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Filière **: Sciences Agronomiques**

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Présentée par :

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Thème

Evaluation de l'utilisation d'*Azolla* dans la formulation d'aliments pour les animaux monogastriques

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Soutenu le, ……..

Devant le Jury :

BENOUIRA.Y	Président	M.C.B.	Univ-Tissemsilt
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TEFIEL.H	Examinateur	M.C.A.	Univ-Tissemsilt

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Specialty: Animal production

Carried out by**:**

BAROUD Chawki

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Topic:

Evaluation of the use of *Azolla* in formulating feed for monogastric animals.

Defended on ……...

Jury composition:

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Dedication

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All the appreciation and respect

HAOUARI BEKKOUCHE

Abstract:

This study aims to evaluate the effect of the *Azolla* plant in formulating feed for monogastric animals, by introducing it in variable proportions in feed for broiler, reproductive and laying hens, and rabbits.

Azolla is an aquatic fern rich with vegetal protein. It is able to fix the nitrogen present in the atmosphere, with its symbiotic relationship with a cyan bacteria called *Anabaena*, the first experiment lasted for about 5 months, and the second for about two months. After cultivating 2 kg of *Azolla*, we obtained a yield of 25 kg of fresh *Azolla*, and after drying on, 5.05 kg of dried *Azolla*. Analyzes of determination of chemical composition for the latter was done. We found significant proportion of protein estimated at 24.8 %. As for the introduction of variable proportions in monogastric animal feed, they are as follows:

Broiler: The results of the nutritional value of the meals given in the stages of beginning, growth and finish by using 10%, 15% and 20%, respectively, of *Azolla* indicated that the use of this later does not change the nutritional value of feed in comparison with standard feeds. A significant decrease was observed in the price of feed estimated at 6.2 dinars per kg, 6.7 dinars per kg, and 4.7 dinars per kg in the stages of start, growth and finish, respectively.

Rabbits: also by using 20% and 30%, respectively, of Azolla in the stages of growth and finish does not change the nutritional value of feed with a significant decrease in the price of feed, estimated at 8.5 dinars per kg and 12.4 dinars per kg, respectively.

Reproductive and laying hens: Also, by using 20% of Azolla in the growth stage, it does not change the nutritional value of the feed with a significant decrease in the price of feed, estimated at 7.6 dinars per kg.

Keywords: Azolla, food, monogastric animals, formulation, price, Broiler, Rabbits, nutritional value.

ملخص:

تهدف هذه الدراسة إلى تقييم تأثير نبات الأزولا في تكوين العلف للحيوانات الغير مجترة, من خلال إدخاله بنسب متغيرة في تغذية دجاج التسمين ودجاج التكاثر والدجاج البياض والأرانب.

الأزولا هو سرخس مائي غني بالبروتين النباتي وقادر على تثبيت النيتروجين الموجود في الغلاف الجوي ,بعلاقته التكافلية مع بكتيريا تسمى *(Anabaena)*، استمرت التجربة الأولى حوالي 5 أشهر في مكان خاص بثنية الحد بتيسمسيلت، والثانية لمدة شهرين تقريبا على مستوى الكلية. عملية زراعة الأزولا تمت في أحواض مائية.

بعد زراعة 2 كلغ من السرخس ، حصلنا على محصول إجمالي مقدر ب 25 كلغ من الأزولا الطازجة ، وبعد التجفيف تحصلنا على 5.05 كلغ من الأزولا المجففة ، ثم قمنا بإجراء تحاليل كيميائية للأخيرة ، ووجدنا نسبة معتبرة من البروتين تقدر بـ 24.8 %. أما عن إدخال النسب المتغيرة في أعلاف الحيوانات فهي كالتالي:

دجاج التسمين: أظهرت نتائج القيمة الغذائية للوجبات المعطاة في مراحل البداية والنمو والانتهاء باستخدام 10٪ و 15٪ و 20٪ على التوالي من الأزولا أن استخدامها لا يغير من القيمة الغذائية للوجبات المعطاة مقارنة مع الأعلاف بدون الأزولا. مع انخفاض في سعر العلف الذي سجل بنحو 6.2 دينار ، و 6.7 دينار، و 4.7 دينار للكيلوغرام في مراحل البدء والنمو والانتهاء على التوالي.

الأرانب: أيضا باستخدام 20٪ و 30٪ على التوالي من الأزولا في مراحل النمو والانتهاء لا يغير من القيمة الغذائية للأكل مع انخفاض معتبر في سعر العلف المقدر بـ 8.5 دينار للكيلو. 12.4 دينار للكيلوغرام على التوالي.

دجاج التكاثر والدجاج البياض: إن استخدام 20٪ من نبات الأزولا في مرحلة النمو لا يغير القيمة الغذائية للأكل مع انخفاض معتبر في سعر العلف المقدر بـ 7.6 دينار للكيلوغرام.

الكلمات المفتاحية: الأزولا، الأعلاف، الحيوانات الغير مشترة، تركيبة الأغذية، السعر. دجاج التسمين، الأرانب، القيمة الغذائية.

Résumé:

Cette étude vise à évaluer l'effet de la plante Azolla dans la formulation des aliments pour les animaux monogastriques, en l'introduisant en proportions variables dans l'alimentation des poulets de chair, des poules reproductrices, des poules pondeuses et des lapins.

Azolla est une fougère aquatique riche en protéines végétales et capable de fixer l'azote présent dans l'atmosphère, grâce à sa relation symbiotique avec une bactérie cyan appelée Anabaena, la première expérience a duré environ 5 mois, et la seconde environ deux mois. Après avoir cultivé 2 kg d'azolla, nous avons obtenu un rendement de 25 kg d'Azolla frais, et après séchage sur 5,05 kg d'Azolla séché, puis nous avons fait des analyses chimiques pour ce dernier. Les résultats d'analyses ont montré une proportion importante de protéines estimées à 24,8 %. Quant à l'introduction de proportions variables dans l'alimentation des animaux monogastriques, elles sont les suivantes :

Poulets de chair : les résultats de la valeur nutritionnelle des aliments formulés donnés aux stades de démarrage, de croissance et de finition en utilisant respectivement 10 %, 15 % et 20 % d'Azolla ont indiqué que l'utilisation de ce dernier ne modifie pas la valeur nutritive des aliments en comparaison avec les aliments standards avec une baisse significative du prix des aliments estimés à 6,2 dinars, 6,7 dinars et 4,7 dinars par kg aux stades de démarrage, de croissance et de finition, respectivement.

Lapins : aussi en utilisant respectivement 20% et 30% d'Azolla dans les stades de croissance et de finition ne modifie pas la valeur nutritionnelle des aliments avec une baisse importante du prix de l'aliment, estimé à 8,5 dinars le kg. 12,4 dinars par kg, respectivement.

Poulets reproducteurs, et poules pondeuses : Aussi, en utilisant 20% d'Azolla, cela ne modifie pas la valeur nutritionnelle des aliments avec une baisse significative du prix de l'aliment, estimé à 7,6 dinars le kg.

Mots clés : Azolla, aliment, animaux monogastriques, formulation, prix, poulet de chair, lapins, valeur nutritive.

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List of abbreviations

ADF = Acid-Detergent Fiber.

AVG = Average.

C = Celsius.

Ca = Calcium.

CO2 = Carbon dioxide.

DE = Digestible energy.

DM =Dry Matter.

ED = Energy digested.

EE = Ether extract.

Fe = Iron.

g= gram.

GF = Grosse fibre.

GP = Grosse Protein.

K = Kalium.

Kg = kilogram.

M = meter.

Max = Maximal.

ME = Metabolisable Energy.

MG = Grasse matter.

Min= Minimal.

OM = Organic Matter.

N2 = Nitrogen.

Nb = Note.

ND = Digestible Nitrogen.

NDF = Neutral Detergent Fiber.

P = Phosphorus.

PDI = Digestible Protiens in Intestine.

PH= Potential Hydrogen.

UFL= Net Energy for lactation.

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CONCLISION

INTRODUCTION

Animal production and specially monogastric animals as poultry and rabbits may be the solution to sustainably feeding a projected human population. Poultry production have the potential to enhance and sustain food and nutrition security with considerable impact on livelihoods of the most at risk through production of safe, high-quality food within a comparably short period.

However, higher price and scarcity of conventional feed ingredients are ruining its prospects. Feed consumption accounts for the major cost of production which is responsible for more than 70% of the total cost. Prices for staple feed ingredients are up to 100% more expensive than they were before some years in Algeria. Although conventional feed ingredients are becoming increasingly expensive, evidence shows alternative resources can maintain productivity and profitability in animal production.

Therefore, nutritionists are in search of economical and sustainable feed ingredients for animal feeding. Many researchers are working on different materials to be used for economic animal's feed ration formulation (Fasuyi *et al*., 2007). *Azolla* has shown a great potential to be included as a source of protein for poultry ration (Bacerra *et al*., 1995) and egg type chicks (Alalade and Iyayi, 2006).

There are multiple reasons for considering *Azolla* as a potential feed resource for sustainable, viable and profitable small-scale in animal production. In terms of production benefits, *Azolla* is rapidly growing, self-perpetuating, high N_2 fixer, high biomass per unit area, takes up much less space and water for propagation than the traditional feed resources (Kumar and Chander, 2017). Evidence existed that *Azolla* is rich source of nutrients containing protein, lipids, minerals and vitamins (Alalade and Iyayi, 2006). *Azolla* is of interest as a protein feed due to the high protein content of its biomass, which was reported to be between 20–40% of the dry weight (Kumar and Chander, 2017). Therefore, the bio-composition of *Azolla* makes it one of the most economic, efficient and sustainable feed substitute for animals (Kamalasanana *et al*., 2002). Incorporation of *Azolla* as an alternative protein ingredient in monogastric animal's ration could make animal production economical.

In this regard, this work discusses the interest that the *Azolla* adds to the formation of manufactured feed for monogastric animals, and how we can address the void and high value cost of imported conventional feed with such available solutions that we have in hand which is the Azolla plant that we introduced in different proportions in Rabbits, broilers, lying hens and reproductive hens.

Chapter I:

General on Azolla

I.1. Entrance*:*

Azolla is a fast-growing aquatic fern which spread on the surface of water, as small compact and flat green mass. *Azolla* produces more than 4 to 5 times protein of excellent quality compared to *Alfalfa* .In addition; biomass production is approximately 4 to 10 times greater than *Alfalfa* (Chander *et al*,. 2017).

Azolla is naturally grown in ponds, ditches and wetlands of warm temperate and topical areas around the world. It is one of the fastest growing plants on the planet because of its symbiotic relationship with *cyanobacteria* "blue-green algae "called *Anabaena*. This latter attracts atmospheric nitrogen (N2) which fertile *Azolla*, and provides an environment filled with nitrogen for *Anabaena* in its foliar cavities.

This allows the plant to double its biomass as little as two days floating easily on water as an inch of 2.4 cm (Granda and Guaman. 2022).

The rapid growth of *Azolla* makes it a potentially important absorbent of the carbon dioxide (CO2) that is converted directly into *Azolla* biomass. This provides feed for local livestock, bio-fertilizers, human food and bio-fuels. Wherever, *Azolla* is grown or cultivated so this remarkable plant has the potential to help us cross environmental threats such climate change caused by humans and shortages of food and land. (Granda and Guaman. 2022).

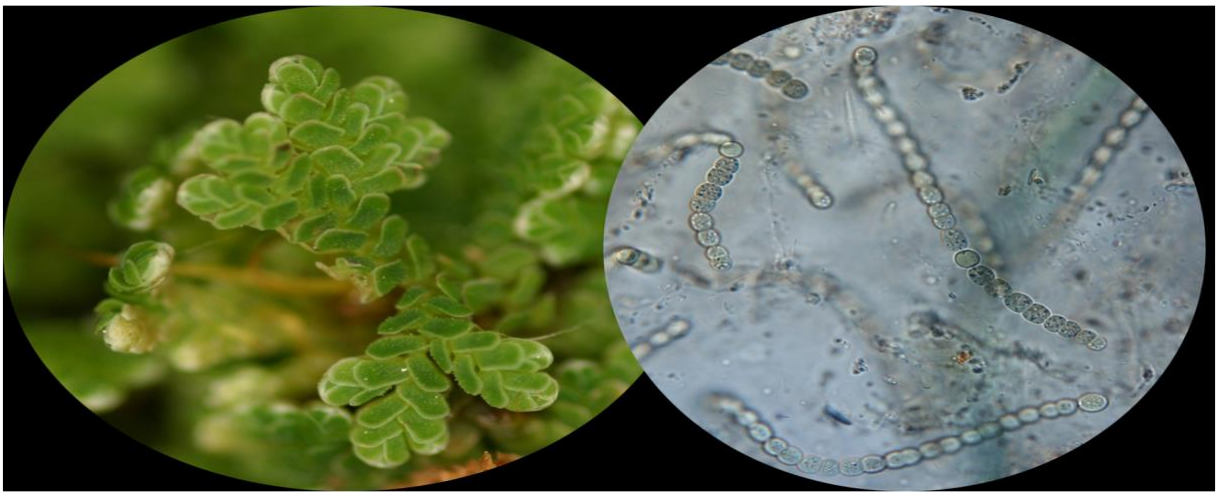

Figure 1: Azolla (left) its endosymbiont Anabaena (right), Picture courtesy of the University of Wisconsin's Department of Botany Website. 2022.

I.2. Classification of Azolla:

Despite its long history of agriculture use, the classification of *Azolla* is controversial and our knowledge on the subject is limited, which probably means that a new order is necessary.

In 1993, using *cladismis* (classification method), Saunders and Flower proposed another super-specific classification. They consider that the differences that separate *A.Nilotica* from all other species of *Azolla* are sufficient to establish a new subgenus. The subgenus, called *Tetrasporocarpia* (*sporocarpes* grouped in four), include only *A.Nilocita*. Then, the subgenus *Azolla* is divided into two sections, *Azolla* and *Rhizosperma*, the last one included *A.Pinnata*; this later with 3 subspecies (Carrapiço, 2000).It is classified as followed:

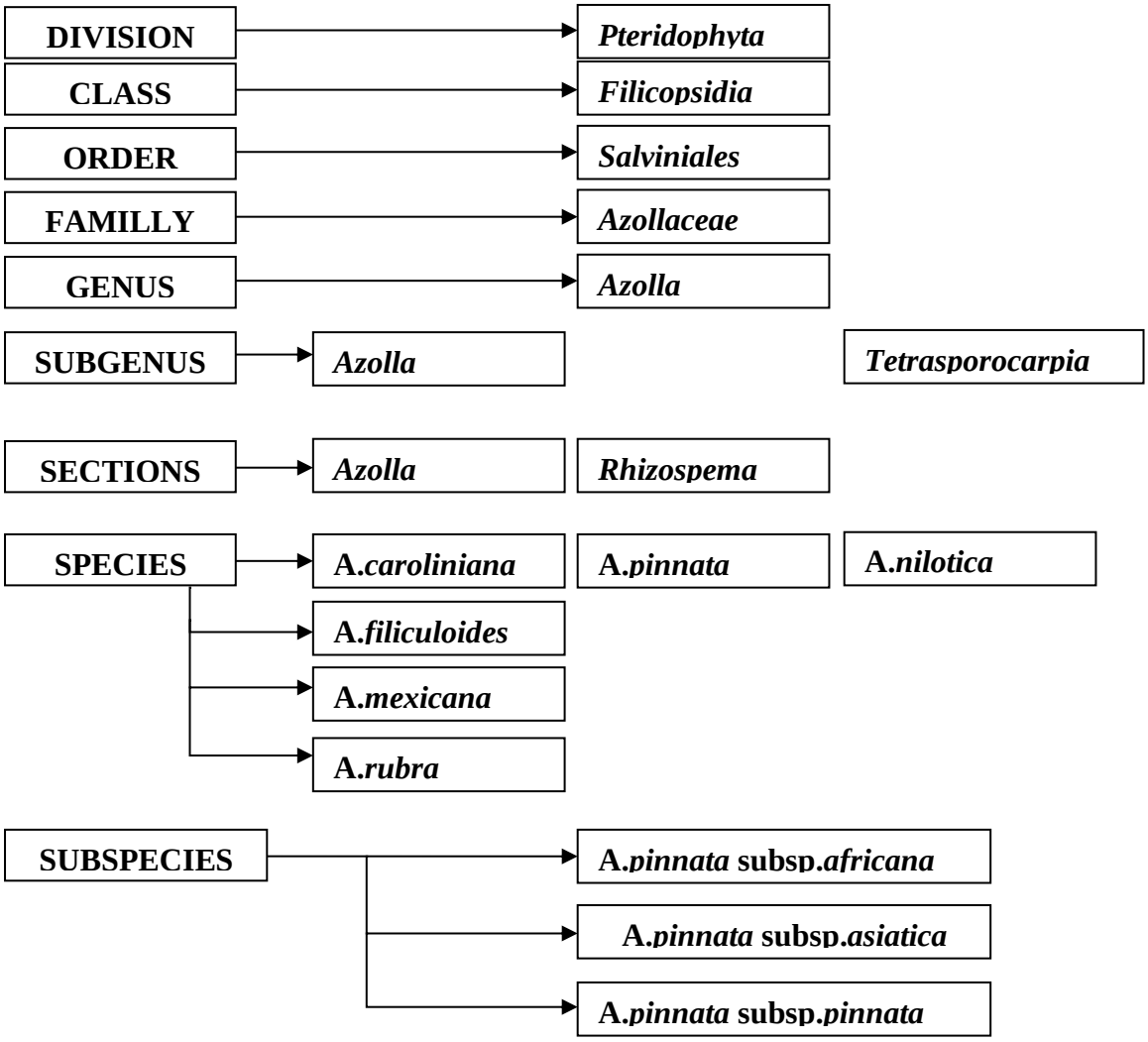

Figure 2: Classification of *Azolla* (Saunders and fowler, 1993).

I.3. Azolla In the world:

Azolla is located in the freshwater habitats of tropical and subtropical regions of warm temperate around the globe (Wagner, 1997).

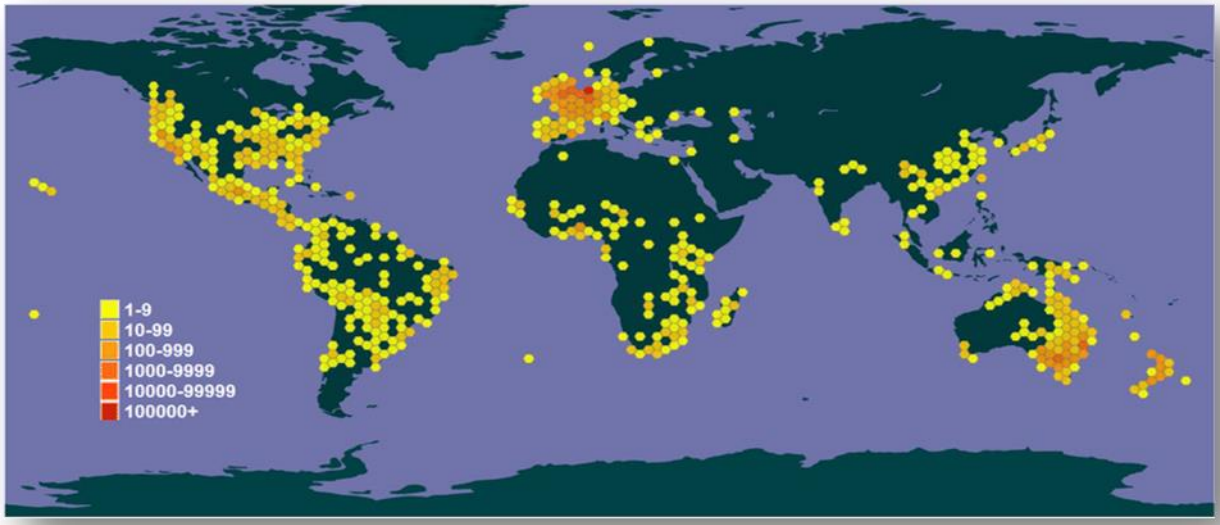

Figure 3: Occurrence (incidence) and distribution of *Azolla* species (adapted from GBIF 2020)

I.4. Morphology:

An *Azolla* plant consists of main length rod rarely 3 or 4 cm (Vanhove, 1989).The fronds of *Azolla pinnata* see Africana measure from 1 to 3 cm long. They float on the surface of the water and are covered with small alternating leaves tightly nested hiding the rod. it forms at regular intervals of the secondary rods having the same general characters as the main rod in turn producing third-order rods ,these are the fourth order rods (Vanhove , 1989).

This architecture gives the plant a more or less triangular or circular shape according to the other species of plants. The adventitious roots are 1 to 3 cm long. They are covered with absorbent hairs arranged in tufts (Reynaud and franche, 1986). The roots are oriented towards the water and sink vertically.

Each leaf consists of two parts called lobes. One of them is arranged above the rod, thicker and chlorophyllian, it contains an internal cavity, housing a population of *cyanobacteria* (blue algae) called *Anabaena Azolla* (Vanhove, 1989).

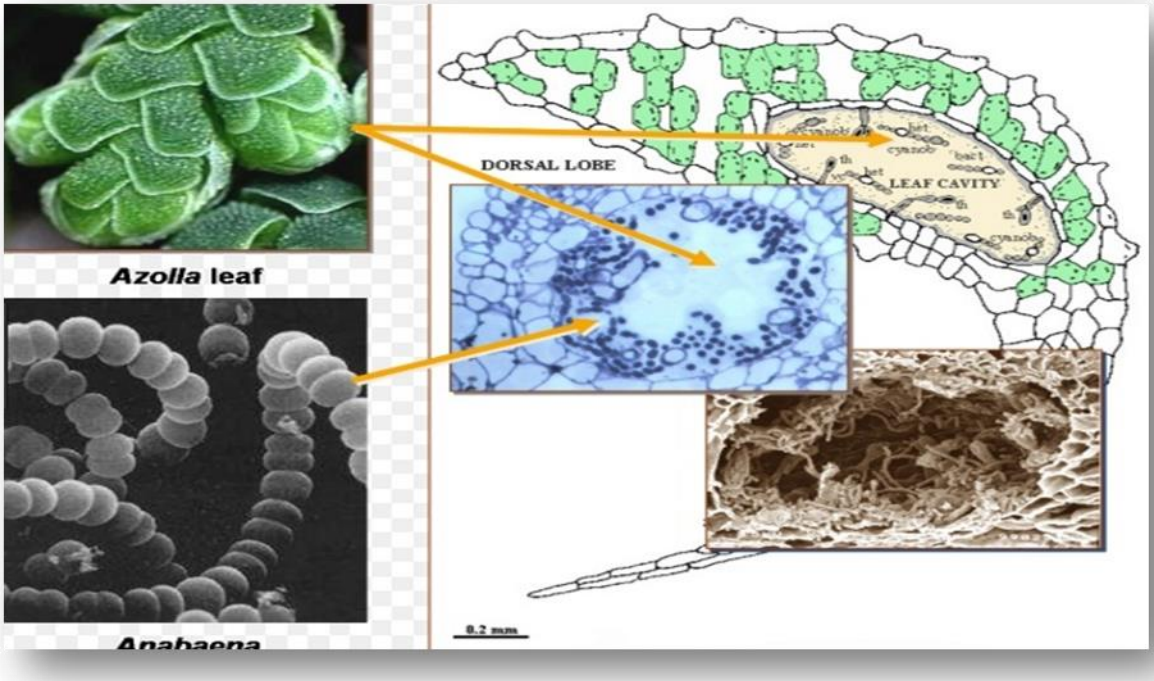

Figure 4: An aspect of the morphology of *Azolla* (Picture from Copyright 2023 Agriculturistmusa.com website)

I.5. Multiplication:

In *Azolla* there are 2 reproduction modes:

Sexual or (generative reproduction), and vegetative reproduction or (multiplication), the reproduction is made by vegetative in favorable climatic conditions and by way of adverse season characterized by intense heat or cold (Becking, 1979).

Symbiosis is maintained during the reproductive cycle. The cells of *anabaena*, in particular the akinetes (*akinetospores*) are locked up inside the macrosporocarpes. They are embedded in a cavity under the cap of the macrosporangium indusium, after fertilization of the oosphere, a zygote forms and develops into sporophyte with its associated algae.

Azolla is generally reproduced by vegetative way by fragmented the abscission layer, at the base of each branch. Sexual reproduction is not very common and seems to be influenced by environmental factors, namely stress. (Carrapiço, 2000).

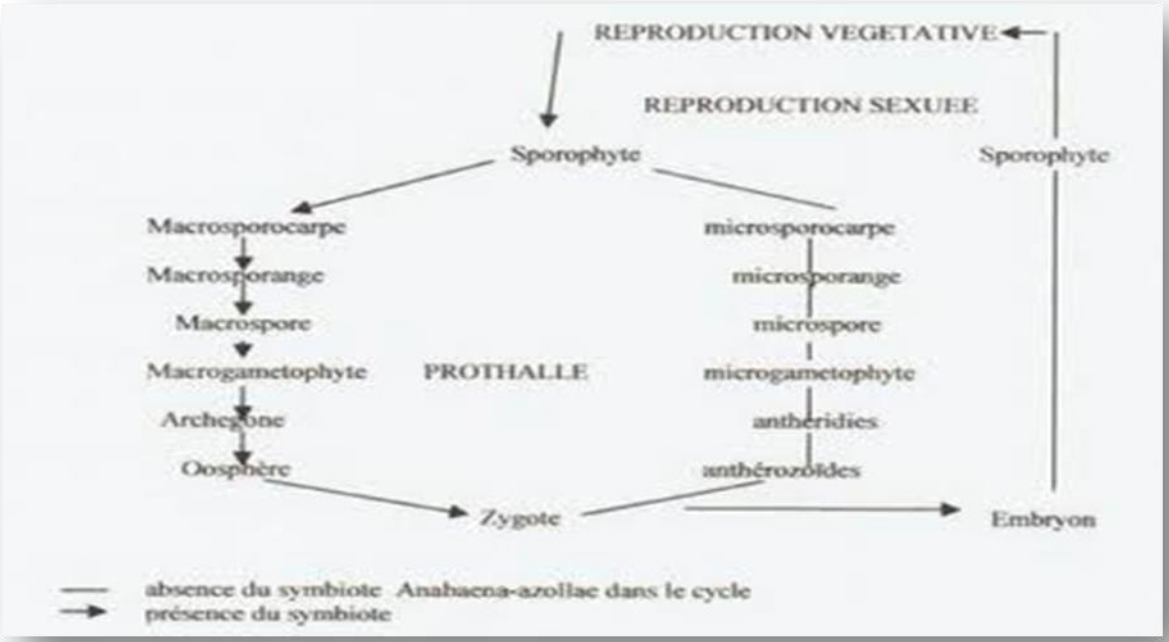

Figure 5: Diagram of the sexual and vegetative reproduction of *Azolla*, (Becking, 1979).

I.6. Environmental requirements for the growth of Azolla:

There are various unconventional foods that can be incorporated into the diet animal. One of these foods is *Azolla*. Thanks to this fern, consumption and feed digestibility is improved, thus animals get more nutrients from the ration given to them. Green *Azolla* (*Azolla pinnata*) can be an alternative to green fodder and as an additional protein feed due to its high taste and of its increased performance. It is considered the most promising because of its ease of cultivation, minimal water use, and its high productivity and good nutritional value (Kathirvelan *et al.,* 2015).

I.6.1. Ecophysiology of Azolla:

According to Rahagarison (2005), *Azolla* is a plant that requires a number of factors to live, to develop and to grow. These factors include: water, temperature, light and pH of the water.

I.6.2. Water requirement:

According to Van Hove *et al*. (1983), the growth of *Azolla* is favored by a layer of water not exceeding a few centimeters: 5 to 10 cm. This promotes mineral nutrition since the roots are close to the ground. Water is the basic condition for growth and multiplication of the *Azolla*, this plant is extremely sensitive to lack of water. The maintaining an adequate water level is essential (Rajesh, 2020). The fern cannot colonize large lakes, or turbulent waters, because the

wave effect and the turbulence of water cause the excessive fragmentation of the fronds thus decreasing its growth (Ashton and Walmsley, 1976).

I.6.3. Humidity rate:

Being a purely aquatic plant, the *Azolla* cannot withstand a humidity level less than 60%. It is a plant very sensitive to drought and dies in a few hours if the soil dries up (Becking, 1979). The optimum relative humidity is 85-90% (Rajesh, 2020).

I.6.4. Salinity:

Being a freshwater plant, the *Azolla* only supports a certain degree of salinity, ranging from 0.05 to 0.1% (Nandabalan and Kannaiyan, 1986). Its growth stops in a solution containing 1.3% salts (Haller et al., 1974).

I.6.5. Temperature:

The geographical distribution of the genus *Azolla* indicates that this plant adapts to conditions extremely varied climatic conditions (Rahagarison, 2005). The optimum temperature for its growth is between 20 and 30°C. Some strains were able to temporarily survive temperatures of -5 and 45°C. Other strains are very affected by lower temperature at 10°C, but can withstand values exceeding 35°C, as is the case with many ecotypes, as in the case of *Azolla pinnata* (Van Hove, 1989). However, Temperatures above 37°C will seriously affect fern multiplication (Rajesh, 2020).

I.6.6. Light:

The *Azolla* grows best in full or partial shade between 25-50% of full sun. There growth decreases rapidly under significant shade (less than 1500 lux) and more than 50 % of full sun reduces photosynthesis (Lumpkin *et al.,* 1980).

I.6.7. PH:

Azolla is particularly tolerant with regard to the pH of the environment. She survives in a pH range from 3.5 to 10, its growth is practically identical with a pH of 4.5 to 7 (Ashton, 1974; Watanabe *et al.,* 1977; Peters *et al.,* 1980; Lumpkin and Plucknett, 1980).

I.6.8. The nutritional needs of Azolla:

Azolla's mineral requirements include the macro elements: Phosphorus, Potassium, Calcium, Manganese and the microelements Fe, Mo, Co (Tuan and Thuyet, 1979; Becking, 1979; Lumpkin and Plucknett, 1980). According to Becking (1979), deficiencies in these elements lead to reductions in growth.

Chapter II:

Modes of use of Azolla

II.1. Chemical composition of the Azolla:

The chemical composition of *Azolla* species varies according to ecotypes and ecological conditions and the growth phase. The DM content is generally low in the range of 5 to 7%. The protein content is about 19 to 30% of DM in optimal growth conditions. The amino-acid profile of *Azolla* depends on the species. But the lysine content value is relatively high (4-6% of the protein). Unlike water lenses, the *Azolla* is relatively rich in fiber: the NDF may be greater than 50% of DM. the gross fiber is about 15% of DM and the lignin content is about 10-13% of DM .Like the most aquatic plants, *Azolla* is rich in minerals (10-20% DM) and can be used as a source of macro and micro minerals. However, the high fiber and mineral content explains why the *Azolla* should generally be included in limited quantities in monogastric meals, as high inclusion rates tend to decrease performances. The fresh *Azolla* is another limit to its use in the feeding of animals and it is often better to be dried, (Feedpedia, 2019).

Table 1: *Azolla* composition (Feedpedia, 2019).

Main analysis	Unite	AVG	South Dakota	Min	Max	Nb
DM	% fed	6.7	1.3	5.1	8.7	8
Gross protein	% DM	20.6	3.5	13.9	28.1	15
Gross fiber	% DM	15	3.5	11.3	22.8	9
NDF	% DM	43.8	5.9	35.4	52.3	6
ADF	% DM	31.8	6.4	24	38.9	5
The lignin	% DM	11.4	1.7	9.3	13.5	5
Ether extract	% DM	3.8	1.3	1.9	5.1	9
Ash	% DM	15.9	3.5	9.8	21.6	12
Starch	% DM	4.1		2.7	5.5	2
Gross energy	MJ / kg DM	17				

Table 2 : Essential minerals of *Azolla* (Feedpedia, 2019).

The minerals	Unit	AVG	South Dakota	Min	Max	Nb
Calcium	g / kg DM	1	4.1	5.8	17	8
Phosphorus	g / kg DM	6.1	5.5	0.3	15.5	1
Potassium	g / kg DM	17.4	3.7	10.9	22.5	7
Sodium	g / kg DM	9	4.3	2.8	12.5	4
Magnesium	g / kg DM	5	0.8	3.9	6.1	5
Manganese	mg / kg DM	762	43	208	1429	5
Zinc	mg / kg DM	38	28	11	77	5
Copper	mg / kg DM	16	7	10	28	5
Iron	mg / kg DM	3794	3794	711	8200	5

II.2.The form of presentation of Azolla:

Azolla can be used in 3 forms: fresh, dry and silage:

II.2.1. Fresh Azolla:

According to Van Hove (1989), this method of presentation does not require any transformation operation and do not distort component (offer to consumption in its natural form after harvest).

II.2.2. Dried Azolla:

Drying (in the sun) conducted in good conditions only changes the nutritional value of the plant. The drying must be done in a well-ventilated, non-wet and well-spreading place the *Azolla* in fine layer. The drying time varies from 3 to 4 depending on the ambient temperature, (Van Hove 1989). The chemical analysis of the *Azolla pinnata* dried in the sun for 3 days is given in the table.

Table 3: chemical composition of dried *Azolla pinnata* (Shamna, 2013).

Chemical composition	DM	GP	Either extract	Gross fiber	Free Nitrogen	Total Ash	Ca	P
A. *pinnata* dried %	88.08	25.46	2.66	14.08	41.58	15.5	2.25	0.4

II.2.3. Silage Azolla:

Blume *et al* (2016) showed that the silage of the *Azolla* can be practiced mainly to treat the excess biomass produced and used as a food for animals. The silo is then covered. Fermentation takes less than a month and the product can be kept for 2 years.

II.2.4. Azolla Pinnata's flour:

There is little work that has studied the Nutritional value of *Azolla* in animal feed, nevertheless, depending on the species to consider the chemical composition of the *Azolla Pinnata* has been reported in the next table:

Table 4: chemical composition of A.*pinnata* flour, (Alalade *et al*. 2006).

Chemical composition %	CP	CF	EE	Ash	Carbohydrates
***A.pinnata* flour**	21.4	12.7	2.7	16.2	47

Chapter III:

The use of *Azolla* in Animal feeding

Azolla is one of the most nutritious aquatic plants, thanks to its high content of crude protein, caroteneids and amino acids. It can be incorporated into the diet of animals and even humans (Van Hove and Lejeune, 2002).

III.1.The use of Azolla in human food:

This plant is used for human food. *Azolla* does not seem toxic, and some preparations with this plant are pleasant, its use in human food comes up against the difficulty of getting rid of the impurities often associated with its root system (Lumpkin and Plucknett, 1982).

III.2.The use of Azolla in animal feed:

Azolla can be fed to animals in fresh or dried form. It can be administered directly or mixed with foods to cattle, poultry, sheep's, goats, pigs and rabbits. It takes a few days for the animals to get used to the taste of the *Azolla*, so it is best to feed it the concentrates in the early stages. When dung is used as fertilizer in *Azolla* ponds, the plant should be washed thoroughly with fresh water to remove the smell of dung (Giridhar and Rajendran, 2013). In addition it has been shown that chickens receiving diets supplied with 5% more *Azolla* had faster growth than ingested the commercial food alone. Thus the food conversion of animals and energy efficiency have been significantly improved (Rajesh, 2020).

III.3.The use of Azolla in ruminant feed:

Most feeding trials with *Azolla* in dairy cattle, growing buffalo, sheep and goats have been carried out in India since 2000 (Pillai *et al*., 2004). The production of *Azolla* to increase milk production has been found to have the second highest benefit/cost ratio after the production of worms for vermin composting (Deshmukh *et al*., 2013). *Azolla*, fresh and dried, can be used in cattle feed (Figure.06), sheep and goats. Despite the long recorded use of *Azolla* in ruminants, data are still insufficient. Trials in India indicate that fresh or dried *Azolla* can be a partial substitute for more conventional protein sources such as peanut flour (Tran, 2015).

Figure 6: Cattle fed with *Azolla* (Tran, 2015)

III.4.The use of Azolla in poultry feed:

III.4.1.The use of Azolla in broilers and pullets:

In poultry, there is a general method about the use of *Azolla* that dried *Azolla* in broiler diets should be limited to 5%, as higher levels tend to reduce nutrient utilization and performance (Parthasarathy *et al*., 2002; Basak *et al*., 2002). In pullet chicks, *Azolla* could be safely included at up to 10% (Alalade and Iyayi, 2006). Fresh *Azolla* (Figure.7), could replace 20% or more of commercial broiler feed (Subudhi and Singh, 1978; Namra *et al*., 2010).

Figure 7: Chickens consuming fresh *Azolla* (www.theazollafoundation.org)

III.4.2.The use of Azolla in laying hens:

Dried *Azolla* can represent up to 15% of the diet of laying hens (Alalade *et al*., 2007; Khatun *et al*., 2008). It had a positive effect on yolk color (Khatun *et al*., 2008).

III.4.3.The use of Azolla in Japanese quail:

Azolla (*Azolla pinnata*) was found to have potential as a feed for Japanese quail due to its high nutrient content, but incorporation rates above 5% reduced growth performances and feed conversion (Sujatha *et al*., 2013).

III.4.4.The use of Azolla in the diet of geese

In China, *Azolla* has been used as green forage for geese. The daily weight gain of geese fed *Azolla* was closed to that of geese fed vegetables (Zhang ZhuangTa *et al*., 1987).

III.4.5.The use of Azolla in rabbits:

Azolla appears to be a suitable food for rabbits. In previous study, where 6-week-old rabbits were fed diets containing 0 to 36% dried *Azolla*, it was concluded that growing rabbits can be safely fed rations containing 24% of *Azolla* dried, which has beneficial effects on most production traits (Abou-Zeid *et al*., 2001). In another trial with breeding does, replacing 25% of soybean meal protein with sun-dried *Azolla* maintained feed conversion, litter size at weaning and female weight, as well as economic performances. However, conception rate, litter size at birth and milk production decreased (Sabra *et al*., 2006).

III.5.The use of Azolla in fish:

Aquarium studies indicate that *cichlids* (*Oreochromis*, *Tilapia* and *Cichlasoma*) as well as a grass carp x bighead carp hybrid tend to prefer *Azolla caroliniana* over other *Azolla* species (Antoine *et al*., 1986; Lahser, 1967; Fiogbé *et al*., 2004; Micha *et al*., 1988).

Chapter IV:

Raw materials used in Formulating feeds

IV.1.The energy sources:

The energy sources used in animal feed are mostly grains and which form a large proportion of energy stock in the animal body.

IV.2.grains:

IV.2.1.Barley:

Barley is the fourth most cultivated cereal in the world after corn, rice and wheat (Faostat, 2018), with an annual average production of 132 million tons. Barley also plays a key role not only in human consumption, but also as a livestock feed in winter when the forage deficit is large and the price of fodder is high (Grando *et al*., 2005). The barley (Hordeum Vulgare L) is used in animal feeds either cut or grant like fodder in winter and then harvest grain rewards; this practice is common in the North African (great Maghreb). According to (Ben Yousef *et al*.2001), this cereal also plays a welding role in the forage calendars between October and February, which allows it to cover a period when the majority of rain worm species are not yet productive. According to several authors, Jiao *et al* (2018) classified the types of barley according to the degree of fertility of the spikelet's and the compactness of the spikelet in two groups:

- The group of six-row barley.
- The group of barges with 2 rows.

❖ **The biochemical constituents of barley:**

Barley is essentially composed of nitrogen, minerals, starch and fiber; the latter exhibit the greatest interest in barley (Grando and Gómez, 2005). It also contains proteins with limited lysine content and lipids, the third of which is located in the germ. These constituents are distributed very differently according to the tissues. The various families of biochemical constituents of a barley grain are shown in following Table.

Table5 : Chemical composition of barely (Allosio-Ouarnier, 1999.)

Chemical compositions	Content in % of dried weight
Carbohydrates	78-85
Starch	63-65
Sucrose	1-2
Reducer sugars	0.1-0.2
Soluble polysaccharides in water (gums)	1-1.5
Soluble polysaccharides in organic solvents (hemicelluloses)	8-10
Cellulose	4-5
Others	1
Lipids	2-3
Proteins	8-11
Albumins	0.5
Globulins	3
Hordeins	3-4
Glutets	3-4
Amino acids and peptides	0.5
Nucleic acids	0.2-0.3
Mineral salts	0.2
Others including lignin	5-6

IV.2.2.Corn:

The grain of corn is essentially intended for human, animal food and as raw material in many agribusiness industries. In the northern regions, corn is used, like millet and sorghum, for the manufacture of local beer. The chemical composition of corn may vary depending on variety, soil and climatic conditions (Cromwell *et al*., 1999), which influences the nutritional characteristics of the grains (O'quinn *et al*., 2000). Nutritionally, it has low protein content (on average from 9 to 10%). As a result, the nutritional quality of the maize grain is relatively lower than those of oats (13%) and wheat (12%).This low nutritional value has caused serious nutrient deficiencies in the poorest populations that had adopted corn as unique food (Consoli, 2000). However, the creation of hybrids has improved the content of corn protein (Bruns and Abbas, 2005).

- **Composition of corn:**

Table 06 indicates that corn is interesting as an energy supply but that it is completed in human or animal nutrition with foods rich in protein and mineral salts.

Note that corn has an energy value that is highest among all cereals it comes up to its starch richness. However, its proteins present a very unbalanced amino acid profile, more particularly, for lysine and tryptophan with excess leucine (Amroune N. 2020).

Table6 : Composition of corn grain (Rouanet 1984).

Constitutions	Composition
Carbon hydrate	80%
Proteins	10%
Lipids	4.80%
Fibers	3.50%
Minerals	2.00%

IV.2.3.Wheat:

Wheat is a cereal appetizing, highly digestible, low fiber and especially rich in starch quickly fermentable. On a dry matter basis, the energy value of this cereal (expressed in metabolizable energy, [EM]) is generally similar to that of corn and greater energy found in other cereals, such as oats and barley (Johanne, 2005).

- **Chemical composition of the different parts of the wheat grain:**

The grain is mainly consisting of starch (about 70%), protein (10 to 15%) according to varieties and culture conditions), the other constituents, weights minors (only a few %), are Lipids, cellulose, free sugars, minerals and vitamins.

Table7: Chemical composition of wheat (Feillet, 2000).

Composition nature	Content (% DM)
Proteins	10-15
Starch	67-71
Pentosans	8-10
Cellulose	2-4
Free sugar	2-3
Lipids	2-3
Minerals	1.5-2.5

IV.3.Protein sources:

IV.3.1.Soy:

Soy is an excellent source of good quality protein, is a raw material widely used in animal feed. (Benabdeljelil, 1999), Soy cakes is a source of amino acids par excellence, they account for 50% of world production. (Johan, 2005), Compared to other sources of vegetable protein, soybean meal contains little fiber and a lot of energy. Thus, when properly processed, contains no toxins or antinutrients (Darwin and Britzman, 1994).

According to NOPA (1997), the different soy products are obtained by separation or extraction of the different soy components, we distinguish:

1. Full seed soy.
2. Crushed soybeans.
3. Soy cake mechanically extracts.
4. Kibbled soybean meal.
5. Cracked soy cake extracted by solvent.
6. Soybean oils.

❖ **The components of soybean cake**:

Soy cake is widely used in poultry feed, generally associated with corn. For the rabbit is incorporated with levels ranging from 10 to 20%.

IV.3.2.Faba bean seed:

Faba bean seeds are made up of a tegument (shell), and a cotyledon (kernel), the shell represents 15.50 to 14.7% compared to the whole seed depending on the cultivar (Wang and Uberschar, 1990; Kaysi and Melcion, 1992).

Faba bean seed is an interesting nitrogen source in animal feed because of its high rate of crude protein which is in genetic and environmental variability, it is significant in spring bean (average of 28% and with a variation of 25 to 36% of TNM) than in the winter types (average 25% with a variation of 22 to 30%), (Gallais and Bannerot. 1992).

Its proteins rich in lysine, but low in sulfur amino acids and tryptophan which limits their use, it is also rich in starch (Larbier and Leclerrcq 1992). There are winter beans and spring beans, the spring varieties are richer protein than winter.

Table8: Total nitrogen matter content of winter beans and that in spring (Kotwika *et al.*2021)

Faba beans	Extreme	Average
Winter beans	22.7 – 31.5	26.7
Spring beans	24.2 – 34.1	27.3

- **Chemical composition and nutritive value:**

Table9: Nutritive value and chemical composition of faba bean (Sauvant *et al.* 2004), (Prolfa. 2009)

Nutritive value of rabbit	Faba beans
DE (kcal/kg)	3073
ED (%)	79
ND(%)	80
Chemical composition(%)	
Proteins	29
Starch	44
Cellulose	9
Fat	2
Minerals	4
Other fibers and soluble sugars	12

IV.4.Protein seeds:

IV.4.1. Description of protein seeds:

- **Appearance and chemical composition:**

Protein seeds come from plants belonging to the legume family. (Fabaceae).This family of dicotyledonous seeded flowering plants contains about 765 genera, which include more than 19,000 species. Among the most cultivated legumes, for their interest as a source of vegetable protein both for animals and for man, are found soybeans, faba beans, peas and lupins, but also beans, vetch, grass pea, lentil, chickpea, alfalfa, clover, etc. The large number of species of legumes leads to a wide diversity of appearance of these plants, which can be perennial or annuals, can grow in the upright form or in lianas, shrubs or even in TREE. The root system is often taproot. Of all the legumes, interest is focused here on grain legumes, and in particular faba bean, lupine and pea (Terres Univia, 2019).

Table10: Chemical composition of lupin, faba beans, and peas seeds ([1] INRA, 2018/ [2] Heuzè et al, 2018a).

	Lupinus albus[1]	Lapinus angustifolius[1]	Lapinus luteus[2]	Vicia faba[1]	Pisun sativum[1]
DM (%)	88.1	90.2	88.8	86.6	87.2
OM (g/kg DM)	960	965	954	962	965
MAT (g/kg DM)	380	337	422	295	233
NDF (g/kg DM)	219	239	243	155	145
ADF (g/kg DM)	165	191	197	112	72
MG (g/kg DM)	98	60	54	15	13
Starch (g/kg DM)	81	48	61	442	513
PDI (g/kg DM)	124	126		112	99
UFL (UF/kg DM)	1.43	1.34		1.26	1.25
Lysine (%)	6.54	6.58		7.41	7.78
Methionine (%)	1.44	1.47		1.48	1.72

IV.5.Oil seeds:

Seeds contain on average 18, 33, 42 and 44% fat respectively. The richness in energy is associated with relatively high levels of protein, hence their name of oilseeds and protein crops. (Terres Univia, 2019).

IV.4.1.Rapeseed:

Rapeseed varieties grown in France and used in animal feed are varieties called double zero or "00". These do not contain erucic-acide and have low levels of glucosinolates. In theory, Rapeseed can therefore be used in the diet of monogastrics and ruminants. Their incorporation into rations is however limited by their high lipid content. Indeed, too high proportions of fat in feed can lead to problems with cohesion of the feed as well as palatability problems for some animals. (Terres Univia, 2019)

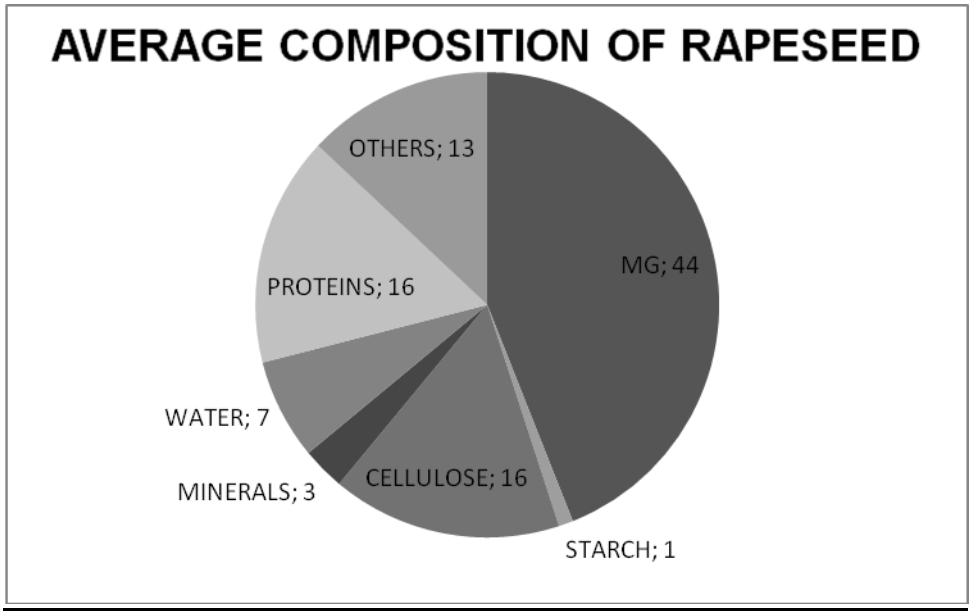

Figure 8: Average composition of rapeseed grain (Feedbase 2023).

IV.4.2.Sunflower:

Sunflower seeds are made up of thick shells that are rich in indigestible fiber. This characteristic explains the lower energy content compared to other oilseeds. As a result, sunflower seeds are little used as a feed for fast-growing animals such as broilers and slaughter pigs. On the other hand, the high fiber content of these seeds makes their use interesting for feeding sows, rabbits and certain ruminants. Sunflower seeds are also widely used in the bird industry. (Terres Univia, 2019).

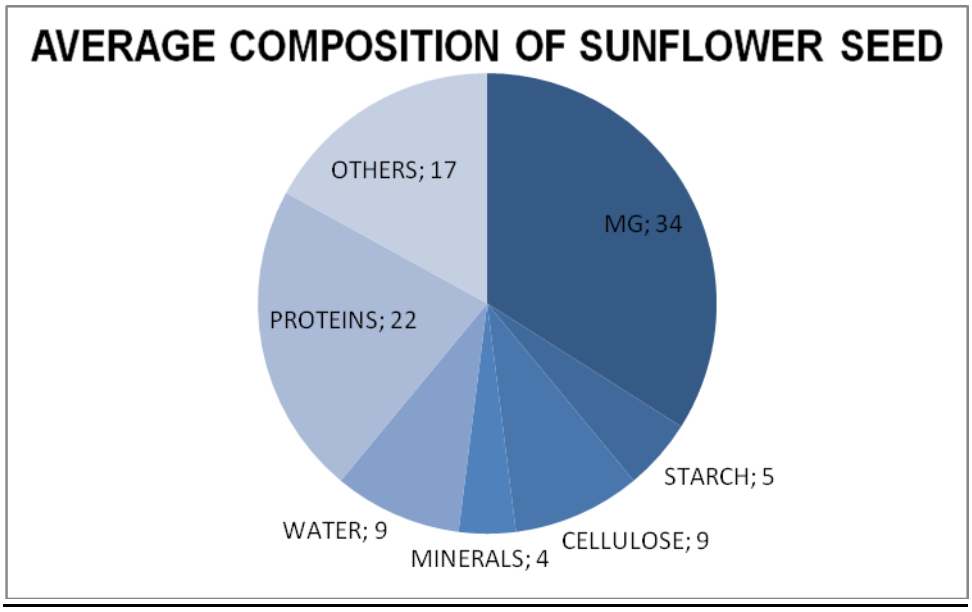

Figure 9: Average composition of sunflower grain (Feedbase 2023).

IV.4.3.Soybean:

Soybean contains varying amounts of trypsin inhibitors, an antinutritional factor that negatively affects protein digestion and animal growth. For this reason, soybean cannot be used as such for food. They require at least a heat treatment capable of inactivating these factors for feeding monogastrics (poultry, pigs, and fish) or to improve the digestibility of proteins by ruminants. (Terres Univia, 2019).

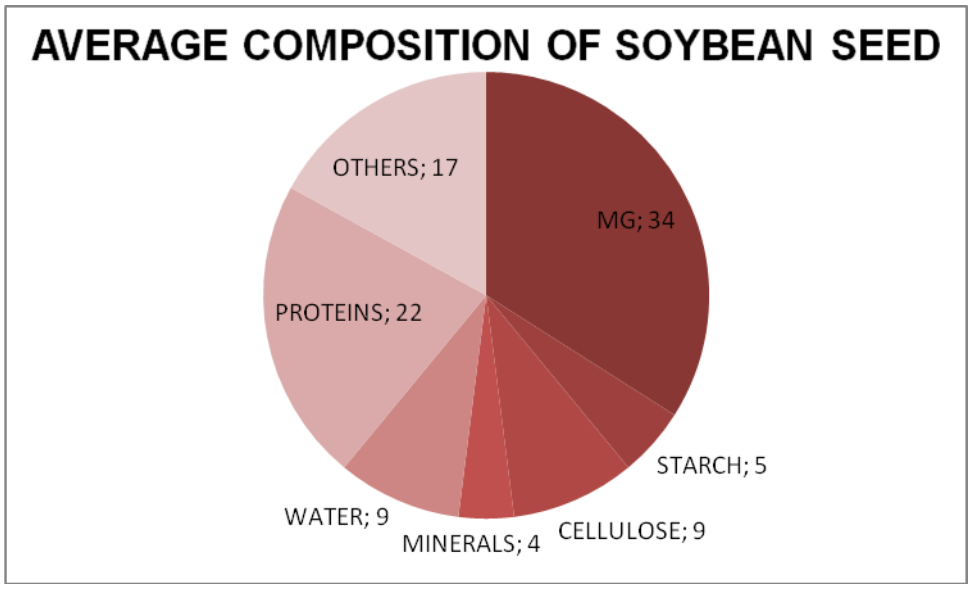

Figure 10: Average composition of soybean grain (Feedbase 2023).

IV.4.4.Linen:

Flaxseeds are characterized by their richness in omega 3, in particular an essential fatty acid called alpha-linolenic acid. The incorporation of flaxseed into animal feed rations has a positive effect on the omega 3 content of the meat and eggs produced. As with soy, however, flax requires heat treatment prior to its use in food to eliminate certain factors harmful to animal health. (Terres Univia, 2019).

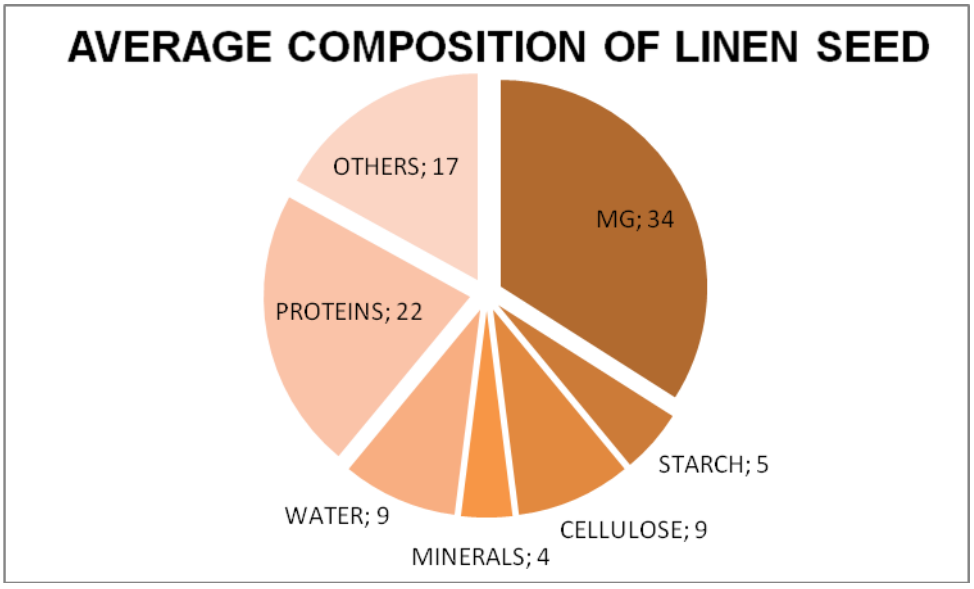

Figure 11: Average composition of flaxseed grain (Feedbase, 2023).

IV.5.The Oilcakes:

Compared to soybean meal, source reference nitrogen in animal feed, rapeseed and sunflower cakes are lower in protein and higher in crude fiber (Table 1). Rapeseed meal is however well balanced in amino acids primary limiting agents (lysine, methionine) then that soy is deficient in methionine, while sunflower is deficient in lysine. The concentration of digestible lysine and methionine in rapeseed is high and close to the recommended thresholds for dairy cows. Finally, the rapeseed meal is rich in calcium and phosphorus, (Base de donnés lo7 – Assosiation française de Zootechnie, 2004)

Table11: Average composition of oilcakes (Cetiom Onidol, 2004).

	Rapeseed	Unshelled sunflower	Hulled sunflower	Soy 48
Proteins (%)	34	29	33	45
Cellulose (%)	12	24	21	6
Fat (%)	2.3	1.9	1.7	1.9
Calcium (g/kg)	8.3	3.9	4.1	3.4
Phosphorus (g/kg)	11.4	10.1	10.8	6.2
Lys available (%)	6.8	5.9	5.8	6.9
Met available (%)	2	2.1	2.1	1.5
Glucosinolates (µmoles/g DM)	5-20 [a]	-	-	-

Chapter V:

Materials and methods

V.1. Study objectives:

The aim of our experiment was to study these different objectives:

1. The interest of using *Azolla* as an ingredient in the formulation of feed for monogastric animals.
2. To determine its nutritional value.
3. To estimate the quality and productivity of this plant in animal nutrition.
4. To estimate the economical value it adds by replacing conventional feed.

Two experiments were carried out in order to cultivate the *Azolla* in two different periods and sites, the first experiment was done outdoors using a basin of 6 m² (classical method of culture) and another in a closed environment under greenhouse in a basin of 3.5 m².

V.2. Experiment 01:

V.2.1. Area and duration of the study:

The installation of the basin of culture of *Azolla* was carried out between, (08 Jun to 02 November 2022, at the level of a plot which is located at a farm in Theniet El Had – Tissemsilt.

This area is characterized by a cold weather in winter (11 °C in November), and high temperatures in summer (21.5 °C in June). Source: (Notional park of Theniat-El Had, 2012).

V.2.2. The material used:

V.2.2.1. Vegetal material:

The cultivated *Azolla* species is *Azolla pinnata*, the plant comes from an *Azolla* production unit in the Wilaya of Ain-Defla, the plant was harvested in spring and it was transferred to Tissemsilet in 2 hours.

Figure 12: *Azolla pinnata*.

V.2.2.2. Measurement equipment (for both experiments 1 and 2):

The material that was used to track the various parameters is as follows:

- **Thermometer:**

Figure 13 : Thermometer.

V.2.2.3. Equipment used for setting up the basins:

Fertile soil, horse manure, bricks, black tarpaulin, cartoon sheets, shovel, axe, colander, water tank to reserve water, water house.

V.2.3. The experimental protocol:

For the first experiment it was necessary to prepare the ground before the installation of our basin. The plot where the basins were set up has been mowed, this step is important because the grass can compromise the experience by perforating the tarp, thus causing a water leak. In

order to avoid this, after mowing, it is preferable to use boxes on the delimited surface of the basin.

V.2.4. Basin installation:

We chose a plot in the backyard of the house located in Thniet el had – Tissemsilt, and surrounded it by iron fence, to protect it from animals and human hands, first step was to brief an area of 4 meters length and 1.5 meters width, in constriction stones (Bricks).On a depth of 30 cm.

Second step was to put the black plastic sheet of tarpaulin on a double layer (using black color was to prevent sunlight from passing to the ground and contribute to the emergence of herbs and harmful plants and thus puncturing the double layer and cause water licking.

As organic matter we have used old horse manure (more than 6 months old) and placed on the plastic tarpaulin with a height of 5 cm. We added 5 cm of moist soil and the last step was adding water to the basin, reaching a height of approximately 20cm.

Than we left the basin for 24 hours to allow us to remove any excess waste that floated on the surface of the water, (which would hinder the growth and reproduction of *Azolla* in the future).

Figure 15: Day 01 after implantation	10 after implantation

V.3. Experiment 02:

V.3.1. Area and duration of the study:

The installation of the water basin took place in the following period of time (from 22 February 2023 to 01 May 2023), in a greenhouse inside Tissemsilt University behind the faculty of sciences of nature and life, in the Wilaya of Tissemsilt.

V.3.2.The material used:

V.3.2.1. Vegetal material:

The cultivated *Azolla* species is *Azolla*.*Microphylla*, the plant comes from an *Azolla* production unit in the wilaya of Ain-Defla, the plant was harvested in spring and it was transferred to Tissemsilt in 24 hours.

V.3.2.2. Equipment used for setting up the basins:

Fertile soil, organic matter, black tarpaulin, Cartoon sheets, shovel, axe, colander, water tank to reserve water, Transportation vehicle, water house, electronic balance.

V.3.3.The experimental protocol:

We followed a set of steps before installing the water basin, represented in preparing the ground as we did with the first experiment.

V.3.4. Basin installation:

We dug the basin that is 2.5 meters long and 1.4 meters wide, with a height of 0.40 meter, we leveled the boundaries well, then we laid cardboard pieces on the ground as a first step (to prevent puncturing the plastic and thus water leakage).

Then we fixed the black plastic on top of the cardboard pieces, then we spread 3 kg of organic matter, and then added moist soil with a height of 5 cm, we filled it with water to a height of 25 cm, so as to allow the *Azolla* to float freely on the water without touching its roots to the soil and thus hindering its growth.

Then, as we did with the first experiment, we left the basin for only 12 hours (to clean it from any floating debris on the surface of the water).

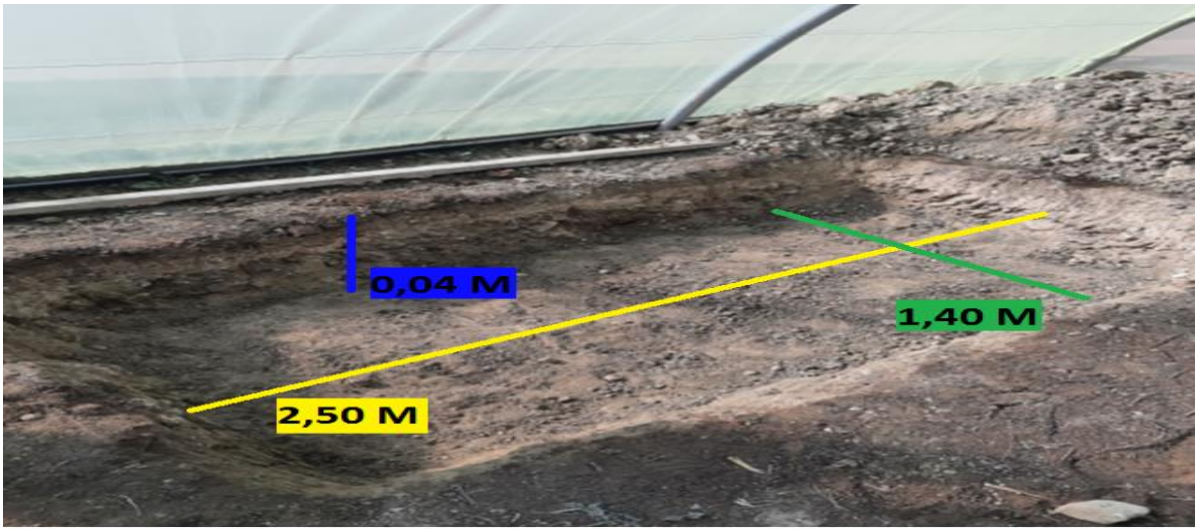

Figure 16 : First step (digging the basin).

Figure 17: Second step (installation of the plastic double layer).

Figure 18: Third step (adding soil+OM).

Figure 19: Fourth step (adding water the basin).

Figure 20: After 1 day of implantation.

V.3.5. Planting the Azolla in the basins:

In both experiments, we planted 2 kg of *Azolla* plant at two different times. We brought the *Azolla* and dispersed it evenly on the surface of the water, so that it can move and reproduce easily and comfortably.

V.3.6. Course of the experiment:

V.3.6.1. Measuring the temperature of the air and water:

In this part, we measured the temperature of the air surrounding the basin inside the greenhouse using a thermometer, as well as the temperature of the water inside the basin.

We inserted the thermometer into the basin and left it for a period of approximetly one hour.

V.3.6.2. Measuring the quantity of water added to the basin daily:

We added water to the basin in the day of 30/03/2023, and in 19 and 28/04/2023. The quantity of 10 liters was added to the basin using a 5L flask.

V.3.6.3. Harvesting and drying Azolla and monitoring the performance of the basins:

For the harvest, it was done three times all along this trial. After cultivating the plant of *Azolla* inside of the basin, we left it a period of time (more than a week) so it can reproduce well enough before starting the process of harvesting.

For harvesting we have used a colander of kitchen (figure 21). We used an electronic balance to measure the weight of the harvested *Azolla* before and after drying. Drying azolla was done outside under sun. (Figure 23)

Figure 21: Using a colander for harvesting the Azolla

Figure22: Using a balance to measure the harvested Azolla.

Figure 23: Using a balance to measure the dried Azolla

Figure 24: Drying *Azolla* in air.

V.4. Laboratory analyses:

V.4.1. Determination of chemical composition of Azolla:

Analyzes of *Azolla* dried had been done in a privet laboratory in the willaya of Oran. Methods of the AOAC (2000) were used to analyze DM (934.01), CP (2001.11). Ether extract was determined after acid-hydrolysis treatment (EC 1998). Dietary NDF (without sodium sulphite), and ADF were sequentially determined using the filter bag system(Ankom Technology, New York) according to Mertens *et al* (2002) and AOAC procedure(2000, 973.187). The gross energy was measured with an adiabatic bomb calorimeter.

V.4.2. Formulation of standard broiler, rabbit, laying and reproductive hens feed without Azolla:

As a first step in the process of trying to incorporate *Azolla* plant in the composition of animal feed such as broilers, rabbits, laying and reproductive hens we have prepared a formula for each of these animals in their various stages of developments and growth.

V.4.3. Ingredients and their chemical composition used in the formulation of different foods:

Different information about ingredients and their chemical composition were extracted from the animal feed production unit FAB GRAIN – Tiaret.

Table 12: Chemical composition of ingredients used in formulation (%).

	Dry matter	Crude protein	Ether Extract	Crude fiber	Starch	ME, Kcal/Kg	Ca	P
Soymeal	92	44	4.7	1.8	31	3200	0.3	0.75
Soybean oil	.	.	.	.	.	9000	.	.
Barley	88	10.3	2	4.6	51	3030	0.06	0.36
Alfalfa	87	15.3	3.2	26.1	0.2	1770	1.5	0.26
Wheat straw	90	3.6	1.2	39.6	0.5	660	0.38	0.08
Calcium carbonate	85	.	.	.	.	.	35	.
Mono-calcium phosphate	88	.	.	.	.	.	18	24
Wheat bran	84	14.9	3.5	8	23	1930	0.12	0.92
Corn	89.7	8.63	3.6	2.3	63.3	3280	0.03	0.25
Corrector[1]	.	.	.	.	.	.	.	.

[1] Provided by Sarl vitavite (Skikda, Algeria), (mg/kg): Mn: 4100; Zn: 11740; Cu: 2000; I: 250; CO: 99; Fe: 16000; Niacin: 4000; Betaine: 10830; Choline: 27500; Vitamin K: 200; Vitamin B1: 200; Vitamin B2: 400; Vitamin B6: 200; Vitamin A: 1700000 UI/Kg; Vitamin D3: 150000UI/Kg; Vitamin E (α-tocopherol).

V.4.4. Formulation of standard feeds of monogastric animals:

Formulation of different feeds was done using a logical of formulation **ALLIX 03** which is used in the animal food production unit **FAB GRAINS**, according to the requirements of

each species of animals as it is shown in table 13. Formulas of different feeds and their nutritional value are shown in tables 14, 15 and 16.

Table 13: Animal's feed requirements.

	Broiler			**Rabbits**		**laying hens**	**reproductive hens**
	Start	**growth**	**Finish**	**Growth**	**fatening**		
ME(Kcal/Kg)	3000	3060	3120	2250	2450	2730	2800
CP	20.8	19.5	17.6	16.5	15.5	15.8	16.4
EE	3.4	3.2	3.1	3	2	1.5	4.5
CF	2.8	3	3	16.5	15.5	3.6	3.5
Ca	0.93	0.84	0.75	0.8	0.8	4.2	3.1
P	0.44	0.41	0.36	0.4	0.4	0.32	0.53

Refferences	**FEDNA 2017**	**De Blas and Mateos (2010)**	**FEDNA 2017**

Table 14: Ingredients and chemical composition of broiler diets (%).

	Start	**Growth**	**Finish**
Corn	53.4	53.4	50.5
Soymeal	32	29	25
Soybean oil	0	1	2
Calcium carbonate	2.4	2.4	2.5
Dicalcium phosphate	1.2	1.2	1
Wheat bran	10	12	18
Corrector[1]	1	1	1
Chemical composition %/ DM			
Dry matter	89.4	89.6	87.5
Crude protein	20.18	19.15	18.04
Ether Extract	3.7	3.7	3.62
Crude fiber	2.6	2.7	3.05
Calcium	1.16	1.16	1.25
Phosphate	0.72	0.72	0.69
Starch	46.02	45.5	43.8
Metabolisable energie, Kcal/Kg	2968	2998	3100

Table 15: Ingredients and chemical composition of rabbit's diets (%).

	Start	**fatening**
barley	21	25
Corn	4.5	4.5
Soymeal	18.5	16
Soybean oil	2.5	2.5
Calcium carbonate	0.5	0.5
Dicalcium phosphate	0.5	0.5
Wheat bran	12.9	12.9
Wheat straw	14	14
Alfalfa	25	22.5
Corrector[1]	0.5	0.5
Chemical composition %/ DM		
Dry matter	89.1	88.2
Crude protein	16.99	15.2
Ether Extract	4.71	1.65
Crude fiber	15.51	15.7
Calcium	0.84	0.79

Phosphate	0.48	0.46
Starch	15.93	16.2
Metabolisable energie, Kcal/Kg	2350	2456

Table 16: Ingredients and chemical composition of laying and reproductive hen's diets (%).

	Laying hens	**Reproductive hens**
Corne	57.75	59.6
Soymeal	21	22
Soybean oil	1.5	1
Calcium carbonate	7.5	7
Dicalcium phosphate	1.25	1.4
Wheat bran	10	8
Corrector[1]	1	1
Chemical composition %/ DM		
Dry matter	57.7	88.5
Crude protein	15.42	16.62
Ether Extract	2.51	4.23
Crude fiber	3.17	3.47
Calcium	4.31	3.3
Phosphate	0.48	0.68
Metabolisable energie, Kcal/Kg	2841	2865

V.4.5. Estimation of the price of different feeds formulated:

The estimation of prices of different foods formulated was done using a list of prices of different ingredients used. The prices of ingredients were also brought from the animal food production unit Fab Grain – Tiaret as it is shown in the following table:

Table 17: Price of different ingredients used in formulation of foods (DA/ Kg).

Ingredients	**Price DA/Kg**
Corn	53
Soymeal	108
Soybean oil	150
Calcium carbonate	5
Dicalcium phosphate	270
Wheat bran	32
Corrector of brioler	370

Corrector of rabbits	280
Corrector of laying and reproductive hens	320

V.4.6. Estimation of the price of production of azolla:

The price of *Azolla* was estimated using the different charges used in realizing and cultivating of *Azolla* in our experiment (the price of different equipments and materials) as it is shown in table19. At the last of experiment we have estimated the yield of production of *Azolla* in the basin in Kg in order to estimate the cost of production of 1 Kg of *Azolla*.

Table 18: The price of different materials used in cultivating of *Azolla*.

Equipements	Price in DA	Quantity used	Cost (DA)
Vegetal materials(*Azolla*)	1000DA/Kg	2Kg	2000DA
Plastic	120DA/ m²	4 m²	480DA
Organic matter	150 DA/Kg	3Kg	450DA

V.4.7. Incorporating *Azolla* in different formulas of monogastric animals:

Azolla was incorporated in the different formulas by different rates so that does not affect the nutritional value of food. For the formulas of broiler *Azolla* was used at the rates of 10%, 15% and 20% for start, growth and finish food respectively, for rabbits formulas it was used at the rates of 20% and 30% for growth and fattening foods respectively. However, for laying and reproductive hens *Azolla* was used at the rates of 20 % for both of them.

Chapter VI

Results and discussions

VI.1.Evaluation of temperature:

After our experiments (1 and 2), we are demonstrating only the results of the second experiment, and are shown next in the following graphs:

V.1.1.For the measurement of the temperature in water and in air:

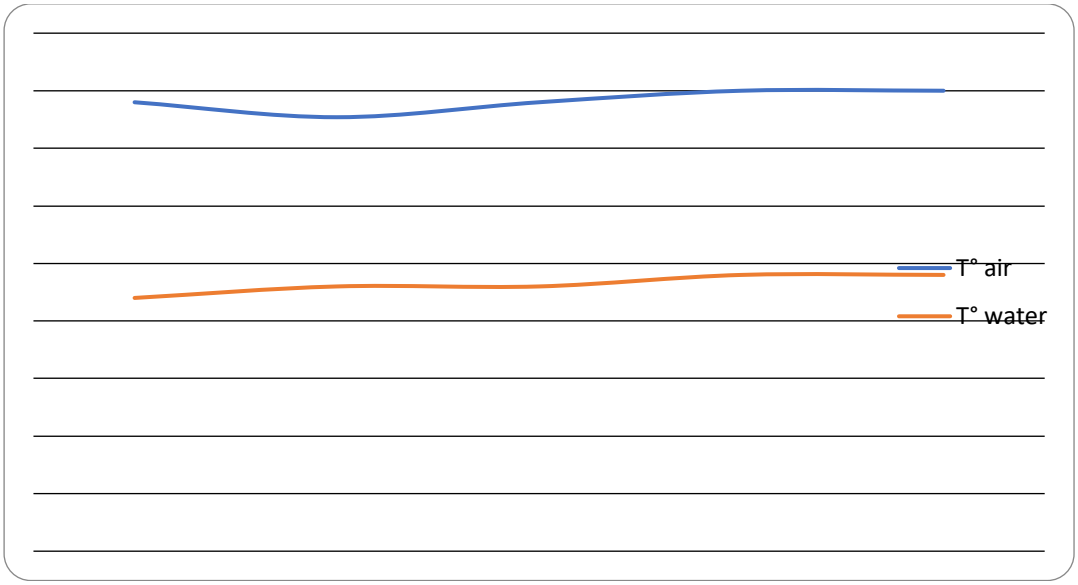

Figure 25: Water and air temperature measurement.

We have observed a relative change in the indoor air temperature of the green house, in the department of natural sciences and life. On the first week, the temperature was measured at 39°C, followed by a decrease of 1.3 °C on the second week. On the third week, the temperature increased to 39 °C and returned to its original value. On the fourth week, the temperature increased by one degree Celsius until the fifth week.

The temperatures within the water basin were relatively uniform. On the first and second week, the temperature was measured at 22 degrees. It increased by one degree on the third week, reaching 23 degree, and similarly increased by one degree on the fourth week to reach 24 degrees, thereafter, the temperature remained constant at this value on the fifth week.

According to Van Hove (1989), the ambient temperature is the main element that promotes the growth of the plant which is measured between 20 and 30 C˚, including the case of the *Azolla pinnata*, it can withstand values exceeding 35 C˚, high heat or extreme cold shows down the development of fodder.

VI.2. Results regarding the harvesting and after drying weights and the days of every process:

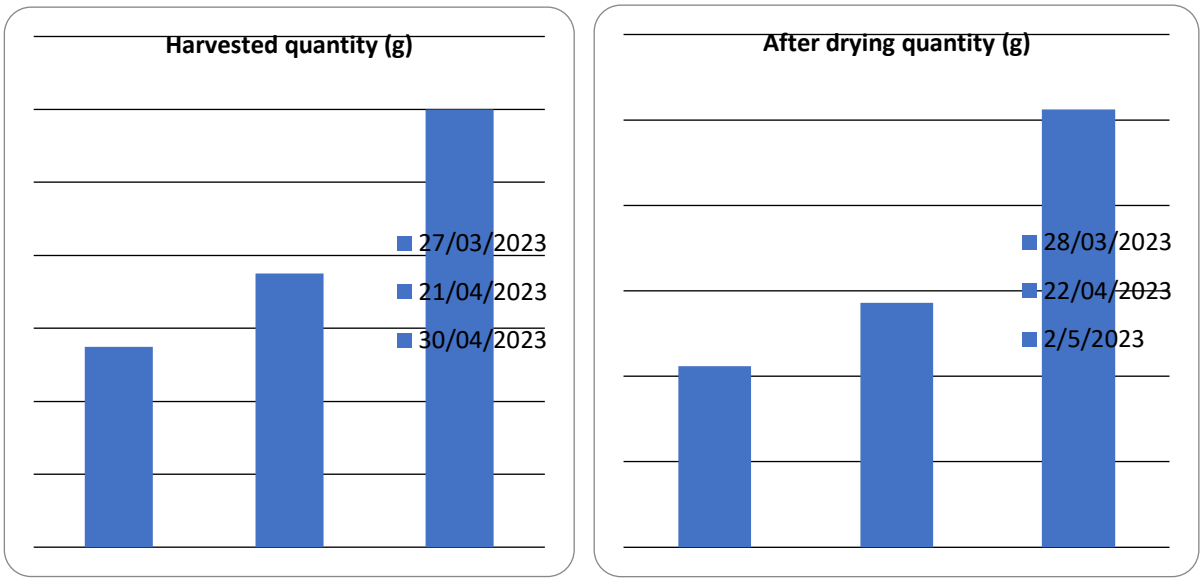

Figure 26: days of harvest and drying Azolla samples, and their weights.

In the first day of harvest (27/03/2023), the quantity harvested was estimated by 5.5Kg (5500 g), this harvested quantity per day is close to the result by Leterme *et al.* (2009) and after that by exactly 24 days, we noticed a tangible progress in the harvested Q, reaching a 7.5 Kg ,and just after 9 days we observed a high development of the mass of *Azolla* in the basin, so we harvested for the third and last time 12 Kg, of fresh *Azolla*, with a progress reaching a 4.5 Kg of mass.

Furthermore, in the second graph (after drying) it is obvious that there is a very high progress to the mass dried in each process, so that in the first day of drying 28/03/2023, we had 1060 g of harvested fresh *Azolla* of a mass (5500 g), which represent 19 % of the mass, and on day 2 (22/04/2023), we gained 1430 g representing 19 % of the mass as well, and in the third day (02/05/2023), we gained 2560 g of dried *Azolla* from 12000 g of harvested mass, which is 21 % of *Azolla's* mass.

We notice that the *Azolla* after drying, decrease in weight, and we explain that the plant contain a very high percentage of water. The total quantity of harvested *Azolla* was estimated by: 25000g of fresh matter (25 Kg). The total quantity of dried Azolla was estimated by: 5050g of dry matter (5.05Kg).

After tracking yield in basin 2, we deduced that the Azolla at a high multiplication frequency, the more time passes, the more its speed of growth increases. One of the advantages of the Azolla that Kumar and Chander, (2017) has also confirmed.

VI.3.Results of chemical composition of dried Azolla:

The (table 19), represent the chemical composition of the harvested and dried *Azolla*, we notice that crude protein is the main element of high value, taking 24.8 % of DM, and also crude fiber (18.52% of DM), EM (2750 Kcal/Kg).

Table 19: Chemical composition of Azolla (% DM).

Parameter	Quantity (%)
Dry matter (DM)	9.2
Crude protien (CP)	24.8
Ether Exctract (EE)	3.53
Crude fiber (CF)	8.52
Neutral Detergent Fiber (NDF)	39.94
Grosse energy Kcal/Kg	2750
Ca	1.6
P	0.3

The results of CP value obtained in the present study was found to be similar to values reported by Querubin *et al.,* (1986) in *A.microphylla* (23.40%) and Alalade and Lyayi (2006) in *A.pinnata* (21.40%). However, Becerra *et al.,* (1995) and Indira *et al.,* (2009) reported higher CP content 26.7% in *Azolla microphylla* and 28.24% in *Azolla pinnata*, respectively. Whereas, Kumar *et al.,* (2014) reported the CP content was found to be 15.4% in sun-dried Azolla and 17.3% in *Azolla microphylla* which were lower than values found in the present study. The possible reason of variability in CP content is the response of *Azolla* strains to environmental conditions like temperature, light intensity, water availability and soil nutrients which affect chemical composition.

The crude fiber content on % DM basis in *Azolla* was found to be 8.52%. Closest results were recorded by Becerra *et al.,* (1995) in *Azolla microphylla* and Ghodake *et al.,* (2012) in *Azolla pinnata* were 11.2% and 9.07%, respectively. However, our results were lower than those recorded by Arvindraj (2012), Sharma (2013) and Kumar *et al.,* (2014) found to be 12.45%, 12.63% and 12.02% CF in *A. microphylla*, respectively.

The ether extract values (3.53%) recorded in present study was similar to the findings reported by, Basak *et al.,* (2002), Sharma (2013) and Kumar *et al.,* (2014). However, Buckingham *et al.,* (1978), Becerra *et al.,* (1995) and Arvindraj (2012) recorded higher values of 5.05%, 4.6% and 4.06% in *A. microphylla*, respectively.

In the present study neutral detergent fiber (NDF) content of *Azolla pinnata* was found to be 41.84% which was comparable with the values recorded by Sharma (2013) and Kumar *et al.,* (2014) in *A. microphylla*. However, Indira *et al.,* (2009) in *A. pinnata* and Arvindraj (2012) in *A. microphylla* recorded higher NDF values of 72.05% and 68.43%, respectively.

Furthermore, calcium and Phosphorous were found to be 1.6% and 0.3%, respectively which was similar for the content in phosphorus to earlier reports of Sujatha *et al.,* (2013), Shamna *et al.,* (2013). But the content of calcium was higher than that was found in their studies recording the values of 0.98%.

Beside, the value of gross energy is 2470 kcal/kg DM, and this is in accordance with that obtained by (Khatun *et al* 2008) who found the value of 2431 kcal/kg. In contrast, our results are higher than previous results of 1759 kcal/kg DM reported by Parashuramulu *et al* (2013).

VI.4.Nutritional value of standard formulas:

Formulation of diets was done to reach all requirements of different animals used according to the data base of FEDNA (2017) for formulas of broiler. For rabbits formulas we have used the requirements reported by De Blas and Mateos (2010). While, in reproductive and laying hens we have used also the data base of FEDNA (2017).

VI.5.Effect of the use of azolla in monogastric animal's feed:

After analyzing *Azolla* it was able to incorporate it in different formulas so that replace some other ingredients without altering the nutritional value of diets. The most important nutrient in formulating foods for monogastric animal is proteins and the main source of proteins as ingredient used in formulation especially in our country Algeria is soy meal with 44 to 46 % of CP (FEDNA 2017). However, *Azolla* with approximately 25 % can be used as a very interested ingredient to replace a part of soy meal in different diets for monogastric animals.

VI.6.Effect of azolla in broiler feed:

The rates of using *Azolla* and the nutritional value of diets of broiler in different stages of life (start, growth and finish) are shown in table 20.

Table 20: Effect of the use of *Azolla* on nutritional value of broiler diets (%).

	Start	**Growth**	**Finish**

Corn	42.4	44	49.5
Soymeal	26	21.5	18
Soybean oil	0	1	2
Calcium carbonate	2.4	2.3	2.5
Dicalcium phosphate	1.2	1.2	1
Wheat bran	17	14	5
Corrector[1]	1	1	1
***Azolla* meal**	**10**	**15**	**20**
Chemical composition %/DM			
Dry matter	81.7	82.6	81.4
Crude protein	20.11	19.06	18.12
Ether Extract	3.69	3.61	3.54
Crude fiber	4.65	5.29	5.81
Calcium	1.31	1.39	1.4
Phosphate	0.75	0.7	0.63
Starch	.	.	.
Gross energie, Kcal/Kg	2904	2978	3145

The results of nutritional value of diets for broiler in start, growth and finish phases of life using 10 %, 15 % and 20 % respectively of *Azolla*, reported that the use of this later don't change the global nutritional value of diets. Similar results for nutritional value of diets for broiler using 15 % of *Azolla* meal were found by Pinkihan (2013). In this later study it was recorded that the use of *Azolla* in broiler diets with rates between 5 and 15 % can improve growth performances and meat quality without altering nutritional value of standard diets. However, Pinkihan (2013) found that incorporating 15 % in broiler chicken feed ration can improve growth of body performance and showed no adverse effect on nutritional value of diet and nutrient digestibility of the bird.

Beside, the incorporation of *Azolla* in broiler diets doesn't affect the feed consumption of birds and can improve the palatability of foods (Blair ., 2018). However, the results found by Pinkihan (2013) affirm that the incorporation of *Azolla* can improve feed conversion in broiler.

Indeed, this previous result leads us and can confirm the positive effect of using azolla in feeding broiler.

VI.7. Effect of Azolla in rabbits feed:

Results of incorporating *Azolla* meal in rabbit's diets in growth and fattening phases at 20% and 30% respectively on nutritional value are shown in table 21.

Table 21: Effect of the use of *Azolla* on nutritional value of rabbit's diets (%).

	Start	**fatening**
Barley	21	22
Corn	0	0
Soymeal	10	5
Soybean oil	2.5	2.5
Calcium carbonate	0.5	0.5
Dicalcium phosphate	0.5	0.5
Wheat bran	13	15
Wheat straw	14	14
Alfalfa	18	10
Corrector[1]	0.5	0.5
Azolla meal	20	30
Chemical composition %/DM		
Dry matter	78.2	75.4
Crude protein	16.67	15.98
Ether Extract	5.28	5.57
Crude fiber	16.78	16.49
Calcium	0.75	0.58
Phosphate	0.46	0.48
Starch	.	.
Metabolisable energie, Kcal/Kg	2464	2542

In formulating rabbit's foods the important nutrients which must be respected at the first place are proteins and fiber. The main source of proteins as in poultry diets is soy meal, while the source of fiber especially NDF in rabbits foods is alfalfa which contain approximately 41% of NDF (feedpedia, 2019). *Azolla* content of NDF is found 39% in our study in addition of its content in protein can be used as potential ingredient in rabbit's food. Therefore, *Azolla* can be used without altering or affecting negatively the nutritional value of diets for rabbits.

According to Van Hove (1989), research on the introduction of *Azolla* in rabbit feed is very recent and has not yet given rise to applications in the farming environment. However, the preliminary results are encouraging and suggest that, given an appropriate choice of species, based on both nutritional value and organoleptic qualities, *Azolla* could be introduced at a rate of 20 to 30% in the food ration.

VI.8.Effect of azolla in laying and reproductive hens feed:

Azolla was incorporated at rate of 20% for both of laying and reproductive hens. Nutritional value of formulas is shown in table**.**

Table 22: Ingredients and chemical composition of laying and reproductive hens diets (%).

	laying hens	**Reproductive hens**
Corn	50	46,1
Soymeal	11	12
Soybean oil	1,5	1,5
Calcium carbonate	7,5	7
Dicalcium phosphate	1	1,4
Wheat bran	8	11
Corrector	1	1
Azolla meal	20	20
Chemical composition %/DM		
Dry matter	81,4	82,6
Crude protein	15,3	16,42
Ether Extract	3,3	3,34
Crude fiber	5,69	5,75
Calcium	4,28	3,28
Phosphate	0,61	0,67
Metabolisable energie, Kcal/Kg	2831	2822

In previous studies, *Azolla* meal improve egg output by hens in terms of daily egg production, egg weight and egg mass. Scientific evidence existed that addition of *Azolla* in layers diets resulted in increasing egg production and egg weight at the expense of feed cost (Seid, 2023). Kumar and Chander (2017) observed an appreciable increase in egg laying capacity (10-15%) in chickens after consumption of *Azolla pinnata*. However, *Azolla* meal evidently play an important role in improving laying performance and feed efficiency without any adverse effect on feed intake and weight changes (Seid, 2023).

VI.9.Estimated cost of different formulas:

The prices of the ingredients are those applied on the market during their acquisition for the conduct of the test, except that of the kilogram of *Azolla* which was estimated at 38 DA/Kg.

Estimated cost of different standard formulas for broiler, rabbits, and laying or reproductive hens are shown in the following tables.

Table 23: Estimated price of ingredients and total cost of broiler diets (DA / Kg).

	Quantity of ingredients in Kg			**Price DA/Kg**	**Cost of incorporated ingredients in DA**		
	Start	**Growth**	**Finish**		**Start**	**Growth**	**Finish**
Corne	53,4	53,4	50,5	53	2830,2	2830,2	2676,5
Soymeal	32	29	25	108	3456	3132	2700
Soybean oil	0	1	2	150	0	150	300
Calcium carbonate	2,4	2,4	2,5	5	12	12	12,5
Dicalcium phosphate	1,2	1,2	1	270	324	324	270
Wheat bran	10	12	18	32	320	384	576
Corrector	1	1	1	370	370	370	370
Totale in Kg	100	100	100	**Totale Price**	7312,2	7202,2	6905
				Price of 1Kg	**73,1**	**72,0**	**69,1**

Table 24 : Estimated price of ingredients and total cost of laying and reproductive hens diets (DA / Kg).

	Quantity of ingredients in Kg		**Price DA/Kg**	**Price of incorporated ingredients in DA**	
	Laying hens	**Reproductive hens**		**Laying hens**	**Reproductive hens**
Corn	57,75	59,6	53	3060,75	3158,8
Soymeal	21	22	108	2268	2376
Soybean oil	1,5	1	150	225	150
Calcium carbonate	7,5	7	5	37,5	35
Dicalcium phosphate	1,25	1,4	270	337,5	378
Wheat bran	10	8	32	320	256
Corrector	1	1	320	320	320
Totale in Kg	100	100	**Totale price**	6568,75	6673,8
			Price of 1Kg	**65,7**	**66,7**

Table 25: Estimated price of ingredients and total cost of rabbits diets (DA / Kg).

	Quantity of ingredients in Kg		**Price DA/Kg**	**Price of incorporated ingredients in DA**	
	Start	**Fatening**		**Start**	**fatening**
Barley	21	25	58	1218	1450
Corn	4,5	4,5	53	238,5	238,5
Soymeal	18,5	16	108	1998	1728
Soybean oil	2,5	2,5	150	375	375
Calcium carbonate	0,5	0,5	5	2,5	2,5
Dicalcium phosphate	0,5	0,5	270	135	135
Wheat bran	13	14	32	416	448
Wheat straw	14	14	6,5	91	91
Alfalfa	25	22,5	65	1625	1462,5
Corrector	0,5	0,5	280	140	140
Totale in Kg	100	100	**Totale Price**	6239	6070,5
			Price of 1Kg	**62,4**	**60,7**

According to estimated cost of different formulas, broiler, rabbits and laying or reproductive hens, we can see that the most expensive ingredient used is soy meal by the price of 108 DA/Kg. However, this ingredient is indispensible in formulating of animals rations because it is the most abundant ingredient used as protein's source, even if it is imported and cost very much. So the research for alternative ingredients to replace or to minimize its use is indispensible to reduce the cost of food.

VI.10. Economic efficiency of azolla in formulating foods for monogastric animals:

Estimated cost of different diets for broiler, rabbits and lying or reproductive hens without or with using azolla are shown in table.

Table 26: Estimated cost of different formulas without and with *Azolla*.

	Cost of formulas in DA	
	standard	**With azolla**
Broiler		
Start	73,1	66.9
Growth	72	65.3
Finish	69,1	64.4
Rabbits		
Growth	62,4	53.9
Fattening	60,7	48.3
Laying hens	65,7	58.1
Reproductive hens	66,7	57.1

In formulas for broiler 10 % of *Azolla* in the start diet of broiler can replace 6 % of soy meal in standard diet. In growth diet it was found that the use of 15 % of *Azolla* can substitute 7.5 % of soy meal. However, the use of 20 % of azolla can replace 7 % of soy meal in the finish diet for broiler. While, in laying and reproductive hens foods *Azolla* replace 10% of soymeal in each of these tow formulas. However, the incorporation of 20 % in start food of rabbits can replace 8.5 % of soymeal and 7 % of Alfalfa in standard food. However, 30 % of *Azolla* in fattening food can replace 11 % of soymeal and 12.5 % of alfalfa in standard food.

In animal production food represent 60% to 80% of the total cost in a system. Of agriculture-livestock integration as indicated by Hêdji *et al*. (2015). For that, the incorporation of *Azolla* as a partial substitute for soymeal constitutes an interesting alternative for cleaning up the environment and reducing the costs associated with animals feed.

In the other hand, it was found that the partial substitution of soymeal by *Azolla* (3% and 6 %) led to a reduction in the price of foods compared to the standard food. This is because

the price of A. *pinnata* was low compared to the price of soymeal on the market (Ouedraogo *et al*., 2021). Our results corroborate those of Deepesh *et al*. (2016) who found that the incorporation of 5% and 7.5% of A. pinnata as a substitute for sesame can reduce broiler production costs.

everal studies showed that the utilization of *Azolla* in animals diets saved the concentrate feed cost (Khatun *et al* 2008; Lakshmanan *et al* 2017; Wasihun *et al* 2020). *Azolla* makes the difference and have to do with lower feed costs. *Azolla* cultivation is non-competitive with human needs. These findings suggest that *Azolla* meal production is both technically and economically feasible strategy without requiring fertile land or high volumes of water for cultivation. The farmer needs no more than stagnant water, cow dung and bed soil of which are mostly free of cost.

CONCLUSION

Conclusion:

The results of this study revealed that:

- Cultivating *Azolla* is very simple and doesn't need a big investment neither lot of money.
- *Azolla* meal had no deleterious effect on nutritional values of formulated diets in comparison with standard formulas.
- *Azolla* is as a high productive plant which can give excellent yield if it is well exploited. Because of its high multiplication and its less expensive cultivation method, less demanding in water and cultivation area compared to other raw materials such as soy meal.
- *Azolla* is rich source of protein and macronutrients. It can be used as unconventional feed for many species including ruminants, poultry and fish.
- Economic analysis results show that feed cost reduced with using different levels of *Azolla* in the diets of monogastric animals.

Recommendations:

From the results and findings of the study, the following recommendations are made:

- A complete nutrient composition analysis of azolla meal in order to provide clearer information of its nutrient composition. This will better enlighten researchers and prospective users on the merits of its use not only for monogastric but also for ruminant's animals.
- Consider a feeding trial on broilers or rabbits using azolla flour at different incorporation rates to see the effect on zoo technical parameters and animal health.
- Thinking about the means of conditioning this plant as pellets or meal is very important in manufacturing feed for animals.

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ANNEXES

Figure 1: Dried *Azolla*

Figure 2: Fresh *Azolla*

Figure 3: Tracking of *Azolla* during culture