



#### **SPRINGER NATURE**

## From First Draft to Article Submission and Data Sharing: Tips and Tricks for Successful Scientific Publication

#### Dr. A. Z. M. Salem

Profesor investigador Facultad de Medicina Veterinaria y Zootecnia – UAEM



### **General introduction**

• Why we need to publish our research work in the international journals?

2. General remarks when writing the research paper.

#### **3.** ARTICLE SECTIONS



### **1.** Why the international journals?

To improve the ranking of our institute among the national and international institutions depending on the international institute quality accreditation

To improve our knowledge about the new information available in our professional research work

To improve our scientific activities as well as the name of the institute around the world in our professional field of research

To improve our possibility of getting funds from the international foundations



### 2. General remarks

The manuscript should be prepared as you wish it to appear in the journal. Formulas, tables and figures should be inserted within the text of the document as you would like them to appear

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### **Principal sections of the research paper**

- I. Title and Authors
- 2. Abstract and Key words
- 3. Introduction and Objectives
- 4. Materials and Methods
- 5. **Results** (description of the data obtained)
- 6. Discussion (interpretations of the results obtained)
- 7. Conclusion/s
- 8. Acknowledgment/s





#### 1. Title and authors 2. Abstract and key words

Title
 1.1. Conditions
 1.2. Examples

Authors and address Conditions Examples

2. Abstract2.1. Conditions2.2. Principal parts

Key words Conditions Examples



### **3. Introduction 4. Material and Methods**

**3. Introduction 3.1.** Conditions **3.2.** Writing the text reference **3.3.** Examples

4. Material and Methods
4.1. Conditions
4.2. Principal parts
4.3. Examples



### 5. Results

#### 6. Discussion

**5. Results5.1.** Conditions**5.2.** Examples

**6. Discussion**5.1. Conditions5.2. Examples



### 7. Conclusions 8. Acknowledgment 9. References

7. Conclusions7.1. Conditions7.2. Examples

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Each journal has specific guidelines for writing the all paper sections

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# **1.1-** Manuscript Title



## **1.1.1-** Title Conditions

Your paper should begin with a **Title** that succinctly describes the *contents* of the paper.

Use descriptive words that you would associate strongly with the content of your paper:

- the molecule studied,
- the organism used or studied,
- the treatment, the location of a field site,
- ✓ the response measured, etc.

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## **1.1.1- Title Conditions (specific)**

The manuscript title should use specific, unambiguous descriptive words that will ensure electronic retrieval

The title should be gives and general idea about the research work done

The scientific name should be writ in the correct form (*Acacia saligna* not ACACIA SALIGANA or *Acacia Saligana*) this is very important notice

Use word substitutes for formulas, symbols, superscripts, Greek letters, or other non-alphabetical symbols in the title

If your title contains symbols or non-Roman letters, please suggest appropriate translations using Roman letters and provide them as keywords



## **1.1.2- Title Examples**

### Example 1.

Impact of source of ruminal inoculum and season of harvest on chemical composition and ruminal digestibility of some Mexican tree browse species

### Example 2.

Influence of exogenous enzymes of anaerobic bacterium on extent of ruminal fermentation activities, nutrient digestibility, and milk production and composition in dairy cows



### Example 3.

Nutritive evaluations of some browse tree foliages during the dry season: Secondary compounds, feed intake and *in vivo* digestibility in sheep and goats

# Example 4. Nutritive evaluation of different Gliricidia (*Gliricidia sepium*) ecotypes as a forage sources in sheep



# **1.2-AUTHORS and ADDRESS**



#### **1.1.2-AUTHORS and ADDRESS Conditions**

Should be write according to the guidelines of the scientific journal and should be add star (\*) with the corresponding author or authors of the paper



### **1.1.3- AUTHORS and ADDRESS examples**

## Example 1.

A.Z.M. Salem<sup>a,\*,</sup> M.Z.M. Salem<sup>b</sup>, M.M. El-Adawy<sup>a</sup>, P.H. Robinson<sup>c</sup>

<sup>a</sup> Department of Animal Production, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt
<sup>b</sup> Department of Timber Trees and Wood Technology, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt
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### **1.1.3- AUTHORS and ADDRESS examples**

## Example 1.

H.M.Gado<sup>1</sup>, M.Hassan<sup>2</sup>, A.Z.M.Salem<sup>\*3</sup>, P.H. Robinson<sup>4</sup>

 <sup>1</sup>Department of Animal Productions, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
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 *E-mail address:* asalem70@yahoo.com (A.Z.M. Salem).



## **2. ABSTRACT**



#### Manuscript ABSTRACT

## **2.1-ABSTRACT Conditions**

Should not have the manuscript details

Should have the idea about the objectives, materials and methods used a short description of the very important results obtained

Should have a general conclusion from the results experiment done

It could have from 200 to 500 words.

You need also to write an abstract at the time to submit it to the symposia or international meeting



## **2.1-ABSTRACT Conditions**

An abstract summarizes, in one paragraph (usually), the major aspects of the entire paper in the following prescribed sequence:

The *question(s) you investigated* (or purpose), (from <u>Introduction</u>) state the purpose very clearly in the first or second sentence.



- The *experimental design and methods* used, (from <u>Methods</u>) clearly express the basic design of the study.
- ✓ Name or briefly describe the basic methodology used without going into excessive detail-be sure to indicate the key techniques used.
- 3. The *major findings* including *key quantitative results*, or *trends* (from <u>Results</u>)
  - report those results which answer the questions you were askingidentify trends, relative change or differences, etc.



A brief summary of your *interpetations* and *conclusions*. (from **Discussion**)

clearly state the implications of the answers your results gave you.



**Manuscript ABSTRACT** 

## 2.2 - Principal parts ABSTRACT





#### 2.3- <u>ABSTRACT</u> examples

Example 1. Salem et al., (2006) Animal Feed Science and Technology 127 (2006) 251–267

Four browse tree foliages (Cassia fistula, Schinus molle, Chorisia speciosa and *Eucalyptus camaldulensis*), native to the semi-arid region of north Egypt, were harvested during the dry season and evaluated for nutritional quality by determination of levels of nutrient and secondary compounds, as well as feed intake and apparent digestibility in sheep and goats (**OBJECTIVE/S**). The study consisted of four experiments conducted in sequential 28-day periods that were the same in all respects, except that a different foliage was evaluated in each experiment which used six adult male Rhmani sheep  $(35\pm2.3 \text{ kg body})$ weight (BW) at the start of the study) and six crossbred goats  $(30\pm1.56 \text{ kg BW})$ . Sheep and goats were randomly divided into two groups of three and offered foliage at a level equal to 1.3 of the previous days voluntary intake of fresh matter and a commercial concentrate, with or without 10 g/animal/d of PEG, at 10 g/kg of BW to meet 0.7 of maintenance metabolizable energy requirements (MATERIALS AND METHODS).

#### Example 1. continue

Foliage crude protein (CP) content ranged from 124 (S. molle) and 128 (C. speciosa) to 185 g/kg DM (*C.fistula*). Ether extract was highest (97 g/kg) in *S. molle. C. fistula* had the lowest neutral detergent fiber (NDFom), acid detergent fiber (ADFom) and acid detergent lignin (lignin(sa)), while E. camaldulensis had the highest values. Total phenolics (TP), condensed tannins (CT), saponins (SAP), alkaloids (ALKA), the aqueous fraction (AF) of lectins, polypeptides and starch, and essential oils (EO) were lowest in C. speciosa (29, 21, 3, 0, 4 g/kg DM and 0.40 ml/kg DM, respectively) and highest in E. camaldulensis (102, 68, 15, 5, 3 g/kg DM and 15 ml/kg DM, respectively). Levels of TP, CT, SAP, ALKA and EO were highly positively intercorrelated among foliages, although AF was weakly negatively correlated to all others. Goats consumed 3.9% more foliage dry matter (DM) than sheep per kg BW0.75, and their digestibility was about 8% higher, probably reflecting their better capacity to detoxify secondary compounds in the rumen than sheep. Levels of CT (and due to its correlations, also TP, SAP, ALKA and EO) was a strong predictor of DM intake of PEG unsupplemented foliages within both sheep and goats. PEG increased (P<0.05) intake of DM and its components in sheep and goats. Digestion of DM and NDFom were not affected by feeding PEG, although digestion of OM, EE and CP were higher (P<0.05). TP in tree foliages (and due to its correlations, also CT, SAP, ALKA and EO) was not a predictor of the proportional increase in DM with PEG feeding, which was best predicted by level of CP within foliage (**RESULTS**). Overall, *C. speciosa*, had the highest nutrient value for both sheep and goats, both without and with PEG feeding, S. molle and C. fistula were intermediate and E. camaldulensis had the lowest nutritive value (GENERAL **CONCLUSION**).

Example 2. G. HERVA S et al., 2000; Journal of Agricultural Science, Cambridge (2000), 135, 305±310.

The current experiment was conducted to study the effect of different doses of tannic acid, a hydrolysable tannin, on ruminal degradation and post-ruminal digestion of treated soya bean meals (SBM) in sheep (**OBJECTIVE/S**). Samples of SBM were prepared by spraying 100 g SBM with 100 ml distilled water containing 0, 1, 5, 10, 15 or 25 g of commercial tannic acid (S!, STA", STA#, STA\$, STA% and STA&, respectively). Three ruminally cannulated ewes, that had never consumed tannic acid previously, were used to determine *in situ* degradability of tannic acid-treated SBM. Intestinal digestibility of protein remaining after 16 h rumen incubation was estimated *in vitro* (MATERIALS AND METHODS).



#### Example 2. continue

Extent of rumen degradation of SBMs was significantly ( $P<0\pm05$ ) affected by the tannic acid treatment. All doses of tannic acid used in this experiment, even the lowest one (STA"), significantly decreased the extent of N degradation but only doses higher than that used to treat STA\$ reduced the extent of DM degradation. This reduction in the extent of DM and N degradation was mainly due to a marked decrease in the immediately degradable fraction (a), which was observed in all treated SBM, and to a lower rate of degradation (c), observed in meals STA\$, STA% and STA&. Intestinal digestion of the non-degraded protein was decreased ( $P<0\pm05$ ) by treatment with the two highest doses of tannic acid (those used to treat meals STA% and STA (**RESULTS**). It was therefore concluded that tannic acid can exert a negative effect both on rumen degradation and on intestinal digestion of SBM, this effect being clearly dependent on the dose used to treat the SBM (**GENERAL CONCLUSION/S**).


# **Example 3.** Effect of ZADO®, as enzymes from anaerobic bacterium, on extent of ruminal fermentation kinetics, microbial protein synthesis and milk production in dairy

**COWS.** H. M.Gado<sup>\*1</sup>, M. Hassan<sup>2</sup>, and A. Z. M. Salem<sup>3</sup>, <sup>1</sup>*Ain Shams University, Cairo, Egypt,* <sup>2</sup>*Cairo University, Cairo, Egypt,* <sup>3</sup>*Alexandria University, Alexandria, Egypt.* 

J. Anim. Sci. Vol. 86, E-Suppl. 2/J. Dairy Sci. Vol. 91, E-Suppl. 1 ADSA PSA AMPA ASAS Joint Annual Meeting, Indianapolis, Indiana, July 7-11, 2008, page 590-591, USA, Oral.

A 2 × 2 factorial experiment was conducted to evaluate the effect of ZADO®, as enzymes preparation containing cellulases, xylanases,  $\alpha$ -amylase and proteases from an anaerobic bacterium, on milk production and composition, ruimianl fermentation activities and nutrients digestibility in dairy cows.(**Objectives**) Twenty multiparous lactating Brown Swiss cows (550 kg BW) were randomly assigned in two groups of 10 animals fed a mixed ration (CP 15%, TDN 74%) with or without addition of 40 g/head/d ZADO®. Milk production was recorded daily during 12 weeks of the experiment (**Materials and Methods**).



Total and individual VFA (acetate, propionate, and butyrate), NH3-N concentrations, and microbial protein synthesis were significantly (P < 0.05) increased for cows fed ZADO® diet. Digestibility coefficients of DM, OM, NDF and ADF were significantly (P < 0.05) improved by addition ZADO® in cow diet. Consequently, total milk yield, 3.5 and 4% fat corrected milk and energy corrected milk improved (P<0.05) by 12, 21, 14, and 20% respectively, and there was no affect on milk composition (Results). In conclusion, supplementing dairy cow diets with ZADO® has the potential to enhance milk yield as consequence for improving the nutrients digestibility, ruminal fermentation activities and microbial protein synthesis. ZADO® confirm its roles in improving the fiber digestibility and suggested positive effects on ruminal fibrolytic microorganisms and increased milk production in dairy cows (conclusion).

Key Words: ZADO®, Microbial Protein Synthesis, Milk Yield



#### **Manuscript ABSTRACT**

Example 4. Feed intake, nutrient digestibility and animal growth performance in sheep and goats fed wheat straw *ad lib.* in presence of ZADO as direct feed of anaerobic enzymes and bacteria. A. Salem<sup>\*1</sup>, M. El-Adawy<sup>1</sup>, H. Gado<sup>2</sup>, and M. Khalil<sup>3</sup>, <sup>1</sup>Department of Animal Production, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt, <sup>2</sup>Department of Animal Production, Faculty of Agriculture, Kin Shams University, Cairo, Egypt, <sup>3</sup>Animal Production Research Institute, Ministry of Agriculture, Dokki, Gizza, Egypt. J. Anim. Sci. Vol. 85, Suppl. 1/J. Dairy Sci. Vol. 90, Suppl. 1/Poult. Sci. Vol. 86, Suppl. 12007

ADSA PSA AMPA ASAS Joint Annual Meeting, San Antonio, July 8-12, 2007, pp. 107, USA, Poster

Six crossbred sheep (32 kg BW) and six Baladi goats (18 kg BW) were used to evaluate the effect of ZADO (new probiotic, patent No. 22155) as feed direct microbials on feed intake, apparent digestibility and animal growth performance (**Objectives**).. Sheep and goats were randomly divided into two groups of three animals and fed wheat straw *ad lib*. as a basal diet and commercial concentrate with or without 10g/animal/d of ZADO. A growth performance trial of 65-days was ended by a digestibility trial of 21-days for each individual animal within each group.(**Materials and Methods**).

Feed intake was not affected (P>0.05) by ZADO addition neither in sheep nor goats but it improved the nutrients digestibility coef cients as well as total digestible nutrient of feed in sheep and goats. ZADO signi cantly increased (P<0.001) the neutral detergent ber digestibility of diet. The improvement (P<0.001) was more in goats than sheep. Average daily gain and feed efficiency were improved (P<0.05) by addition of ZADO, and the improvement was more in goats than sheep. Calculated net energy required for one kg gain was decreased (P < 0.05) by inclusion of ZADO in diets and the decease was more in goats than sheep. Improving the animal performance by addition of ZADO was as a consequence to the improvement in digestibility in sheep and goats (**Results**). In conclusion, ZADO had improved the nutritive value of wheat straw, as a basal diet in sheep and goats and suggested that its useful roles in activating the ruminal fiber degrading enzymes (Conclusions).

Key Words: Feed Intake, Growth Performance, Sheep.



Example 4. Effect of Rumensin® and Tylan® in feedlot diets containing wet distillers grains plus solubles fed to beef steers. N. F. Meyer\*1, G. E. Erickson1, T. K. Klopfenstein1, J. R. Benton1, M. K. Luebbe1, and S. B. Laudert1, 1*University of Nebraska, Lincoln, 2Elanco Animal Health, Greenfield, IN.* J. Anim. Sci. Vol. 86, E-Suppl. 2/J. Dairy Sci. Vol. 91, E-Suppl. 1 ADSA PSA AMPA ASAS Joint Annual Meeting, Indianapolis, Indiana, July 7-11, 2008, page 587, USA, Oral.

The objective of this study was to evaluate the effects of Rumensin and Tylan in feedlot diets containing wet distillers grains plus solubles (**Objective**). Eight hundred beef steers ( $329 \pm 25$  kg) were blocked by initial BW and randomly assigned to one of five treatments (20 steers per pen, 8 pens per treatment). Treatments consisted of a corn-based diet with Rumensin and Tylan (**CORN+RT**) and four treatments with 25% wet distillers grains plus solubles (**DG**) and either 36.7 mg/kg (**R**) or 49.0 mg/kg (**HIR**) of Rumensin and Tylan (**T**) at 90 mg•hd–1•d–1.(**Materials and Methods**).



Compared to CORN+RT, steers fed DG+RT gained more, were more efficient (P<0.05), and had similar DMI (10.7 vs. 10.6 kg). Feeding Rumensin increased G:F by 3.1% and Rumensin plus Tylan increased G:F by 4.9% when compared to DG alone (P < 0.05). With the exception of dressing percentage, there were no differences in performance or carcass characteristics when Rumensin was fed at 36.7 compared to 49.0 mg/kg. Total liver abscesses were significantly greater for DG (42.4%) and DG+R (40.8%), compared to treatments containing Tylan, CORN+RT (17.0%), DG+RT (8.3%), and DG+HIRT (8.9%). Severe liver abscesses were also less for diets containing Tylan ( $P \le 0.05$ ) (**Results**). This study indicates that steers fed Rumensin and Tylan in diets containing wet distillers grains plus solubles results in improved feed efficiency and decreased liver abscesses compared to similar diets without these feed additives(Conclusion).

Key Words: Cattle, Feed Additives, Wet Distillers Grains Plus Solubles.



# **3- KEY WORDS**



# **3.1- KEY WORDS Conditions**

Keywords may be added to enhance the title. Space is provided on the agreement form for you to suggest keywords. Also the key word will help the researchers to reach to your article by searching in internet

We have to select the words which repeated many times in the paper as a key words



#### **Manuscript ABSTRACT**

## **3.2- KEY WORDS examples**

## Example 1

Browse; Cows; Digestibility; Goats; Dry season; Rainy season

#### Example 2

Browse; Cow; gas production; Metabolizable energy; Goats; Dry season; Rainy season

#### Example 3

Gas production, Dry matter degradability, ZADO®, Ceacal activity, Probiotics, Rabbits



# **INTRODUCTION**



## **1.1- INTRODUCTION Conditions**

In this section you will descript the **major problems** which you would to resolve it by your research work done in this paper and some **details** about thesis problems.

You will use the **previous studies** published in that area of your paper.

At the final part of the introduction you need to add your **aim/s** from your study in this paper.



# **1.1- INTRODUCTION Conditions**

The Introduction must answer the following questions:

- **1.** What was I studying?
- **2.** Why was it an important question?
- **3.** What did we know about it before I did this study?

4. How will this study advance our knowledge?"



# **1.1- INTRODUCTION organization**

Establish the context of the work being reported. This is accomplished by discussing the relevant primary research <u>literature</u> (with <u>citations</u>) and summarizing our current understanding of the problem you are investigating;

<u>State the purpose</u> of the work in the form of the hypothesis, question, or problem you investigated; and,

Briefly explain your <u>rationale</u> and approach and, whenever possible, the possible outcomes your study can reveal.



# **1.1- INTRODUCTION** (during writing)

The information should flow in your Introduction

- **1.** Begin your Introduction by clearly identifying the subject area of interest
- 2. Establish the *context* by providing a brief and balanced review of the pertinent published literature that is available on the subject
- **3.** What literature should you look for in your review of what we know about the problem?
- **4.** Be sure to clearly state the purpose and /or hypothesis that you investigated
- **5.** Provide a clear statement of the rationale for your approach to the problem studied



**Manuscript INTRODUCTION** 

# **1.2 - Principal parts INTRODUCTION**



# **2. PROBLEM/S DETAILS >>>>**

# **3.** AIM/S of resolving the problem/s



## **1.3-** Writing the text reference "INTRODUCTION"



## 2.3- **INTRODUCTION** examples

Example 1. Salem et al., (2006) Animal Feed Science and Technology 127 (2006) 251–267.

Salem et al., (2006) Animal Feed Science and Technology 127 (2006) 251–267

A major cause of low productivity of livestock in tropical regions, such as Egypt, is inadequate amounts, and poor nutritional quality, of many locally available feeds (MAJOR PROBLEMS). Browse fodder is a potentially inexpensive locally produced protein supplement for ruminants, particularly during the critical periods of the year when the quantity and quality of herbage is limited. However, most tropical browse species contain substantial amounts of phenolic compounds, mainly tannins (Makkar and Becker, 1998; Salem, 2005) as well as other secondary compounds (Salem et al., 2004b). This can reduce their nutritional value, as most tannins bind to feed proteins thereby making them unavailable to ruminal microorganisms. Thus, the use of high tannin browse species as supplements to crop residue-based diets may not increase the productivity of animals, as ruminally available N frequently limits ruminal microbial growth and subsequent degradation of structural carbohydrates. However, several fodder shrubs and trees have been shown to be able to partially or totally replace concentrate feeds without decreasing digestion or growth of sheep and goats.

For example, Ondiek et al. (2000) concluded that *Leucaena leucocephala* and *Gliricidia* sepium foliage could contribute N in diet supplements without detrimental effects on production of dairy goats. Liu et al. (2001) showed that mulberry (Morus alba) leaves could be used as a protein supplement in an ammoniated rice straw diet to fully substitute for rapeseed meal. Goats are effective browsers, have the ability to utilize woody species and low-quality forages better than cattle and sheep, and can adapt to harsh environments (Tisserand et al., 1991; Silanikove, 2000a, 2000b; Salem et al., 2004a). Extensive shrublands of evergreens and small trees, known as garrigue or maquis, that are often high in tannins and other secondary compounds are the basic component of diets of goats in the Mediterranean area. Attempts have been made to deactivate tannins, and other secondary compounds, in temperate and tropical forages. These attempts include use of polyethylene glycol (PEG), a synthetic polymer for which tannins have a greater binding affinity than proteins (Makkar, 2003a). Therefore, PEG releases forage proteins from tannin–protein complexes and improves their nutritional value. Degen et al. (1998, 2000) used Acacia saligna, a tannin rich leguminous shrub species, and suggested that effects of PEG may persist for up to 14 days in sheep and goats after PEG feeding is terminated (PROBLEMS DETAILS).



This study was designed to determine the nutritive value of four browse tree species in terms of nutrient and secondary compounds, and to assess the capability of PEG added to the diet to mitigate adverse effects of secondary compounds on feed intake and nutrient digestibility in sheep and goats (AIMS).



## **Example 2:**

G. HERVA S et al., 2000; *Journal of Agricultural Science, Cambridge* (2000), **135**, 305±310.

Effects of tannins recorded in ruminants include formation of tannin-protein complexes which are stable over a wide range of pH, but dissociate at pH values of less than  $3\pm 5$  or more than  $8\pm 5$  (McLeod 1974) (MAJOR PROBLEMS). Therefore, in the presence of tannins in the rumen, plant proteins may be bound and protected from microbial degradation, but are released in the bomasums, enabling protein digestion and absorption of amino acids in the small intestine (Barry & Manley 1984; Barry & McNabb 1999). On the other hand, antinutritional effects of tannins have also been extensively reported (Griffiths 1979; Horigome et al. 1988; Silanikove et al. 1994; Salawu et al. 1999). However, none of the above observations can be stated as being common for all tannins. Conventional classification of tannins recognizes two major groups: hydrolysable tannins, which consist of a carbohydrate core with phenolic carboxylic acids bound by ester linkages, and condensed tannins, which consist of oligomers of favan-3-ols and related favanol residues which typically produce anthocyanidins on acid degradation (Mueller-Harvey & McAllan 1992).

In addition, each of these two conventional groups consists of a complex array of tannins whose biological activity may differ considerably depending on their chemical structure and molecular weight (Clausen et al. 1990; Hagerman et al. 1992). Condensed tannins are widely accepted to affect digestibility (Salawu et al. 1997; Barry & McNabb 1999). Some authors have reported similar effects with tannic acid (Driedger & Hateld 1972; Pace et al. 1993) although hydrolysable tannins have often been shown to interact weakly with proteins and even to have no effect on digestibility because they are comprised largely of low molecular weight fractions that may be metabolized (Hagerman et al. 1992; Van Soest 1994) (**PROBLEMS DETAILS**). With the aim of contributing to clarifying this controversy, the present experiment was conducted to study the effect of different doses of tannic acid on ruminal degradation and post-ruminal digestion of treated soya bean meals (AIMS).

# **MATERIALS and METHODS**



#### **2.1- MATERIAL AND METHODS Conditions**

All the M&M used in this research work **should be descript** in details in this section of the paper.

The details of the M&M section should be allow to any one to repeat this experiment at his location.



**Dividing** the M&M section to a sub-sections will be more useful for the researchers when red it.

**References** of the reported assays or methodology which used in this experiment/s should be added clearly.



#### **2.1- MATERIAL AND METHODS Conditions (specific)**

In this section you explain *clearly* how you carried out your study in the following *general* structure and organization (details follow below):

the <u>the organism(s) studied</u> (plant, animal, human, etc.) and their pre-experiment handling and care, and when and where the study was carried out (*only* if location and time are important factors).

<u>if a field study</u>, a <u>description of the study site</u>, including the significant physical and biological features, and precise location (latitude and longitude, map, etc);



#### **2.1- MATERIAL and METHODS Conditions (specific)**

the <u>experimental or sampling design</u> (i.e., how the experiment or study was structured. For example, controls, treatments, the variable(s) measured, how many samples were collected, replication, etc.);

the **protocol for collecting data**, i.e., how the experimental procedures were carried out,

how the data were analyzed (qualitative analyses and/or statistical procedures used).



**Manuscript MATERIAL and METHODS** 

## 2.2 - Principal parts MATERIAL and METHODS





## 2.3- MATERIAL AND METHODS examples

Example 1. Salem et al., (2006) Animal Feed Science and Technology 127 (2006) 251–267

The study was completed at the experimental station of the Faculty of Agriculture of Alexandria University in northern Egypt during May–August 2004 (EXPERIMENTAL LOCATION and PERIOD).

#### 2.1. Tree foliage species

Consumable parts (*i.e.*, leaves and twigs of about 1 year of age) of each foliage species used (*i.e.*, *Cassia fistula*; *Schinus molle*; *Chorisia speciosa*; *Eucalyptus camaldulensis*) were randomly harvested, by hand plucking from 8 to 10 trees of each species, every second day.



#### 2.2. Animals, management and feeding

This study consisted of four experiments completed in sequential 28 day periods that were the same in all respects, except that a different tree foliage was evaluated in each experiment which used six adult male Rahmani sheeps and six crossbred goats weighing 35±2.3 and 30±1.56 kg body weight (BW), respectively, at the start of the study. Sheep and goats were randomly divided into two groups of three to create the two experimental groups. All were offered foliage at a level equal to 1.3 of the previous days voluntary intake of fresh matter, and a commercial concentrate (with or without 10 g of PEG/animal/d; MW4000, Analytical grade, Sigma®-Aldrich, El-Safua Co., Alexandria, Egypt) at 10 g/kg of BW to meet 0.7 of their calculated maintenance metabolizable energy (ME) requirements (NRC, 1985).

The concentrate used was formulated to contain undecorticated cotton seed meal (300 g/kg), ground yellowcorn (355 g/kg), wheat bran (300 g/kg), limestone (30 g/kg), salt (10 g/kg) and 5 g/kg of a trace mineral/vitamin premix (all values/kg of DM: Vitamin A, 2,000,000 IU; Vitamin D3, 150,000 IU; Vitamin K, 0.33 mg; Vitamin B1, 0.33 g; Vitamin B2, 1.0 g; Vitamin B6, 0.33 g; Vitamin B12, 1.7 mg; pantathenic acid, 3.33 g; biotin, 33.0 mg; Folic acid, 0.83 g; choline chloride, 200 mg; Zn, 11.7 g; Mn, 5.0 g; Fe, 12.5 g; Mg, 66.7 mg; Se, 16.6 mg; Co, 1.33 mg; Cu, 0.5 g; I, 16.6 mg; antioxidant, 10.0 g). The concentrate was fed at 9.00 h and animals were fed the foliage 10.00 h and allowed access to it until 2 h before the next feeding of concentrate, at which time uneaten foliage was removed and weighed. All offered concentrate was consumed by all sheep and goats within 60 min of offer on all occasions, and so orts were assumed to be foliage.

2.3. Metabolism trial (feed intake and apparent digestibility determinations)

During each experiment, after the 15 day adaptation to dietary treatments, a digestion study of 10 days duration, involving quantitative collection of feeds, refusals and faeces was conducted to determine the apparent digestibility of the diets. Animals were acclimatized to the metabolism cages for 3 days after the 15 day adaptation period and prior to the 10 day collection period. Faeces voided during each successive 24 h period were collected and weighed. Representative samples of foliage, concentrate, refusals and faeces were collected daily and dried at 105 °C to determine daily intake of DM for each animal. Other representative samples of each material, by animal for refusals and faeces, were collected daily over the 10 day collection period, bulked, mixed, sub-sampled and ground to pass a 1mm sieve for subsequent laboratory analysis. (EXPERIMENTAL CONDITIONS and PROCEDURES)

#### 2.4. Analytical methods

Ground samples of feeds, refusals and faeces were analyzed for dry matter (DM) by drying samples at 105 °Cfor 24 h in forced air oven. Ash contentwas measured after igniting samples in a muffle furnace at 550 °C for 4 h. The crude protein (CP) was determined by Kjeldahl method (AOAC, 1990; ID 954.01). Ether extract (EE) was determined by Soxhlet method (AOAC, 1990; ID 920.39). Neutral detergent fiber (NDFom), acid detergent fiber (ADFom) and acid detergent lignin (lignin(sa)) were determined by methods of Van Soest et al. (1991). NDFom was assayed without the use of an alpha amylase but with use of sodium sulfite. Both NDFom and ADFom are expressed without residual ash.

Samples of each tree foliage were dried at 40 °C for 72 h and ground to pass a 1mm sieve. All samples were thoroughly mixed and sub-sampled into four representative bulk samples of each foliage for further analysis of secondary compounds. Approximately 200 mg (DM) of ground samples of each foliage were extracted in 10 ml of aqueous acetone (7:3 v/v) in a water bath maintained at 39–40 °C for 90 min (Makkar, 2000). Total extractable phenolics (TP) were assayed by Folin-Ciocalteu-reagent 2N (Sigma®–Aldrich, El-Safua Co., Alexandria, Egypt) based on known concentrations of tannic acid as the calibration curve (Sigma®–Aldrich) according to Makkar and Becker (1993).

Condensed tannins (CT) were determined according to Porter et al. (1986) with the modification of Makkar (2000, 2003b) using butanol/HCl (95:5 v/v) and ferric ammonium sulfate (20 g/l 2M HCl) as reagents, and a solution of purified quebracho tannin (1 mg/ml aqueous acetone, 700 ml/l) as the standard. Absorbance was measured against a blank at 550 nm.

Saponins (SAP) were extracted and isolated according to Ahmad et al. (1990), wherein dried samples are extracted with methanol several times. The combined methanol extract was evaporated and partitioned between ethanol acetate and H2O.

For the alkaloid (ALKA) extract, dried samples were first extracted with ethanol and then dissolved in dilute HCl. This solution was filtered and extracted with petroleum ether to remove fat (Arambewela and Ranatunge, 1991).



The aqueous fraction (AF) of lectins, polypeptides and starch (see review of Cowan, 1999) was determined according to Hussein et al. (1999) using fractionation by column chromatography of extracted samples by saturating the extract with distilled H2O and 500 g/l methanol. For essential oil (EO) analysis, fresh leaves of tree foliage were cut into small pieces (0.2–0.4 cm length) with a small chopper and steam distilled. The distillate was then extracted with petroleum ether, and the resulting extract was dried on anhydrous sodium sulfate. Petroleum ether was removed carefully and EO was obtained as the liquid . (ANALYTICAL METHODS)



#### 2.5. Statistical analysis

Tree foliage nutrient and secondary compound contents were statistically analyzed using the 'PROC GLM' procedure of SAS (1999), with methods of Steel and Torrie (1980), and differences among foliage species were determined using Duncan's multiple-range test (Duncan, 1955). Data on nutrient components of total feed intake, foliage consumed and digestibility were analyzed as  $2 \times 2$  factorial experiments (2 animal species (sheep and goats)  $\times 2$ treatments (with or without PEG)) within each tree foliage for each experiment using 'PROC GLM' (SAS, 1999), with methods of Steel and Torrie (1980), to determine differences due to animal species and PEG. In the case of significant interactions (*i.e.*, P<0.05), Duncan's multiple-range test (Duncan, 1955) was used to separate means within animal species. Correlations between foliage secondary compounds (Table 6) used simple linear regression (SAS, 1999), whereas multiple regressions (Table 7) used the 'PROC STEPWISE' procedure of SAS (1999). (STATISTICAL ANALYSIS)



# **1. RESULTS**



## **1.1- RESULTS Conditions**

- the Results section is to objectively present your key results, without interpretation, in an orderly and logical sequence using both illustrative materials (Tables and Figures) and text.
- Summaries of the statistical analyses may appear either in the text (usually parenthetically) or in the relevant Tables or Figures (in the legend or as footnotes to the Table or Figure).
- The Results section should be <u>organized</u> around a series of <u>Tables and/or Figures</u> sequenced to present your key findings in a logical order.


The text of the Results section follows this sequence and highlights the answers to the questions/hypotheses you investigated.

Important negative results should be reported, too.

Authors usually write the text of the results section based upon this sequence of Tables and Figures.

Use the past tense.

Avoid repetitive paragraph structures

Do not interpret the data here.



Always enter the appropriate <u>units</u> when reporting data or summary statistics, according to the following cases:

for an *individual value* you would write, "the mean length was 10 m", or, "the maximum time was 140 min."

When including a measure of variability, place the unit *after* the error value, e.g., "...was  $10 \pm 2.3$  m".

Likewise place the unit after the last in a *series of numbers* all having the same unit. For example: "lengths of 5, 10, 15, and 20 m", or "no differences were observed after 2, 4, 6, or 8 min. of incubation".



## Some problems should be avoid

- × **Do not** reiterate each value from a Figure or Table only the key result or trends that each conveys.
- Do not present the same data in both a Table and Figure
  this is considered redundant and a waste of space and energy. Decide which format best shows the result and go with it.
- × **Do not** report raw data values when they can be summarized as means, percents, etc.



## **1.1- RESULTS Conditions (General)**

Researchers should prepare the data in tables, figures, or diagrams according to the best method of presenting the data

Description the data obtained in the text should be depending on the statistical analysis (significant or no significant differences).



Researcher should be focus in their description on the very important results obtained.



#### **Manuscript RESULTS**

If we have a data numerically higher, but not have significant differences, we can mention it in the text but with it *P value*.

#### Example:

"Addition of exogenous enzymes tended to (P=0.231) increase VFA concentrations in the rumen of T1 than in T2 group"

Results description could be divided in to sub-sections.





## 2.3- <u>RESULTS</u> Examples

Example 1. Salem et al., (2006) Animal Feed Science and Technology 127 (2006) 251–267

3.1. Chemical composition and secondary compounds of the tree foliages

The crude protein (CP) content of the foliages (Table 1) ranged from 124 (*S. molle*) and 128 (*C. speciosa*) to 185 g/kg DM (*C. fistula*), with *E. camaldulensis* intermediate (154 g/kg). Ether extract was highest (97 g/kg) in *S. molle*, with the others containing less than half that level. *C. fistula* had the lowest NDFom, ADFom and lignin(sa), *E. camaldulensis* had the highest values, and *S. molle* and *C. speciosa* were intermediate.

Total phenolics, condensed tannins, saponins, alkaloids, the aqueous fraction of lectins, polypeptides and starch, and essential oils were lowest in *C. speciosa* (29, 21, 3, 0, 4 g/kg DM and 0.40 ml/kg DM, respectively) and highest in *E. camaldulensis* (102, 68, 15, 5, 3 and 15). *C. fistula* and *S. molle* had intermediate values, although *S. molle* had higher levels of TP and CT. Tannins (*i.e.*, TP and CT) were higher than 50 g/kg of DM in *S. molle* (70 and 50) and *E. camaldulensis* (110 and 70), which is considered to be their upper beneficial level in ruminant nutrition.

3.2. Effects of tree foliage species on intake and digestion

#### 3.2.1. C. fistula

Water consumption was higher (P<0.05) in sheep, although the actual values are not convincing. Sheep also consumed more (P<0.01) total and foliage DM (absolutely and relative to BW) than goats (Table 2), as well as all measured nutrients, although their digestion of nutrients, except NDFom, was lower (P<0.05). Addition of PEG had no impact on water intake, but increased (P<0.05) intake of DM and its components in sheep and goats. Digestion of DM and NDFom were not affected by feeding PEG, although digestion of OM, EE and CP were higher (P<0.05).

#### 3.2.2. S. molle

Water consumption was higher (P<0.05) in sheep, which consumed more (P<0.01) total, but not foliage, DM (absolute and relative to BW) than goats (Table 3), as well as all measured nutrients, although their digestion of nutrients, except NDFom, was lower (P<0.05). Addition of PEG had no impact on water intake, but increased (P<0.05) intake of DM and its components absolutely, although relative to BW the increase in DM intake and digestibility was greater within goats (P=0.04). Digestion of DM and NDFom were not affected by feeding PEG, although digestion of OM, EE and CP were higher (P<0.05) with PEG feeding.

3.2.3. C. speciosa

Water consumption was higher (P=0.02) in sheep, which **consumed more (P<0.01)** total and foliage DM than goats absolutely (but less (P<0.01) foliage than goats relative to BW) (Table 4), as well as all measured nutrients, although their digestion of nutrients, except CP and NDFom, was lower (P<0.05) except OM (P=0.06). Addition of PEG had no impact on water intake, but increased (P<0.05) intake of DM and its components both absolutely and relative to BW, although relative to BW the increase in total DM intake was greater within goats (P=0.01). Digestion of CP and NDFom were not affected by feeding PEG, although digestion of DM, OM and EE were higher (P<0.05) with PEG feeding.



#### 3.2.4. E. camaldulensis

Water consumption was unaffected by animal species, but sheep consumed more (P<0.01) total, but not foliage, DM than goats absolutely (although goats consumed more (P=0.01) foliage DM relative to BW) (Table 5), as well as all measured nutrients, although their digestion of nutrients was lower (P<0.05) except **EE (P=0.06).** Addition of PEG <u>tended (P=0.06) to increase</u> water consumption, although the actual values are not convincing. Addition of PEG only increased (P<0.05) intake of NDFom, although intake of DM and all other measured components **tended** (P<0.10) to be higher. Digestion of EE and NDFom were not affected by PEG, although digestion of DM, OM and CP were higher (P<0.05) except DM (P=0.07) with PEG.



# **2. DISCUSSION** (interpretations the results obtained)



#### **2.1. DISCUSSION Conditions**

the Discussion is to interpret your results in light of <u>what</u> <u>was already known</u> about the subject of the investigation, and to explain our new understanding of the problem after taking your results into consideration.

✓ The Discussion will always connect to the <u>Introduction</u> by way of the question(s) or hypotheses you posed and the literature you cited, but it does not simply repeat or rearrange the Introduction.

Instead, it tells how your study has moved us forward from the place you left us at the end of the Introduction.



#### **2.1. DISCUSSION Conditions**

Organize the Discussion to address each of the experiments or studies for which you presented results; discuss each in the **same sequence** as presented in the Results, providing your interpretation of what they mean in the larger context of the problem.

**Do not waste** entire sentences restating your results; if you need to remind the reader of the result to be discussed, use "**bridge sentences**" that relate the result to the interpretation:

Example:

"The slow response of the lead-exposed animals relative to controls suggests that...[interpretation]".



You will necessarily make <u>reference to the findings of</u> <u>others</u> in order to support your interpretations.

Use <u>subheadings</u>, if need be, to help organize your presentation.

✓ Be wary of mistaking the reiteration of a result for an interpretation, and make sure that <u>no new results</u> are presented here that rightly belong in the results..

You must relate your work to the findings of other studies - including previous studies you may have done and those of other investigators.

✓ In either case you should discuss reasons for similarities and differences between yours and others' findings..



Consider how the results of other studies may be combined with yours to derive a new or perhaps better substantiated understanding of the problem.

Be sure to state the conclusions that can be drawn from your results in light of these considerations.

You may also choose to briefly mention further studies you would do to clarify your working hypotheses.

Make sure to <u>reference any outside sources</u> as shown in the Introduction section..

**Do not introduce new results in the Discussion.** 



#### **2.1. DISCUSSION (Conditions General)**

Researchers should be select the very important results obtained to interpret them in this section using the confirmation of the previous studies at the same line of research.

Discussion section could be divided to sub-sections.



## **General Notes in DISCUSSION writing**

Usually using the <u>possibility</u> words such "may be, might be, could be" and then due to >>> the explanation which you need to add it.

Using of the previous studies to confirm the current results obtained, for example: Our results >>>> a finding consistent with Gilboa et al. (1995) who found that goats .....



## We can add Our explanation >>>>>(References as confirmation)

*Example 1:* Goats, as browsers, may have selected the parts of the foliage with a lower proportion of secondary compounds, *versus* sheep as grazers (Kababya et al., 1998; Salem, 2002; Salem et al., 2003).).

## Example 2:

Feeding PEG has been shown to improve intake of foliage containing secondary compounds in goats (Silanikove et al., 1997; Decandia et al., 2000) and sheep (Silanikove et al., 1994; Salawu et al., 1997).



## 2.2- **DISCUSSION** examples

## Example 1. Salem et al., (2006) Animal Feed Science and Technology 127 (2006) 251–267

#### 4.1. Composition of the tree foliages

High CP, and low NDFom and ADFom levels, suggest browse with potential as N supplements to ruminants fed low quality forages during the dry season in semi-arid regions. Use of multipurpose trees and shrubs has become a useful alternative ruminant feed in harsh semi-arid environments (FAO, 1992; Topps, 1992). Differences in CP contents between these browses are probably due to differences in protein accumulation in them during growth. The reported nutrient levels are comparable to those found by Le Hou'erou (1980), Topps (1992) and Rubanza et al. (2003), although some inconsistencies (e.g., Rubanza et al. reported values ranging from 115 to 205, 52 to 126, 182 to 619, 68 to 196 and 44 to 130 g/kg DM for CP, ash, NDFom, ADFom, and lignin(sa), respectively, in browse legume tree leaves native to Tanzania) are likely <u>due to</u> <u>differences</u> in the stage of growth and type (*i.e.*, twigs, leaves or soft stem) of foliage sampled. Inconsistencies could also be due to sampling site and climatic influences on foliage growth and plant nutrient accumulation.

High secondary compound contents in foliages <u>are mainly a property</u> of plant genotypic factors controlling physiological synthesis and accumulation of secondary compounds (Okuda et al., 1993; Kelman et al., 1997). Other factors associated with high rates of polyphenolic synthesis include high environmental temperatures, drought stress, and plant defensive mechanisms against pests, pathogens and predators (Mangan, 1988). Shayo and Uden (1999) and Abdulrazak et al. (2000) also reported high phenolic and tannin levels in some East African browses. High polyphenolic components were <u>also reported</u> in semi-arid of north Egypt (Salem, 2005) and arid regions of Sudan (Fadel Elseed et al., 2002). There were differences between levels of TP and CT in the tree foliages studied compared to similar tree foliages reported by others (e.g., Rubanza et al. (2003) reported TP and CT were between 65–237 and 6–74 g/kg DM, respectively). This may, at least partly, be due to different assays and assay standards, although variability in chemical composition of polyphenolics among foliages (Makkar and Becker, 1993; Pino et al., 2005) may also be a factor. Some differences might also have been <u>due to</u> stage of plant growth and/or season of collection (Salem, 2005), site of sampling (Makkar and Becker, 1998), and/or proportions of foliage materials sampled (Salem, 2005).

In the current study, the secondary compounds SAP, ALKA, AF and EO were determined for the first time in these tree foliage species. However, their interpretive value relative to prediction of negative impacts of plant secondary compound levels on voluntary DM intake and animal performance <u>may be</u> modest, particularly for SAP, ALKA and EO, which were very strongly positively correlated to TP and CT in these tree foliages (Table 6). In contrast, AF was weakly negatively correlated to TP and CT, as well as SAP, ALKA and EO, <u>suggesting that it may have</u> value in predicting voluntary DM intake and performance of animals fed tree foliages.



#### 4.2. Effect of animal species

Inter-animal species differences in voluntary intake of these foliages, without addition of PEG, were inconsistent among the foliages. While sheep ate more grams per day of C. fistula, S. Molle and C. speciosa than goats, intake of E. *camaldulensis* was slightly higher in goats. In contrast, sheep ate more C. *fistula* than goats relative to BW, but goats ate more of the other three foliages. Over all PEG unsupplemented foliages, goats consumed 3.9% more foliage DM than sheep per kg of BW0.75 (Fig. 1), a finding consistent with Gilboa et al. (1995) who found that goats were able to consume larger amounts of tanninrich browse than sheep under similar conditions, probably due, at least **partially**, to the ability of goats to detoxify higher amounts of tannins or secondary compounds *versus* other ruminants (Silanikove et al., 1996). In **addition**, goats, as browsers, may have selected the parts of the foliage with a higher proportion of CP, and lower proportion of fiber and/or secondary compounds, versus sheep as grazers (Kababya et al., 1998; Salem, 2002; Salem et al., 2003).

However, this is speculatory, as the composition of the uneaten feed was not determined. Salem et al. (2004a) observed an increase in the number of eating bouts of short duration in goats fed alfalfa hay treated with 50 g quebracho/kg DM, versus sheep fed the same hay, and suggested that this may be a mechanism used by goats to minimize negative effects of secondary compounds in foliages. The mobile upper lip of goats allows them to browse a variety of plants to obtain nutrients under harsh conditions. In many studies (Tisserand et al., 1991; Silanikove, 2000a, 2000b; Salem et al., 2004a), goats had the ability to utilize woody species and low quality forages better than cattle and sheep, and were able to adapt better to harsh environments, such as the extensive shrub lands of evergreen shrubs and small trees, that are the basic component of the diets of goats raised in the Mediterranean basin.



In spite of the higher PEG unsupplemented foliage DM intake relative to BW of goats versus sheep in three of the four foliages, an event that would be expected to suppress digestibility (NRC, 2001), digestion of DM, and its measured components (except NDFom which was only numerically higher in three of the four foliages) were consistently higher in goats. For example, average DM digestibility was 509 g/kg in sheep and 551 g/kg in goats unsupplemented with PEG, suggesting an approximate increase of 8% in the energetic value of these foliages to goats *versus* sheep. In addition to the advantages of goats versus sheep noted above, their ability to consume larger amounts of tannin-rich browse (Gilboa et al., 1995) and ability detoxify higher amounts of tannins (or other secondary compounds) versus other ruminants (Silanikove et al., 1996), may occur by development of adaptive mechanisms in response to the presence of secondary compounds in the diet (Provenza and Malechek, 1984; Silanikove et al., 1996; Kababya et al., 1998; Salem et al., <u>2004a</u>). Such an adaptive mechanism may be due to the existence of ruminal bacteria, such as *Streptococcus caprins*, in goats that has the ability to degrade tannin-protein complexes (Brooker et al., 1994).



In addition, goats, as browsers, <u>may have selected</u> the parts of the foliage with a lower proportion of secondary compounds, *versus* sheep as grazers (Kababya et al., 1998; Salem, 2002; Salem et al., 2003).

#### 4.3. Effect of PEG supply

we add our explanation and then References for confirmation

Polyethylene glycol is widely used to neutralize tannins and other secondary compounds in foliages. Formation of complexes between PEG and secondary compounds, particularly tannins, from leaves of trees and shrubs was investigated by Makkar et al. (1995a), and the affinity of tannins for PEG at various pH's was demonstrated. Positive effects of PEG feeding on feed intake, digestibility, rumen fermentation, microbial synthesis, daily gain and wool growth by sheep and goats fed tannin rich forages have been widely demonstrated (Pritchard et al., 1992; Miller et al., 1997; Silanikove et al., 1997; Degen et al., 1998; Ben Salem et al., 2000; Decandia et al., 2000; Barry et al., 2001), but the nature and magnitude of the positive impact is thought to depend on factors such as tannin structure, level of tannin in the foliage, PEG dose level and means of administration, animal species and diet composition.



In the current study, the level of CT (as well as the levels of TP, SAP and ALKA due to their high correlations to CT levels as shown in Table 6) was a strong predictor of foliage DM intake (g/d), explaining 0.81 and 0.60 of the variation (*i.e.*, r2) in sheep and goat DM intake, respectively (Table 7). Levels of AF and CP in the foliages were poor predictors (r2 from <0.01 to 0.20) of DM intake, but when CP was added to CT, 0.87 and 0.78 of the variation in sheep and goat DM intake, respectively, was explained and if AF was added to CT, predictions were essentially perfect. Clearly four foliage observations are insufficient to support firm conclusions, <u>but it suggests that</u> negative effects of CT on DM intake can be counteracted to only a slight degree by lower levels of CP, but to a substantive extent by higher levels of AF.

In general, these results are consistent with findings of others. For example, secondary compounds, particularly phenolics, could act by lowering foliage palatability by their negative effects in the mouth, such as by astringent bitterness (Jackson et al., 1996), binding to salivary proteins in the mouth (Wong, 1973; Salem et al., 2000), or by negative effects on gustative receptors (McLeod, 1974).



#### we add our explanation and then References for confirmation

Higher levels of secondary compounds in foliages, particularly in *E. camaldulensis*, during eating could have negatively affected salivation rate, which could have increased the astringent taste and so decreased feed intake (Salem et al., 2000, 2001). Reduced salivation <u>might also have</u> negatively affected ruminal microbial activity (Salem et al., 2002) and inhibited enzyme production (Dawson et al., 1999; Barry and McNabb, 1999; Salem et al., 2002). In addition, secondary compounds perturb intestinal wall permeability through reactions with intestinal membrane proteins (McLeod, 1974; Zimmer and Cordesse, 1996; Fondevila et al., 2002).

Studies on tannin–saponin interactions <u>which suggested that</u> effects of both tannins and saponins to decrease *in vitro* digestibilities and gas production were additive (Makkar et al., 1995b; Makkar, 2003a), do not support the hypothesis that simultaneous presence of tannins and saponins might alleviate the adverse effect of each other. For example, Johnson et al. (1986) <u>found that</u> some saponins increase the permeability of intestinal mucosal cells *in vitro*, inhibit active mucosal transport and facilitate intestinal absorption of compounds that are normally not absorbed.

#### we add our explanation and then References for confirmation

The EO, which are the volatile components responsible for some of the characteristic aroma of foliage species, <u>may also have</u> negative effects on DM intake. EO appear to have selective antibacterial activity (Janssen et al., 1986; Demetzos et al., 1997; Newbold et al., 2004), and Nagy and Tengerdy (1968) <u>found that</u> addition of EO extracted from Sagebush (*Atemisa tridentate*) altered the rumen bacterial population composition.

Feeding PEG <u>has been shown</u> to improve intake of foliage containing secondary compounds in goats (Silanikove et al., 1997; Decandia et al., 2000) and sheep (Silanikove et al., 1994; Salawu et al., 1997). It has also been shown to increase availability of nutrients in the gastrointestinal tract and so increase digestibility (Ben Salem et al., 2005). However, the actual chemical linkages between tannins and PEG that neutralize the negative effects of secondary compounds of foliages to allow increased feed intake and digestibility are not clear. Consistent with results of others, supplementation of PEG to sheep and goats in the current study increased foliage DM intake and digestion to variable extents in both animal species fed all foliages.



The foliages used in the current study had very different levels of secondary compounds (e.g., the CT of E. camaldulensis was 3.27 times that of C. speciosa), and it might have been expected that PEG feeding would have a larger positive impact on DM intake in foliages with higher level of secondary compounds. However, this was not the case. The level of CT (as well as the levels of TP, SAP and ALKA due to their high correlations to CT levels as shown in Table 6) was not a predictor of the percentage increase in foliage DM intake due to feeding PEG in either sheep or goats, explaining only <0.01 and 0.04 of the variation for sheep and goats, respectively, increase in foliage DM intake due to PEG (Table 7). The best single (negative) predictor of the percentage increase in foliage DM intake due to PEG feeding was the CP level of the foliage, explaining 0.62 and 0.64 of the variation for sheep and goats, respectively. If CT was added to CP as a predictor, the variation explained did not change (*i.e.*, 0.62 and 0.76), however addition of AF (positive) to CP increased the variation explained to 0.87 and 0.99, respectively, for sheep and goats.



The inability of CT (and by correlation the other secondary compounds) to explain the percentage increase in DM due to PEG feeding contrasts to the ability of CT to predict the absolute DM intake of these foliages. However, as previously noted, four foliage observations are insufficient for firm conclusions, although it does <u>suggest that the positive effects of PEG on DM intake may not</u> be related to its levels of CT, or other secondary compounds, but <u>due to</u> associations with CP and AF that overcome the negative affects of secondary compounds on DM intake and digestion.



## **1. CONCLUSIONS**



## **1.1- CONCLUSIONS Conditions**

In this section it should be get a general statement reflex the objective from this study, and this will be the conclusion.



Be careful to writ a specific conclusion depending on the results obtained not a possibility case.

It is not acceptable to add references in this section

Add the conclusion without any details or repetition of methodology or the results sections



## **2.3- CONCLUSIONS Examples**

Example 1. Salem et al., (2006) Animal Feed Science and Technology 127 (2006) 251–267

The nutritional quality of the browse tree foliages C. fistula, S. molle, C. speciosa and E. camaldulensis, native to the semi-arid region of north Egypt, were evaluated by determining levels of nutrients and secondary compounds, as well as feed intake and apparent digestibility in sheep and goats. Goats consumed 3.9% more DM than sheep per kg BW0.75, and their digestibility was about 8% higher. Levels of CT (and due to its correlations, also TP, SAP, ALKA and EO) was a strong predictor of DM intake of PEG unsupplemented foliages in both sheep and goats. PEG increased intake of DM and its components in both sheep and goats, but levels of TP (and due to its correlations, also CT, SAP, ALKA and EO) was not a predictor of the proportional increase in DM with PEG feeding, which was best predicted by the level of CP within foliage (negative), which was improved by adding AF (positive) to the prediction. C. speciosa, had the highest nutrient value for both sheep and goats, both without and with PEG feeding, S. molle and C. fistula were intermediate and *E. camaldulensis* had the lowest nutritive value.

## **Example 2:**

G. HERVA S et al., 2000; *Journal of Agricultural Science*, *Cambridge* (2000), **135**, 305±310.

The current results show that **tannic acid can exert a negative effect** both **on rumen degradation** and **on intestinal digestion of the SBM**, **this effect being clearly dependent on the dose used to treat the SBM**. It is considered that this effect is also dependent on the adaptation of the rumen microbial population to the presence of tannic acid, which would increase its ability to degrade this compound.



## 2. ACKNOWLEDGMENT (include as needed)



## **2.1-ACKNOWLEDGMENT Conditions**

If, in your experiment, you received any **significant help** in thinking up, designing, or carrying out the work, or **received materials from someone** who did you a favor by supplying them, **you must acknowledge their assistance and the service or material provided**.

Authors *always* acknowledge outside reviewers of their drafts (in PI courses, this would be done *only* if an instructor or other individual critiqued the draft prior to evaluation) and any **sources of funding** that supported the research.

Although usual style requirements (e.g., 1st person, objectivity) are relaxed somewhat here, Acknowledgments are **always brief and never flowery.** 



**Manuscript ACKNOWLEDGMENT** 

## **2.1-ACKNOWLEDGMENT Conditions**

## In this section the authors will thanks any person or foundation had assistant in this work to be publish.


# **2.2- ACKNOWLEDGMENT Examples**

Example 1. Camacho et al., (submitted to the Animal Feed Science and Technology)

This work was undertaken with funds from the Universidad Autónoma del Estado de México (project UAEM 2384/2006). Our gratitude also to the Mexican National Council for Science and Technology (Consejo Nacional de Ciencia y Tecnología-CONACYT) for the grant received by Luis Miguel Camacho Díaz. The authors gratefully acknowledge Prof. Dr. Peter H. **Robinson** (Department of Animal Science, University of California, Davis, USA) for his assistance and advice during revision of the manuscript.

# **Example 2:**

G. HERVA S et al., 2000; *Journal of Agricultural Science*, *Cambridge* (2000), **135**, 305±310.

The authors wish to thank Dr S. Lopez for helpful comments and revision of the manuscript. This work was supported by the Inter-ministerial Commission of Science and Technology (CICYT) of Spain (Project AGF98-0874) and the Junta de Castilla y Leon (Project CSI 7}98).



# **3. REFERENCES**



# **3.1- REFERENCES Conditions**

Each journal has a guideline in writing the References in the manuscript, but generally we need to know how we can to writ a Refs. of a research paper, meeting, thesis or book.



# **3.2- REFERENCES Examples**

3.2.1.Research paper reference

1- Author/s name>> 2- year>> 3- paper title>> 4- journal name>> 5- volume>> 6- issue>> 7- pages number.

# 3.2.1.1.Reference of One author:

Salem, A.Z.M. 2005. Impact of season of harvest on *in vitro* gas production and dry matter degradability of *Acacia saligna* leaves with inoculum from three ruminant species. *Anim. Feed Sci. Technol.* 123-124, 67-79.



## 3.2.1.2.Reference of Two authors

Titi, H., Lubbadeh W.F., 2004. Effect of feeding cellulase enzyme on productive responses of pregnant and lactating ewes and goats. Small Rumin. Res. 52, 137–143.

# 3.2.1.3. Reference of Three authors

Varga, G.A., Dann, H.M., Ishler, V.A., 1998. The use of fiber concentrations for ration formulation. J. Dairy Sci. 81, 3063–3074.



## 3.2.1.4. Reference of More than three authors

Ranilla, M.J. Tejido, M.L. Giraldo, L.A. Tricárico, J.M., Carro, M.D., 2008. Effects of an exogenous fibrolytic enzyme preparation on *in vitro* ruminal fermentation of three forages and their isolated cell walls. Anim. Feed. Sci. Technol. 145(1-4), 109-121.

Juskiewicza J., Semaskaiteb A., Zdunczyka Z., Wroblewskaa M., Gruzauskasb R., Juskiewicz M. 2007. Minor effect of the dietary combination of probiotic *Pediococcus acidilactici* with fructooligosaccharides or polysaccharidases on beneficial changes in the cecum of rats. *Nutr. Res. 27, 133–139*.

Silanikove, N., Gilboa, N., Nir, I., Perevolotsky, A., Nitsan, Z., 1996. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannincontaining leaves (*Quercus calliprinos, Pisticia lentiscus* and *Ceratonia siliqua*) by goats. J. Agric. Food Chem. 44, 199–205.



#### 3.2.2. Meeting or international conference reference

 1- Author name/s >>> 2- year>>> 3- paper title>>> 4- conference name>>> 5- location>> 6-paper pages. You can also mention if it is an abstract or full paper

#### 3.2.2.1.References of One author

Salem, A.Z.M., 2010. The effect of feeding alfalfa treated with quebracho on parotid salivation in sheep. In: Van Arendonk, J.A.M. (Ed.), Proceedings of the 51st Annual Meeting of the European Association for Animal Production (EAAP), Session N5.17. Wageningen Press, The Hague, The Netherlands, p. 152.



#### 3.2.2.2. References of\_Two authors

Stokes, M.R., Zheng, S., 1995. The use of carbohydrase enzymes as feed additives for early lactation cows. 23<sup>rd</sup> Biennial Conf. Rumen Function, Chicago, IL, 23:35 (Abstract).

#### 3.2.2.3. References of More than two authors

Salem, A.Z.M., González, J.S., López, S., Ranilla, M.J., 2000. The effect of feeding alfalfa treated with quebracho on parotid salivation in sheep. In: Van Arendonk, J.A.M. (Ed.), Proceedings of the 51st Annual Meeting of the European Association for Animal Production (EAAP), Session N5.17. Wageningen Press, The Hague, The Netherlands, p. 152.



#### 3.2.3. PhD and MSc thesis reference

#### Rules

1- Author name>>> 2- year of thesis>>> 3- Thesis title>>>> 4-Type of thesis>>> 5- The university name>>>> 6- location (city and country)

#### Example:

Salem, A.Z.M., 2002. Parotid saliva production and composition, feeding behavior, rumen fermentation, digestibility, and plasmatic parameters in sheep and goats: evolution of the response to the condensed tannins of quebracho in the diet. PhD Thesis. University of Leon, Leon, Spain.



#### 3.2.4. Book and manual reference

#### Rules

1- Authors >> 2- year>> 3- chapter title>> 4- Book title>> 5-Editors name>> 6- Academic published book>> 7- Location>> 8chapter pages

#### 3.2.4. 1.Use a specific Book chapter as a reference

Stewart, C.S., Flint, H.J., Byrant, M.P., 1997. The rumen bacteria. In: The rumen microbial ecosystem. 2nd ed. *Edited by* P.N. Hobson and C.S. Stewart. Blackie Academic and Professional, New York. pp. 10–55.



Makkar, H.P.S., 2003. Quantification of tannins in tree and shrub foliage. In: Makkar, H.P.S. (Ed.), A Laboratory Manual. Kluwer Academic Publishers/FAO/IAEA, Vienna, Austria, p. 102.

#### 3.2.4. 1.Use a specific Book chapter as a reference

Stewart, C.S., Flint, H.J., Byrant, M.P., 1997. The rumen bacteria. In: The rumen microbial ecosystem. 2nd ed. *Edited by* P.N. Hobson and C.S. Stewart. Blackie Academic and Professional, New York. pp. 10–55.



#### 3.2.4. Use the whole Book as reference

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics, 2nd ed. McGraw-Hill International, New York, NY, USA.

SAS, 1999. SAS/STAT User's Guide, Version 6, 4th ed. SAS Institute, Cary, NC, USA.

Fuller R. 1999. Probiotics for Farm Animals. A Critical Review. G.W. Tannocka (ed.) Horizon Scientific Press, Wymondham, England.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics, 2nd ed. McGraw-Hill International, New York, NY, USA.





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