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A preliminary study on milk yield, composition and economic profitability of alfalfa pellets and long ryegrass fibre (Kit N° 10) in a total mixed ratio in lactating dairy cows

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Abstract
This study was developed to improve the qualitative and quantitative performance of milk production of dairy cows. The aim of this work is to evaluate the impact of feeding Kit N°10 based on alfalfa pellets and long ryegrass fibre produced by "La Romana Farine Sri" on the milk yield and milk composition in dairy cows. Kit N°10 was administered thrice daily to six multiparous Holstein–Friesian cows assigned to two treatments including control and staple feed Kit N°10. Feeding the Kit N°10 as basic ratio improved animal performance by increasing significantly (p<0.05) the daily milk production (52.31%), the protein content (6.17%) and the lactose content (6.35%). Obtained results suggest that using Kit N°10 could be a valuable cost-effective alternative to improve and balance the protein and energy intake of animals. Further, an economic analysis was carried out to calculate the costs of milk production, gross production value gross margin, absolute profit, and relative profit of dairy cattle farms. A single product budget analysis method was used for calculating production costs. The analysis shows that the cost of feeding without Kit N° 10 was unprofitable; with a loss of 932 TND/Dairy Cow. However, the milk production cost of feeding with Kit N° 10 generates a positive result of around 2434 TND/Dairy Cow. The results showed some evidence to prove a specific effect of Kit N°10 on economic profitability and on animal performance.

Keywords: alfalfa pellets, ryegrass, milk production, dairy cows, Tunisia

1. Introduction
Alfalfa (*Medicago sativa* L.) is one of the most widespread and appreciated forages species for ruminant feeding worldwide (Sedaghati *et al*., 2014) [25], due to its high-quality and yielding potential, as well as the adaptability to different environmental conditions, and the high voluntary intake (Andre *et al*., 2021) [1]. Alfalfa contain high protein and vitamin (E, A, K), stimulates cellulose digestion, and has higher energy and lower cell wall contents than other hays (Sezmis and Gursoy, 2020) [26]. Alfalfa, known also as 'Queen of Forage', has a high protein content between 11.3-25.9%, high dry matter intake (DMI) (3.4-5.9%) and highly digestible dry matter (DDM) (57.4-75.8%) (Avci *et al*., 2018; Cacan *et al*., 2018) [3, 6]. However, the availability of these nutrients depends upon the harvesting conditions and the forage conservation method (Andrighetto *et al*., 1993) [2]. Dried alfalfa is acknowledged to be the best conservation method as it preserves its high protein, vitamins, and overall nutritive value (Renaud, 2002) [20]. On the other hand, the ryegrass (*Lolium multiflorum* Lam.) is one of the most important forage crops. It is widely distributed through temperate areas of the world and generally regarded as the basis of grassland improvement because of its high nutritional value, digestibility, and well ensiling characteristics (Breese, 1983) [5].
The high nutritive value of alfalfa can justify the adoption of more expensive alternative conservation systems in order to reduce DMI and quality losses (Putnam *et al*., 2014) [18].

Forage dehydration leads to a fast decrease in free water content, lowering the moisture content below 8%. Dehydrated alfalfa is used as a protein source in dairy cow rations, but little is known about the effects of alfalfa on greenhouse gas produced by ruminants (Doreau *et al*., 2014) [10]. After dehydration, alfalfa can be baled and directly incorporated in total mixed rations or, alternatively, grounded and then pelleted, allowing its easier transport and storage (Scott *et al*., 2018) [24]. The small particle size following the grinding process leads to a higher exposure of forage surface to microbial attachment in the rumen and to a faster ruminal passage rate (Dewhurst *et al*., 2009) [9]. For ruminants, the most efficient energy utilization from fibrous feeds occurs when they are available for microbial fermentation in the fore-stomachs.

Dairy producers strive to formulate rations that allow the cow to produce great quantities of milk cost-effectively. The key problem in high producing dairy animals with forage-based diets is getting adequate net energy intake to meet the requirements of animals (Mahanta *et al*., 2020) [16]. Therefore, effective feeding with forage could improve the additional value of ruminant products and could have positive effects on the health of cows. Maximizing milk production while minimizing feed costs and health problems has been one of the extremely important tasks in dairy cow nutrition in recent years. High-quality alfalfa comes closer to meeting all the above considerations in successful feeding of dairy cows than any other one feed. The objective of the present study was, therefore, to investigate the effect of dehydrated alfalfa pellets and long fibre of Italian ryegrass (*Lilium multiflorum* Lam.) on milk production and composition of lactating Holstein-Friesian cows.

2. Materials and Methods

2.1. Feeding experiment

The experiment was conducted during two months in November and December 2021 at the Higher School of Agriculture of Mograne in Tunisia. Six multiparous Holstein–Friesian cows were divided into two homogeneous groups in terms of average live weight. Two dietary treatments: (group I, basic ration and 7 Kg of commercial concentrate; group II basic ration of kit N° 10 and 7 Kg of commercial Concentrate), were applied for sixty days experimental periods after 14 days of adaptation (Table 1). The rations were offered three times a day in equal portions of 5 kg/diet. Commercial concentrate amount provided per animal is 7 kg spread over three diets. The base diet is composed by Kit N°10 produced by "La Romana Farina srl" containing pure selected stems of alfalfa and long fibre of ryegrass, dehydrated, and compressed into pellets through a mechanical process that involves no chemical additives or glues. The adaptation period allows the digestive flora to be able to digest the corresponding rations.

Table 1: Period of adaptation and corresponding meal quantity.

Period	Quantity/meal (kg)
30-2 November	1
2-5 November	2
5-8 November	3
8-11 November	4
11-14 November	5
total	15

2.2. NIRS analysis

Milk Near infrared spectroscopy analysis (NIRS) was performed using a sample (0.5–1.0 g) which was exposed to an electro-magnetic scanning over a spectral wavelength range of 1100 to 2500 nm (near infrared) (Corson *et al*., 1999) [8].

2.3. Milk Yield and Composition

Cows were milked twice a day (06h am and 15h pm) using a milking machine (MOTECH GVP 200). The milk yield of each individual cow was registered every day during the monitoring period. After milking, a milk sample of 100 mL was collected and stored at (-20 °C) for further analyses. Afterward, milk samples were thawed in a water bath at 37 °C. After homogenizing the samples, 50 mL aliquots were refrigerated at 4 °C and analysed by Milko Scan™ FT1.

2.3. Statistical analysis

Statistical analyses were performed using the general linear model's (LMs) procedure of Statistical Analysis System (SAS, 2002) [23] with significance at $p \leq 0.05$. If the F-test for treatment effect was significant, differences between treatment means were determined using Student–Newman–Keuls (SNK) multiple range test.

3. Results and Discussion

3.1. Chemical composition of the Kit N°10

The chemical compositions of Kit N°10 are presented in Table 2. The dehydrated alfalfa and long fibre ryegrass kit N°10 contained 11.7% of protein. Alfalfa is known as the 'Queen of Forage' as it has high protein content between 11.3-25.9%. The kit N°10 contained 52.05% of neutral detergent fiber (NDF) and 41.49% of acid detergent fiber (ADF). These results are similar with previous findings (Testa *et al*., 2011; Zhang *et al*., 2014; Avci *et al*., 2018; Cacan *et al*., 2018) [28, 28, 30, 3, 6],

In contrast with this study, Basbag *et al*., 2009 [4], Kavut and Avcioglu, 2015 [12], Avci *et al*., 2018 [3] and Cacan *et al*., 2018 [6] found in alfalfa 20.3-49.6% of neutral detergent fiber (NDF) and 16.8-40.4% of acid detergent fiber (ADF).

Table 2: Chemical composition of dehydrated alfalfa and long fibre ryegrass kit N °10.

Parameters	Composition (%)
FUM	0.61
Crude ash	9.50
Crude protein	11.70
Crude lipid	1.41
Organic matter	12.07
Starch	2.34
Neutral Detergent Fibre	52.05
Acid Detergent Fibre	41.49
Neutral Detergent Lignin	7.34
Sugar	5.63
Digestibility NDF 24h (NDF %)	46.01
NDR- Neutral Detergent Residue	56.82
NDIP	3.8
ADIP	1.94
Soluble Protein	4.35
PDIA: g/Kg	40.00
PDIN: g/ Kg	70.00
PDIE: g/ Kg	60.00
Calcium	1.24
Phosphor	0.31
Magnesium	0.22
Potassium	2.33
Sulphur	0.23
Chlore	0.77
Sodium	0.90
Fer (mg/kg)	1.36
Digestibility NDF (%NDF)	42.33

FUM: Feed unit milk, NDIP: Neutral detergent insoluble protein (NDIN × 6.25), ADIP: Acid detergent insoluble protein, ADIN × 6.25), PDIA: Dietary underrated protein, PDIN: PDI when nitrogen limits microbial growth, PDIE: PDI when energy limits microbial growth.

3.2. Effect of alfalfa pellets and long fibre of ryegrass Kit N°10 on milk production

According to ANOVA analysis, a significant effect (p<0.01) of the Kit N°10 on milk production of the studied cows was observed. In fact, cows receiving dehydrate lucerne and long fibre of ryegrass have mean of 27.03±1.23 L/day milk production while the control cows produced a mean of 12.89 ± 3.00 L/day over 2 months of monitoring period (Figure 1). These findings confirm that use of dehydrate alfalfa and long fibre of ryegrass, with a high percentage of NDF and rich in nutritional elements, significatively improved milk production.

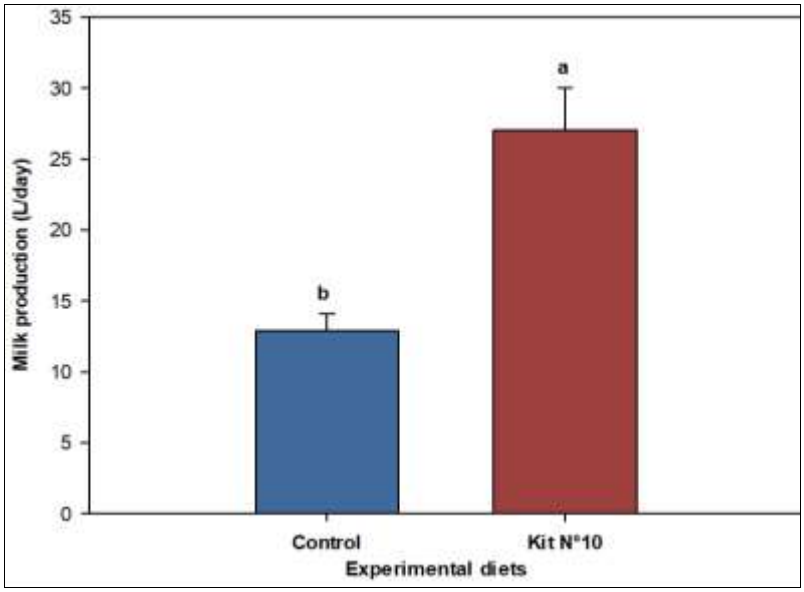

Fig 1: Effect of dehydrate alfalfa and long fibre of ryegrass kit N°10 on the daily milk production of Holstein-Friesian dairy cows

Figure 2 showed the evolution of the mean daily milk production of the dairy cow of group I that received a basic ration composed of Kit N°10 and 7 Kg of commercial concentrate and group II that received 15 Kg of oat hay and 7 Kg of concentrate. The complementary Kit consisting of organic fodder balanced in energy and protein has positively affected milk production.

Diet is considered as one of the main factors influencing milk production. In fact, the dairy cows found in this fodder the required constituents that allow the milk production, which may increase growth and gestation. Similarly, Charpentier *et al*. (2019) [7] showed that supplying 400 g/day of dehydrated lucerne has a much-pronounced positive effect on milk production of dairy goats than an additional grazing period of 4 hours after evening milking. Srisaikham and Rupitak (2020) [27] recommended selecting alfalfa in the form of dehydrated pellets as a diet, which is more practical and convenient for smallholder farmers.

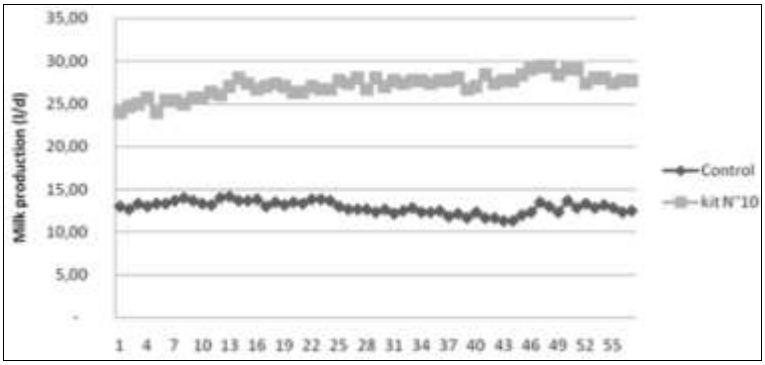

Fig 2: Daily evolution of the milk production of Holstein-Friesian cows over 60 days monitoring period

Regardless of the forage type in the diet, milk production was higher for cows fed Kit N°10 diets than for those fed with control diets. In fact, dehydrated forages may be considered as concentrates when given as pellets, as in this experiment, due to their low rumen filling effect (INRA, 2010) [11]. Furthermore, the increase in feed efficiency for cows fed may be related to the greater intake of readily fermentable carbohydrates (Keim *et al*., 2020) [13]. Our results are consistent with those obtained by Owen (1969) [19] where the milk production has been improved with higher amounts of the dehydrated alfalfa.

The increase in milk production with supplementation was high (+0.82 kg/kg DM of dehydrated alfalfa) and within the range observed for milk production response to concentrate supplementation of grazing dairy goats (Rubino *et al*., 1995; Lefrileux *et al*., 2008) [22, 14].

Alfalfa also contains significant amounts of linolenic acid, and its inclusion in the diet of dairy cows improves milk fatty-

acid (FA) composition (Doreau *et al*., 2014) [10]. Riediger *et al*. (2009) [21] showed that linolenic acid potentially has positive effects on human health. Increasing beneficial FAs in milk by dietary means is limited, but it may contribute to a nutritional improvement of the human diet.

Furthermore, these results showed that the fibre size of ryegrass affected the fermentation and the physical availability of feed particles to microbial attack by stimulating chewing and salivation, rumination, and ruminal motility that is referred to as effective fibre. The large feed particle size could increase resistance to microbial attachment and degradation (Zebeli *et al*., 2012; NASEM, 2016) [31, 17].

3.3. Effect of dehydrated lucerne and long fibre ryegrass on milk composition

The composition of milk recovered from the cows that received Kit N°10 is presented in Table 3. The inclusion of Kit N°10 significantly improved milk composition regarding protein, lactose and total milk composition (p<0.05).

The feeding conditions and diets of cows were important effective factors for milk quality (Lv *et al*., 2021) [15]. It has been acknowledged that variations in milk composition occur; the present results showed that milk composition indicated 3.70% fat, 3.24% protein and 4.88% lactose. However, the composition of milk nationally marketed has been rather constant over the last 15 years, averaging 3.6% fat, 3.2% protein, and 4.7% lactose. This is probably due in part to the prominence of the Holstein breed and the pricing of milk based on fat concentration.

Milk fat and protein production were greater by 11 g/day and 10 g/day respectively of dehydrated lucerne supplementation than access time to pasture of dairy goat (Charpentier *et al*., 2019) [7]. These obtained results are consistent with those obtained by Thénard *et al*. (2001) [29] who showed that dehydrated alfalfa improves the protein level in diets based on grass silage. According to Zebeli *et al*. (2012) [31], greater fat and protein percentage in the milk of cows fed the moderate PS suggests a better nutrient availability, in particular that of microbial protein, likely due to a better distribution of nutrient intake over the day.

Table 3: Effect of dehydrated lucerne and ryegrass long fibre on milk composition.

Variable	Control	Kit 10	F value
Fat (%)	3.33±0.23	3.70±0.16	0.267
SNF (%)	8.31±0.11	8.87±0.08	0.015
Density (%)	28.95±0.41	30.76±0.29	0.023
Protein (%)	3.04±0.04	3.24±0.02	0.014
Lactose (%)	4.57±0.06	4.88±0.04	0.015
Salt (%)	0.67±0.01	0.72±0.007	0.023
Added water (%)	0.00±0.00	0.00±0.00	NS
Freezing point (°C)	-0.527±0.008	-0.572±0.006	0.01

3.4. Effect of dehydrated lucerne and ryegrass long fibre on the milk/concentrate ratio

The effect of dehydrated lucerne and long fibre ryegrass Kit N°10 on the milk/concentrate ratio is presented in figure 3.

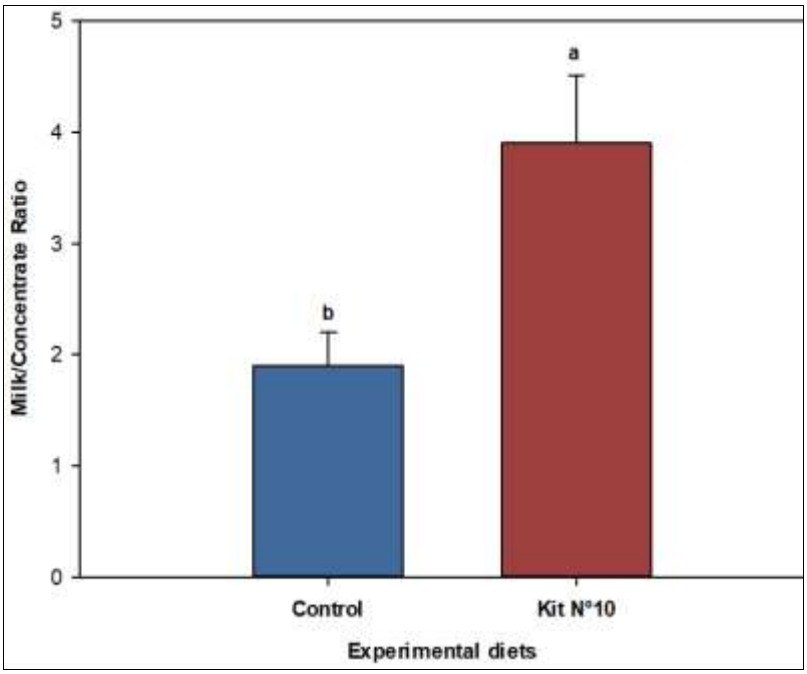

Fig 3: Effect of supplementing Kit N°10 on milk/concentrate ratio

The present results showed that the average milk/concentrate ratio was significantly higher (p<0.05) in group supplementing Kit N°10 than in the control fed. This explained that the concentrate is brought to meet the maintenance needs of the animals. It can therefore induce risks of the appearance of nutritional diseases and in particular, acidosis. Whereas for Kit N° 10, a milk/concentrate ratio of 3.86 was obtained. This proves that this basic ration is balanced and it is possibly provided in order to support milk production.

3.5. Impact of Kit N° 10 on the economic profitability of the dairy farm

For the determination of the cost of milk production, we have adopted the following hypotheses (Table 4).

Table 4: Presentation of the hypotheses of the study

	The hypothesis 1: For holdings without Kit N°10	**The hypothesis 2: For holdings with Kit N°10**
Dairy cow number	3 head	3 head
Production System	Integrated	Integrated
Replacement rate	20%	20%
Mortality rate	2%	2%
Lactation	305 days	305 days
Annual milk production	3931 L	8244 L

With average prices on October 2022 (in Tunisian Dinar TD):
Price heifers born and bred in Tunisia: 9800 TD
Price of a weaning calf: 1200 TD
Price of culling cows: 1600 TD
Price of manure: 30 TD/ton
Number of years of a dairy cow production: 5 years (5 lactation)
Number of cows: 3

For the determination the effect of the introduction of Kit N°10 as a basic ration of dairy cows, a techno-economic analysis was carried out to establish a comparative study between the two rations: without and with Kit N°10. The following tables illustrate the cost of milk production within these two types of farms (Table 5).

The analysis of Table 5 shows that feeding without Kit N° 10 is unprofitable, with a loss of 932 TND/Dairy Cow, while feeding with Kit N° 10 generates a positive result of around 2434 TND/Dairy Cow. Thus, the result improved by 3366 TND/DC, despite the increase in expenses per cow of 2265 TND. This improvement could be explained by the fact that the increase in the quantity of milk produced with the introduction of Kit N° 10 induces to an increase in expenses. For the batch, the overall result was improved by 10099 TND from -2797 to 7302 TND.

Table 5: Determination of the Production Cost (October 2022 Prices) for smallholder dairy farms (Integrated Production Mode) without and with Kit N°10

			Alimentation without Kit N°10		**Alimentation with Kit N°10**		**Variation**
Designation	**Unit**	**Unit price**	**Need/head**	**Cost**	**Need/head**	**Cost**	
Expenses							
Alimentation							2295
Concentrate	Ton/Year	1200	2.5	3000	2.5	3000	
Hay	Bale/Year	13	65	845	0	0	
Straw	Bale/Year	9	40	360	0	0	
Kit N°10	Ton/Year	700	0	0	5	3500	
Total Alimentation				4205		6500	
Workforce							0
Workforce	Day/Year	20	25	500	25	500	
Total Workforce				500		500	
Divers							-30
Mechanisation	Hour/Year	15	10	150	10	150	
Water/Electricity	TND/Head/Year	40	1	40	1	40	
Veterinary/Artificial Insemination	TND/Head/Year	100	1	100	0.7	70	
Others	TND/Head/Year	100	1	100	1	100	
Total Various				390		360	
Amortisation							0
Livestock	Heifer/Year	9800	0.2	1960	0.2	1960	
Mortality	Cow/Year	9800	0.02	196	0.02	196	
Stable cushioning	TND/VL/Year	45	1	45	1	45	
Equipment cushioning	TND/VL/Year	40	1	40	1	40	
Total Amortisation				2241		2241	
Total Expenses				7336		9601	2265

Receipts			Alimentation without Kit N°10		Alimentation with Kit N°10		Variation
Designation	Unit	Unit Price	Production	Receipts	Production	Receipts	
Calves	Calf/Year	1200	0.8	960	1	1200	240
Cull cows	VL/Year	1600	0.2	320	0.2	320	0
Manure	Ton/Year	30	7	210	7	210	0
Milk	Litre/Year	1,25	3931	4914	8244	10305	5391
Dairy Free Recipes (TND/VL)			1490		1730		240
Total Recipes (TND/VL)			6404		12035		5631
Result (TND/VL)			-932		2434		3366
Global Result (NDT)			-2797		7302		10099

Based on the average selling price of milk of 1.250 TND/L, the unit profit, corresponding to the difference between the selling price of milk and the production cost of milk, is 0.245 TND whereas it is -0.287 TND. Thereby, the Kit N° 10 induced an improvement of 0.532 TND per litre of milk produced. In fact, the production cost of a litre of milk is the floor price, known as threshold or equilibrium price, from which the workshop will be profitable. Thus, Kit N° 10 could

reduce the pressure on the purchasing power of the consumers but also on the government compensation fund by reducing the equilibrium price from 1.487 TDN/L to 0.955 TND/L.

The margin on food cost measures the efficiency of the management of the monitored batch. The results showed a marked improvement in the margin on feed cost of 4,676 TND/Dairy Cow/Day after the introduction of Kit N° 10. This could improve the effectiveness of the used concentrate. The gross margin rate in cattle breeding, which must be around 50%, is only 28% for the feeding without Kit; however, with the introduction of the Kit N° 10, it has been improved to reach 43%.

The gross operating surplus is an intermediate management balance indicating the level of wealth generated by dairy cow. The economic efficiency, measuring the share of gross operating surplus in gross product, has been improved by 18% with the introduction of Kit N° 10. Moreover, the Milk/Concentrate Ration, for which the Tunisian national standard is 2 L/Kg, indicated to the poor performance of the studied batch feeding without the introduction of Kit N° 10. However, this ratio improved to reach 3.3 L/Kg with the introduction of the Kit.

Table 6: Economic indicators

	Alimentation without Kit N°10	**Alimentation with Kit N°10**
Milk Production Cost (TND/L): (Total Expenses - Recipes without milk) / Milk Production	1.487	0.955
Food cost margin (TND/DC/Day): (Gross product – feedstock) / quantity of milk produced)/ lactation duration in days	13.471	18.148
Gross margin (GM) (TND/DC): Gross Product (GB) – Variable Expenses	1809	5175
Gross margin rate: (GM/GB)	28%	43%
EBE (TND/DC): GM – Labor charges	1309	4675
Economic efficiency: (EBE/GB)	20%	39%
Milk/Concentrate Ratio (L/kg) (Tunisian standard 2 L/kg)	1.6	3.3

The Table 7 summarizes the economic effects of the introduction of Kit N° 10 in the basic ration of the studied batch of dairy cows.

Table 7: Summary of the economic impact of the introduction of Kit N°10 in the basic ration

	Expenses			**Receipts**		
Impacted Factor	**Veterinary fees**	**Feed cost**	**Cost of Kit N°10**	**Reproduction Parameter**	**Milk production**	**Global Impact**
Impact (TND/DC)	-30	-1205	3500	240	5391	3366
Total Impact (TND)	-90	-3615	10500	720	16174	10099

Conclusion

The use of combination of dehydrated lucerne and ryegrass long fibre KIT N°10, produced by LA ROMANA FARINE, and the concentrated feed as a ration of dairy cows in the Off-land breeding positively affected the production and quality of milk. For this reason, it is recommended to use this innovative method in the feeding of dairy farms and to use the products that have proven successful while ensuring that dairy farmers can obtain supplies easily and regularly. There is thus a marked improvement in the economic profitability of dairy farms adopting KIT N°10 from LA ROMANA FARINE in the ration of dairy cows. The kit N°10 can solve the problem of limited forage in the off-land breeding dairy cows resources and the availability or lack of self-produced forage, the increase of prices and the inconsistent supply of roughages and concentrates in the market.

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