber digestion as fiber concentration in the ration is increased using these carbohydrate sources (Varga, 2006). The NDF digestibility is a function of the potentially digestible fraction and its rate of digestion and rate of passage. Digestibility of NDF measured *in vivo* is confounded by different retention times in the rumen, which can be affected by differences in DM intake (Oba and Allen 1999). In addition, exposure to acidic conditions in the small intestine and fermentation in the large intestine *in vivo* might reduce differences observed for fermentation by rumen microorganisms *in situ*. For this reason, NDF digestibility determined *in situ* is an important measure of forage quality and should be distinguished from NDF digestibility *in vivo* (Varga, 2006).

In this study, GHW had relatively high CP and medium amounts of cell wall. However, OC had low CP and high cell wall fractions. These by-products are non-forage fiber sources similar to beet pulp. GHW was high in '*a'* value of DM, which can increase voluntary intake. It could be an excellent energy source for ruminants; however, the expected high lignin content limits its value as a feed source for ruminants.

OC had low CP and high ADF and NDF values compared to GHW. These values are comparable to a previous report (Abo Omar and Gavoret., 1995; Abo Omar *et al*., 2012). Factors such as plant maturity, cultivation management and type of soil and fertility could affect chemical composition of feed ingredients. Alibes and Berge (1983) and Ohlde and Becker (1982) found that olive cake has high NDF, ADF and lignin contents. However, a large proportion of the protein (80 to 90%) is linked to the ADF (Nefzaoui, 1983) and only 1.5 to 3% of total nitrogen has particularly low solubility (René Sansoucy *et al*., 1985). The nitrogen solubility adds to the low quality of the olive cake which is similar to that low quality roughage.

The GHW had higher degradation rates compared to OC. The nutrient composition of GHW of high CP, NDF and lower ADF levels may explain the higher degradation rates observed. It was shown by previous research that degradation rates were different among types of roughage (Lucci *et al*., 1989). The degradation rates of legumes at 3, 12, 24, 48, 72 and 96 h were higher than those of Rhodes grass hay (Lucci *et al*., 1989). The disappearance of DM and CP for clover in rumen was higher than these of ryegrass (Van Straalen et *al*., 1993). Hoffman et al (1993) reported that degradation rate value of alfalfa was higher than that of brome hay. It was reported that alfalfa hay had greater ‘c’ value than other grasses (Von Keyserlingk *et al*., 1996).

Effective degradability (ED) of DM and CP calculated at 2, 5 and 8% h^{-1} outflow rates from the rumen showed that GHW consistently had significantly higher values than OC. Effective DM and CP degradability decreased with increase in outflow rates. The disappearance of the DM and CP contents in OC and GHW by the end of 48 hrs of incubation, generally considered to be equivalent to *in vivo* digestibility (Ehargava and Orskov, 1987) and being a retention time of fibrous feeds in ruminants (Kimambo and Muya, 1991).

GHW had more than 50% DM loss compared to 46% loss in OC. However, beyond 72 hrs of incubation, both GHW and OC had disappearance values above 50%. The difference between values for DM contents and the disappearance values after 72 hrs of incubation showed a value of range of 22.82 to 39.79% in GHW and OC, respectively. This provides an insight into the level of rumen undegradable DM incubation beyond 72 h. The ruminal degradability coefficients of different types of olive cake were not tested before.

Effective degradability (ED) of CP, ADF and NDF calculated at different flow rates from the rumen (i.e. 2%, 5%, 8%) showed that GHW consistently had higher values compared to that in OC. Effective degradability decreased with increase in outflow rates for all tested nutrients .

The fractions of a, b, degradable and un-degradable fraction, fractional rate of disappearance, and effective degradability for DM, NDF and CP were significantly different for OC. Reducing the fiber content in raw ingredients significantly increased the a, b, degradable fractions, and effective degradability, but decreased un-degradable fraction for DM, crude protein and NDF.

Martin Garcia *et al*. (2003) found that the olive cake was rich in NDF (62.4% of DM) and poor in CP (7.9%) and that a great part of the N is attached to the ADF fraction. According to Nefzaoui (1983), since olive cake is rich in ADF, it has a low degradability in the rumen that the maximum value obtained for exhausted screened olive cake was 32% of DM after 72 h ruminal incubation. In addition, protein degradability was also very low in olive by-products, which may be because 75 to 90% of protein is linked to the ADF fraction Nefzaoui (1978). The DM and nutrients digestibility of different types of olive cake that were measured *in vivo* had a high variation. Using sheep, digestibility of OM, CP, EE and CF were 32.9 (Boza and Varela, 1960), 30.8 to 45.7, 10 to 24.5, 65.5 to 89.2 and 28.4 to 29.6% in crude olive cake, respectively (Theriez and Boule, 1970). Also in sheep, digestibility of OM and CP were 69.4 and 28.0% in exhausted olive cake, respectively (Theriez and Boule, 1970). In addition, Olive cake is particularly rich in lignin which protects carbohydrates that linked to ADF and lignin. Theriez and Boule (1970) explained that many factors such as high ADF, poor digestive degradation and utilization may result in decreasing ruminal microbial activity that may decrease by 40% after ingestion of crude olive cake.

The ammonia production of sheep rumen liquor receiving olive cake also confirmed decreased activity of the rumen micro-organisms (Nefzaoui *et al*., 1982). The influence of different olive cakes on ruminal microbial activity may result in quantity, nature and condition of (conversation). High concentrations of free fatty acids in the rumen can alter digestion and appetite. In addition, olive by-products have some phenolic compounds that would inhibit fermentation, or tannin, which would insolubilize the proteins in the diet or in the olive by-products (Theriez and Boule, 1970). However, exhaustion eliminates large quantities of polyphenols and tannins from by-products. Therefore, higher degradable fraction of DM, crude protein and NDF in partly destined exhausted olive cake, may result in the removal of polyphenols and tannins from by-products. Unfortunately, in the current experiment, we did not measure the phenolic components and tannins in by-products. However, analyses of olive cake by Nefzaoui (1978) showed that tannin rates below 1% were not sufficient to act as a depressant on rumen microflora, and digestibility of protein and levels of polyphenols between 0.15 and 0.75% of DM are not sufficient to inhibit fermentation.

In addition, *in situ* ruminal DM and CP degradability of olive leaves was low that could be due to high fat content and incomplete removal of ruminal microbes from un-degraded residues in the nylon bags by washing and stomaching procedures (Hvelplund and Weisbjerg, 2000). Martin Garcia *et al*. (2003) reported that *in vitro* digestibility either of olive cake and leaves showed low values, especially for CP (9.62 and 1.24% of DM, respectively). Potential degradability was lower in olive cake than in olive leaves (44.95 vs. 74.5% for DM and 55.45 vs. 70.15% of crude protein respectively). Effective degradability was also lower in olive cake than in olive leaves (41.05 vs. 53.55% of DM and 43.4 vs. 45.85% of crude protein respectively).

Conclusions

In this study, we attempted to develop a comparative data set of in situ DM and CP degradation parameters for some agricultural by-products which are used in narrow scale but have the potential to be used for sheep nutrition in Palestine. Extensive differences in ruminal degradation kinetics of DM, CP, ADF and NDF were determined between OC and GHW. The new data presented in this study could be useful for the purposes of ration formulation and sheep performance. Considering these findings, one can propose using both by-products, together or separately as part of ruminant rations. According to its degradability can be introduced in theses rations as part of roughage (wheat and barley straw). However, the GHW can be incorporated in ruminant rations as part of good quality roughage (legume hay).

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Appendices

Appendix 1

Determination of Moisture (A.O.A.C., 1995)

Procedure

1. Heat the crucible for four hours in an oven at 105 c, cool and weigh.
2. Weigh by difference 1g into the can.
3. Place it in the oven at 105 c overnight.
4. Remove the can from the oven then transfer to a desiccators.
5. Allow to cool to room temperature then weigh.

% Moisture = (weight of can + sample before drying)-(weight of can + sample after drying) × 100% weight of wet sample.

Appendix 2

Determination of Ash (A.O.A.C., 1995)

Procedure

1. Heat the crucible for one hour in a muffle furnace at 500c, cool and weigh as quickly as possible.

2. Weigh by difference 1g into the crucible.

3. Place it in a cool furnace and slowly bring the temperature up to 600c, leave to overnight.

4. Remove the crucible from furnace then transfer to a desiccators.

5. Allow to cool to room temperature then weigh.

Calculation

% Ash = (Weight of ash) ×100% Weight of sample (dry matter)

Appendix 3

Crude Protein Determination (Kjeldahl Method, O.A.C., 1995)

Reagents:-

1. Sulfuric acid (concentrated 98%)

2. Boric acid 4% solution. (Dissolve 4g boric acid in 100ml volumetric flask and complete to the mark).

3. Sodium hydroxide dissolves 500g. Sodium hydroxide in 100ml volumetric flask cools and make up to 1000ml.

4. Indicator solution screened methyl red indicator solution.

(Dissolve 2g. methyl red in 100ml of 96% v/v ethanol.Dissolve. 1g. methyl red in 100 ml of 96% v/v ethanol).

5. Digestion mixture add to each digestion flask. 19g of CuSo4 0.5g. H2O and 9.7g. K2SO4 and mix.

6. Anti foaming granules.

7. Hydrochloric acid solution.01N.

Procedure:-

1. Weigh about 1.0g sample into 100 ml Kjeldahl flask.
2. Add 20ml of concentrated sulfuric acid, and then add 10g of digestion mixture and few antifoaming granules into the digestion flask.
3. Digestive the mixture until the solution becomes clear.
4. Transfer the digestion tube to connect the distillation unit, add 50 ml of distilled water into the cooled digestion tube.
5. Add 40ml of sodium hydroxide 50% to digestion tube.
6. Place a receiving flask containing 30ml of 4% boric acid with few drops of mixed indicator.
7. Allow distillation to proceed to assure ammonia is free from the sample.
8. Titrate the ammonia collected in the receiving flask with standard 0.1N HCL solution.

Calculation

% Nitrogen = V0.1 HCL×N.HCL×14.007×100×100 100×Weight of dry sample %Crude protein =%Nitrogen×6.25.

Appendix 4

Neutral Detergent Fiber (Robertson and Van Soest, 1981)

1. Neutral detergent solution

-Dissolve 18.61g ethylene diamine tetra acetate dehydrate and 6.81g sodium borate decahydrate in distilled water.

-Dissolve 30g sodium lauryl sulphate and 10ml 2-ethoxy ethanol in distilled water.

-Dissolve 6.81g disodium hydrogen phosphate in some water.

-Put all the above solution in 1 litter volumetric flask and fill to the mark with distilled water.

-Adjust the ph to range 6.9-7.1.

Procedure:

1. Weigh 1.00g sample and put in a beaker.
2. Add in order, 100ml neutral detergent solution, and 2m Decahydronaphthalene and 0.5g sodium sulfite.
3. Heat to boiling and reflux for 60 minutes from the onset of boiling.
4. Filter using glass crucible and rinse with hot distilled water.
5. Wash twice with acetone.
6. Dry the crucible at 105c overnight and weigh.

Calculation

Neutral Detergent Fiber = M1-M0×100/M2

Where, M0=weight of the crucible.

M1=weight of the crucible and sample after drying.

M2=weight of the sample.

Appendix 5

Acid Detergent Fiber (Robertson and Van Soest, 1981)

Dissolve 20g of cetylmethylammonium bromide in 1L Sulfuric acid (1N).

Procedure:-

1. Weigh 1g sample and put into a 600ml beaker.
2. Add 100ml of acid detergent solution using a measuring cylinder.
3. Add 2ml of decahhyronphalene.
4. Heat to boiling and reflux for 60 minutes from the onset of boiling.
5. Filter using glass crucibles and with hot distilled water.
6. Wash the fiber with acetone.
7. Wash the fiber with hexane.
8. Dry at 105c overnight, cool and weigh.
9. Ash at 600c overnight cools and weigh.

Calculation

Acid Detergent Fiber =M0-M1×100/M2

Where, M0=weight of crucible and fiber.

M1= weight of crucible and ash. M2=weight of sample.

جامعة النجاح الوطنية

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2013

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الملخص

استخدم في هذه التجربة اثنين من المخلفات الزراعية وهما جفت الزيتون الخام، ومخلفات الخضار الناتجة من البيوت البلاستيكية، وقد تم استخدم المخلفان لتقييم مستويات التحطم لمكوناتها من المادة الجافو، والبروتين الخام، وألياف المنظف الحامضي، إضافة إلى ألياف المنظف المتعادل. تم وضع عينات ثنائية من المخلفات في أكياس النايلون الخاصة بالتجربة (5 غم) وتم تحضينها في كرش اثنين من كباش العساف التي زودت بفتحة بالكرش من اجل عملية التحضين. وقد كانت مدة التحضين للعينات 4 و8 و16 و24 و48 و72 ساعة.وبينت النتائج ان لنوع الخلفات اثر معنوي على المكونات الذائبة، والقابلة للتحطم وعلى مستويات التحطم لكل من العناصر التي تناولها البحث، وهي المادة الجافة والبروتين الخام والمنظف الحامضي والقاعدي. وبينت الدراسة أيضا أن نسب التحطم في الكرش للمكونات الذائبة في مخلفات البيوت البلاستيكية كانت 16.19، 15.15، 10.64، و14.06% لكل من المادة الجافة، البروتين الخام، المنظف الحامضي والمنظف المتعادل، على الترتيب. بينما كانت النسب للمكونات القابلة للتحطم في الكرش فيما يخص مخلفات البيوت البلاستيكية 55.82، 62.3، 49.4 و55.09 لكل من المادة الجافة، البروتين الخام، المنظف الحامضي والمنظف المتعادل، على الترتيب. بينما كانت هذه لمكونات الجفت 47.6، 44.26، 46.34 و 48.53% للمكونات السابقة، عل الترتيب. وقد تبين من الدراسة ان مستويات التحطم كانت لكل العناصر المتناولة في الدراسة والخاصة بمخلفات البيوت البلاستيكية كانت الأعلى مقارنة بمكونات الجفت، كما ان مستوى فعالية التحطم كان الأعلى أيضا لمكونات مخلفات البيوت البلاستيكية مقارنة بمكونات الجفت.