Nutrivit

The use of non-protein nitrogen NPN (Urea) slow release in ruminant diets

PROTINAT® PROCESS

(Patent IT/102022000014362 PCT/IB2023056515)

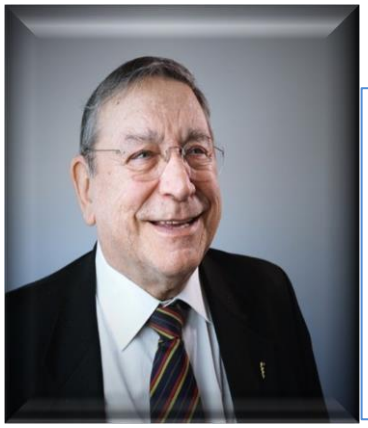
Speakers: Dr. G. Gabaldo, Prof. A.Ubaldi, Dr. M.Bellini








Villafranca di Verona - March 2025



Giulio Gabaldo, a veterinary doctor over seventy years old, from Verona, Italy, specialises in the development of New Technologies in the field of animal feed and nutrition and is the inventor of urea treated with the PROTINAT® PROCESS. He has worked for over fifty years in Italy and most of Europe, sometimes on the American continent as a nutritionist specialising in ruminants. He has worked directly on livestock farms and in the livestock industry (feed and pharmaceutical industry) designing new natural technological solutions for solving zootechnical problems, acquiring great experience in the preparation of feeds and functional foods, additives, supplements, therapeutic products and in particular those of a natural kind such as nutraceuticals, stimulators of the digestive "microbionte", phytotherapeutics, antioxidants, Omega 3, etc. He has also organised numerous refresher and training courses on numerous topics for operators in the livestock sector, such as veterinarians, nutritionists and breeders. He has written numerous articles and technical manuals, some of which have been translated into Spanish, French, English, Catalan, Portuguese, Polish and Russian, and two books, one of which is in English. He has been a speaker at various National and International Conferences and Congresses of Buiatrics and Veterinary Sciences, (Lucania Dairy Expo - Potenza in 2006, National Congress of Buiatrics in Castellaneta in 2009, Word Buiatric Congress in 2008 in Budapest, Word Buiatric Congress in 2010 in Santiago de Chile, Expo Agrotech in Russia in 2014, Agricola Internazional Expo in FERMA in Poland in 2015, International Veterinay Congress in Poznam at University - Bêdlewo - Poland in 2016.
He obtained his PhD for the development of New Technologies for the preparation of Pharmaceuticals and for a few years he Zootechnics and Zoognostics at the Agricultural Technical Institute of Verona. He has been external professor at the Animal Production, Epidemiology and Ecology Section of the University of Turin and as Professor of Dairy Cow Nutrition Physiopathology at the Department of Veterinary Science of the University Parma. He currently works as a consultant for public bodies, Italian and foreign companies in Italy and throughout Europe (France, Spain, Benelux, Switzerland, Poland and Russia).

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Antonio Ubaldi, born in Parma 80 years ago, married with one son, Professor emeritus at the Faculty of Veterinary Medicine of the University of Parma from 1974 until 2015. He has taught as chair of clinical biochemical laboratory diagnostics, veterinary toxicology, residue toxicology in food and in three specialisation schools at the Universities of Parma and Milan. His main research topics are animal metabolism, with a special focus on ruminants, metabolic diseases of intensively farmed animals, toxicology in the animal sector and in particular in production chains (milk, meat and eggs). He has collaborated with various pharmaceutical industries in the testing of new drugs. He develops and disseminates health interventions suitable resolving states of intoxication in animals, such as intoxication by nitrites and nitrates, mycotoxins, arsenic in drinking water, phytohormones and pesticides in fodder and feed. He was President of the World Society of Veterinary Laboratory Diagnostics. He has been Visiting Professor at various Universities, such as Guelph (Canada), Biet Dagan-TelAviv (Israel), Davis-California (USA), Lyon (France), Toulouse (France). He has produced over 260 scientific publications in journals of national and international interest. He has been a speaker at numerous international congresses, as well as organiser, president and member of the scientific committees of two international congresses based in Parma. He has participated as head of operational unit in two European projects for the organisation of new Faculties of Veterinary Medicine in African countries (Cameroon, Gabon, Equatorial Guinea, Chad). He has worked in Ethiopia as an expert in technical collaboration with the Italian Ministry of Foreign Affairs. He currently works as a consultant for companies, industries and research centres. He is the scientific manager of a university spin-off in Parma.

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Marco Bellini from Mantua, 70 years old. He obtained his classical high school diploma at the Liceo Ginnasio Virgilio in Mantua in 1975. He graduated in Agricultural Sciences in 1981 at the Faculty of Agriculture of the University of Padua. In 1986, qualified as a Chartered Agronomist at the University of Milan.
From 1983 to 1988 he worked at the then Mantua Dairy Institute (now ERSAF) as part of the 'Qualità Latte' project. From 1989 to 2023 in employed by the Mantuan Breeders' Association as a doctor of agriculture for advising member companies.
Since 2023 Consultant at the Lombardy Regional Breeders' Association. Since 2016 Inspector at the Agri-food Quality Department in Rome for the liliaries assigned to him.

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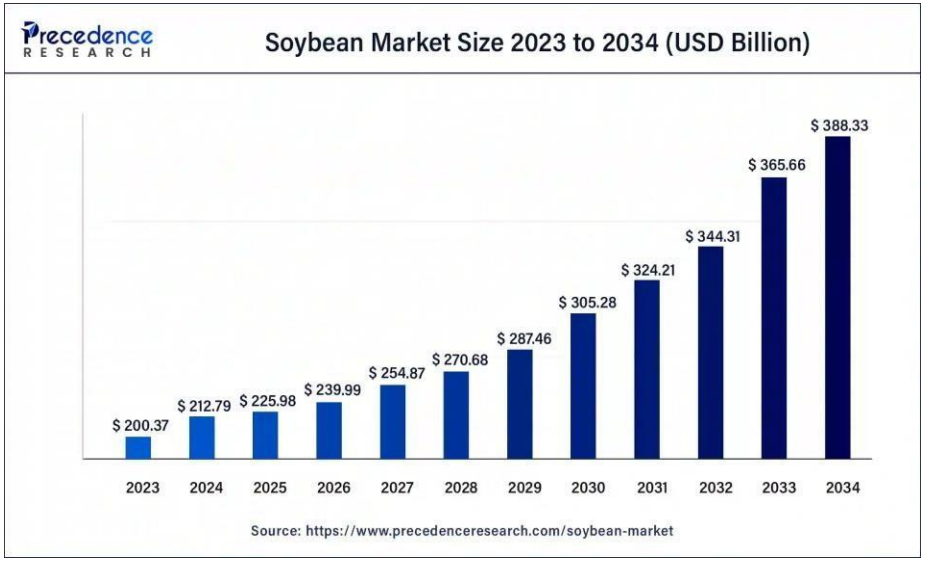
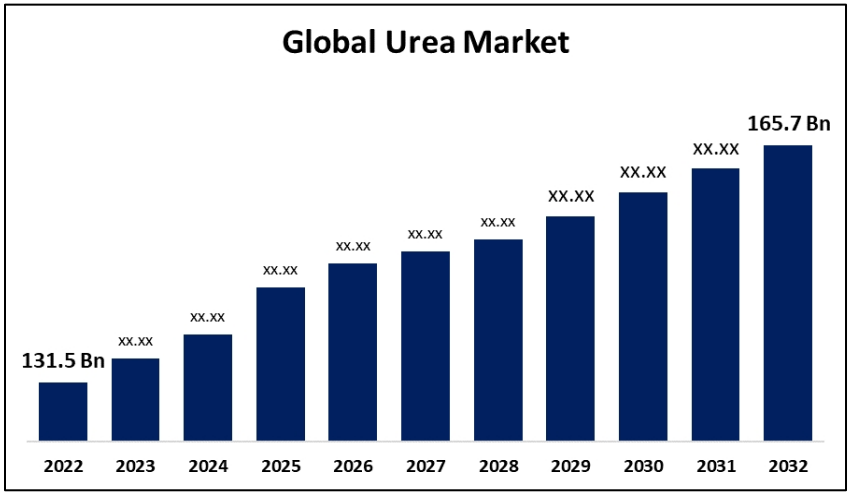
Dr. Giulio Gabaldo - DVM- PhD
Inventor of the Technology and Patent Holder
(Patent -n°102022000014362) PROTINAT® PROCESS

Soy and urea in the diet of adult ruminants

Currently, soya is practically considered the most effective source of protein in the diet of ruminants.





Adult ruminants with a **functioning rumen** can synthesise proteins from **non-protein nitrogen** (NPN) sources such as urea and metabolise nitrogen compounds in the feed into **NH3, which is used for the formation of microbial proteins.**

Nitrogen in ruminants

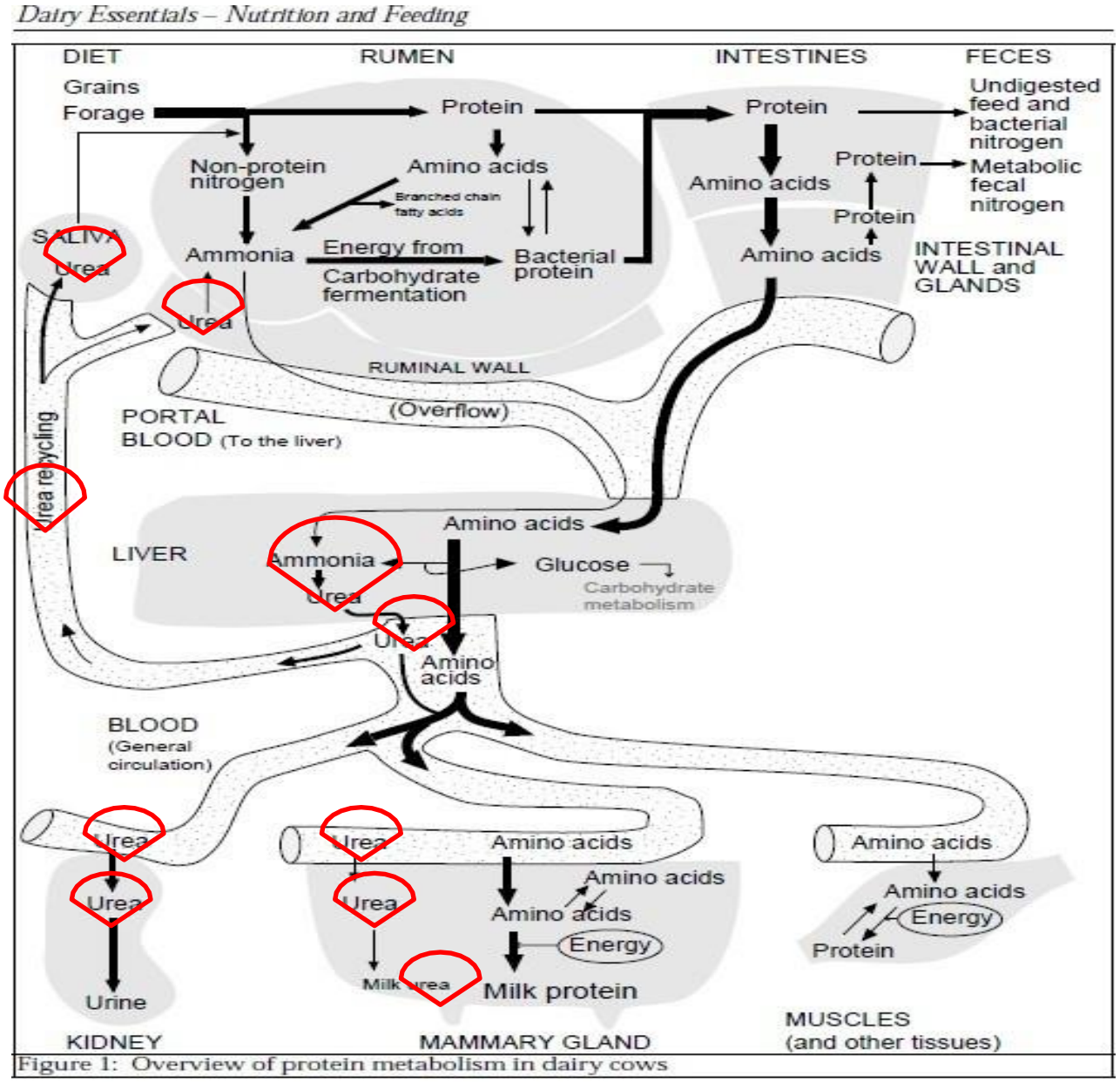


Figure 1: Overview of protein metabolism in dairy cows

(by M.Wattiaux - Babcock Institute for International Dairy Research and Development University of Wisconsin-2014)

Questions to ask.....

Problem No.° 1

Soya is expensive and not always available in all regions of the world. **If not treated well, it could also be toxic?**

Problem No.° 2

In some cases, when soya not is appropriately

treated, **its use in ruminant diets is not recommended**

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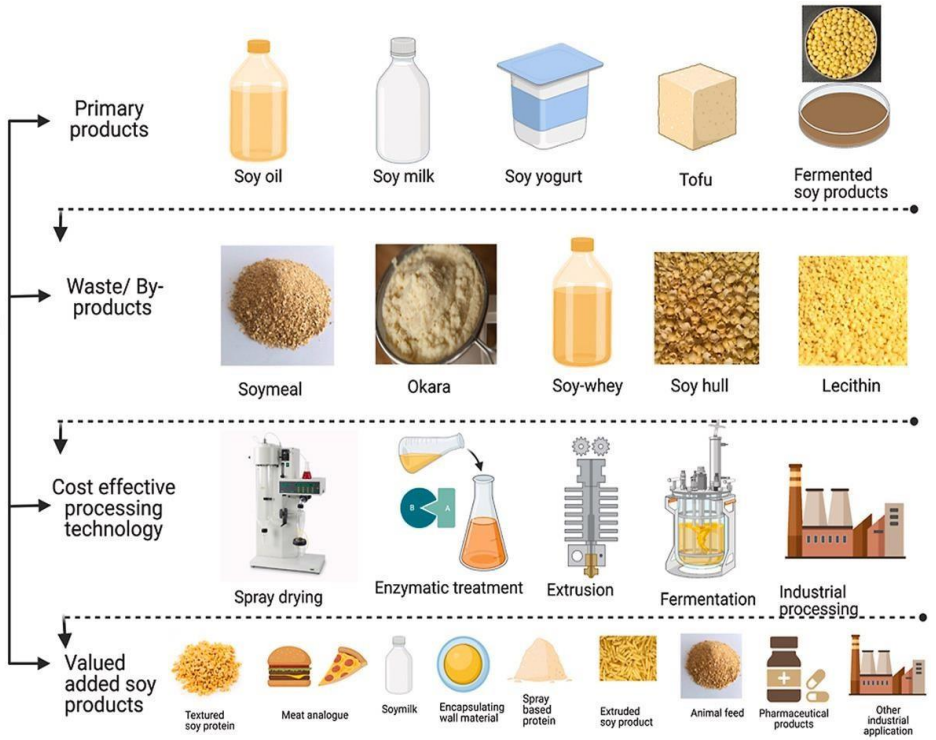
Problem No.° 3

How can we obtain protein in ruminants in a region **where soya is not readily available?**

Soybean Food Processing Industry

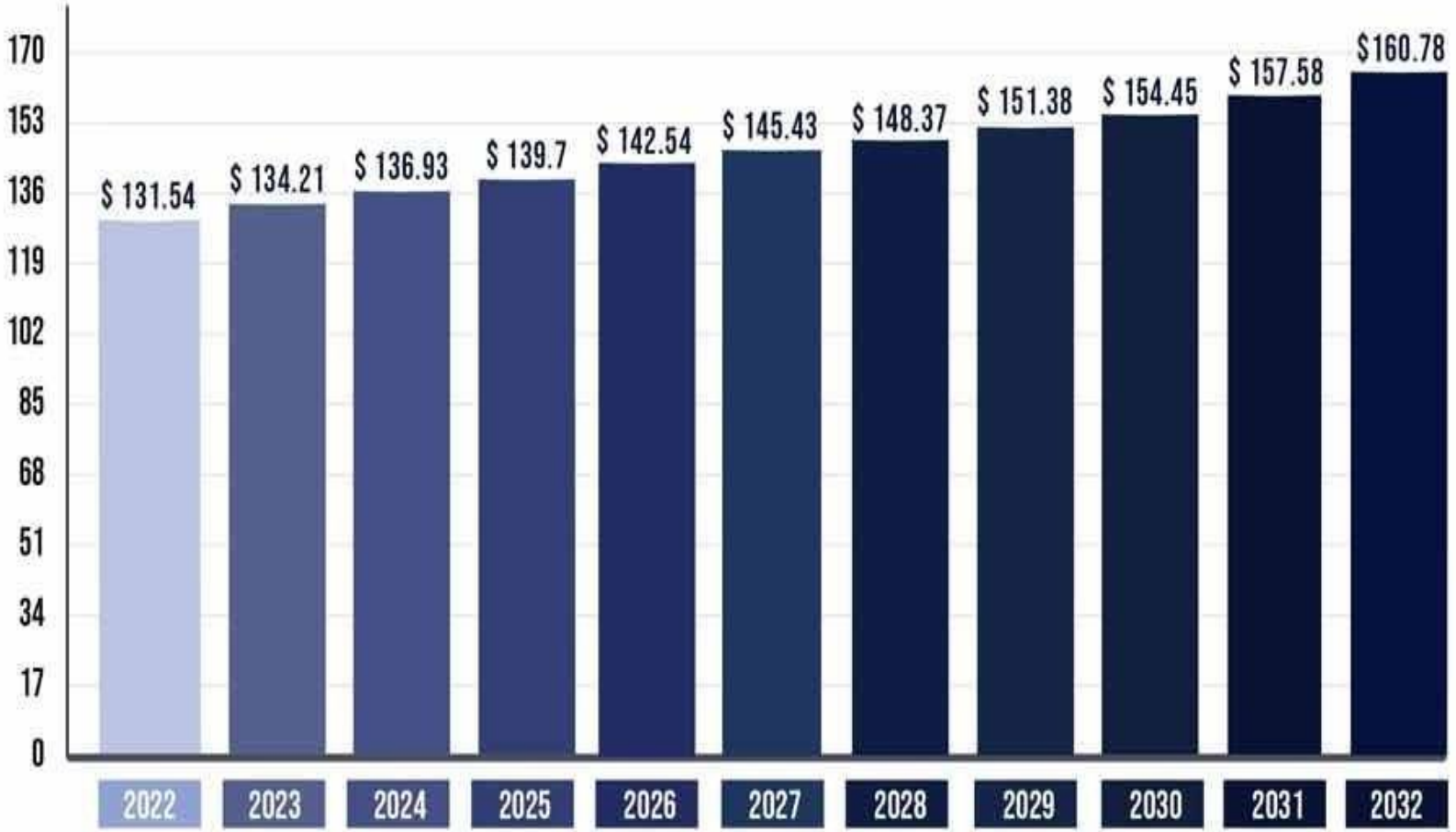


Adult ruminants with a functioning rumen can synthesise protein from non-protein **NPN** nitrogen sources, such as urea, metabolising the nitrogen compounds feed in $^{NH4+}$ and $NH_{(3)\ in}$ a controlled manner such that can be used in the formation of bacterial and possibly protozoan proteins.

Nutrivit

PRECEDENCE RESEARCH

UREA MARKET SIZE, 2023 TO 2032 (USD BILLION)



Source: www.precedenceresearch.com

Crude protein = NPN x 6.25

(By Ruminants Academy)

CRUDE PROTEIN GRAPH

Crude protein (CP)= Nitrogen (NPN)x 6.25

Contains protein and Non-Protein Nitrogen **(NPN)**

Available crude protein

Crude protein not available are:

Bound to the detergent acid fibre increases when overheated.

Indigestible and excreted with faeces.

Degradable in the rumen

Soluble or insoluble.

Used by microbes for protein synthesis.

Not degradable in the rumen:

Largely insoluble.

They pass into the small intestine.

Digestible fractions

They supply the cow with amino acids.

Indigestible fractions

They are excreted in the faeces.

Metabolic hydrolysis of urea in ruminants

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For ruminants, dietary urea recycled by the liver can be absorbed by the microbial population in the rumen, metabolised and transformed into **microbial proteins** that are a good source for milk or muscle protein synthesis (Tadele - 2015).

These pathways require **various enzymatic activities** and accessory proteins; however, compounds metabolisation must reach effective sites, thus requiring transport mechanisms **(specific carriers**).

Advances in research **on urea carriers** and **hydrolysis** are helpful in guiding the use and administration of urea as a protein feed. **Hepatic urea is transported from the rumen to the intestinal epithelia where ureases are located.**

Urea metabolism uses networks of interconnected pathways (Arriaga et al. 2009; Sigurdarson et al. 2018).

The first pathway of metabolic hydrolysis of urea is required:

a) to release nitrogen and make it available.

b) to produce, through the anabolic processes **of assimilation and biosynthesis, the amino acids and peptides** used by cells.

This process plays a vital role in nitrogen utilisation and metabolism in ruminants (Long et al. 2004; Reynolds and Kristensen 2008; Wang et al. 2011; Zhou et al. 2017).

NH_3 from the hydrolysis of urea and other nitrogen-containing compounds are absorbed and transported to the liver (Abdoun et al. 2006).

Here, **NH_3** is used for **endogenous urea synthesis**, which is recycled through the ruminal wall and salivary secretion. However, when **the amount of NH_3** is excessive, a form of **toxic syndrome** is triggered, resulting in **hepatic steatosis**.

Utilisation and waste urea in the feeding of ruminants

Following extensive research demonstrating its safety and usefulness at correct dosages under particular conditions, urea has become an accepted normal ingredient in ruminant diets.

A vast amount of information and knowledge has been documented concerning the mechanisms of utilisation of urea and other non-protein nitrogen compounds by the ruminal **microbiota**.

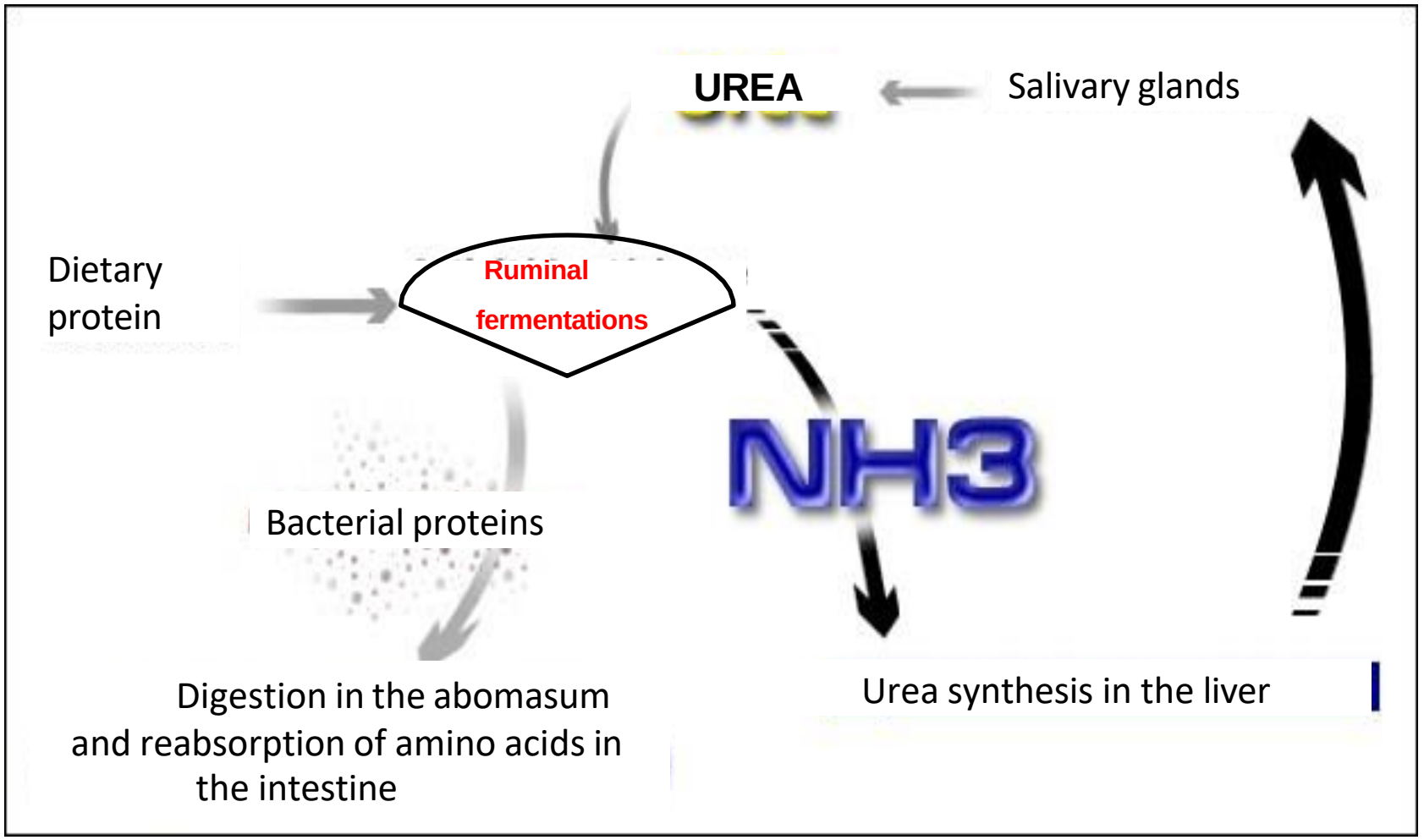
Problems of ammonia/urea toxicity can be easily prevented through proper utilisation of scientific knowledge on feeding urea in the diet of ruminants.

Today there is a production process of my invention composed of additives and physical-chemical treatment techniques to improve the anabolic use of UREA by the rumen microbial population thanks to the remodulation of the urea-N recycled in the rumen. (Patent IT/102022000014362 PCT/IB2023056515).

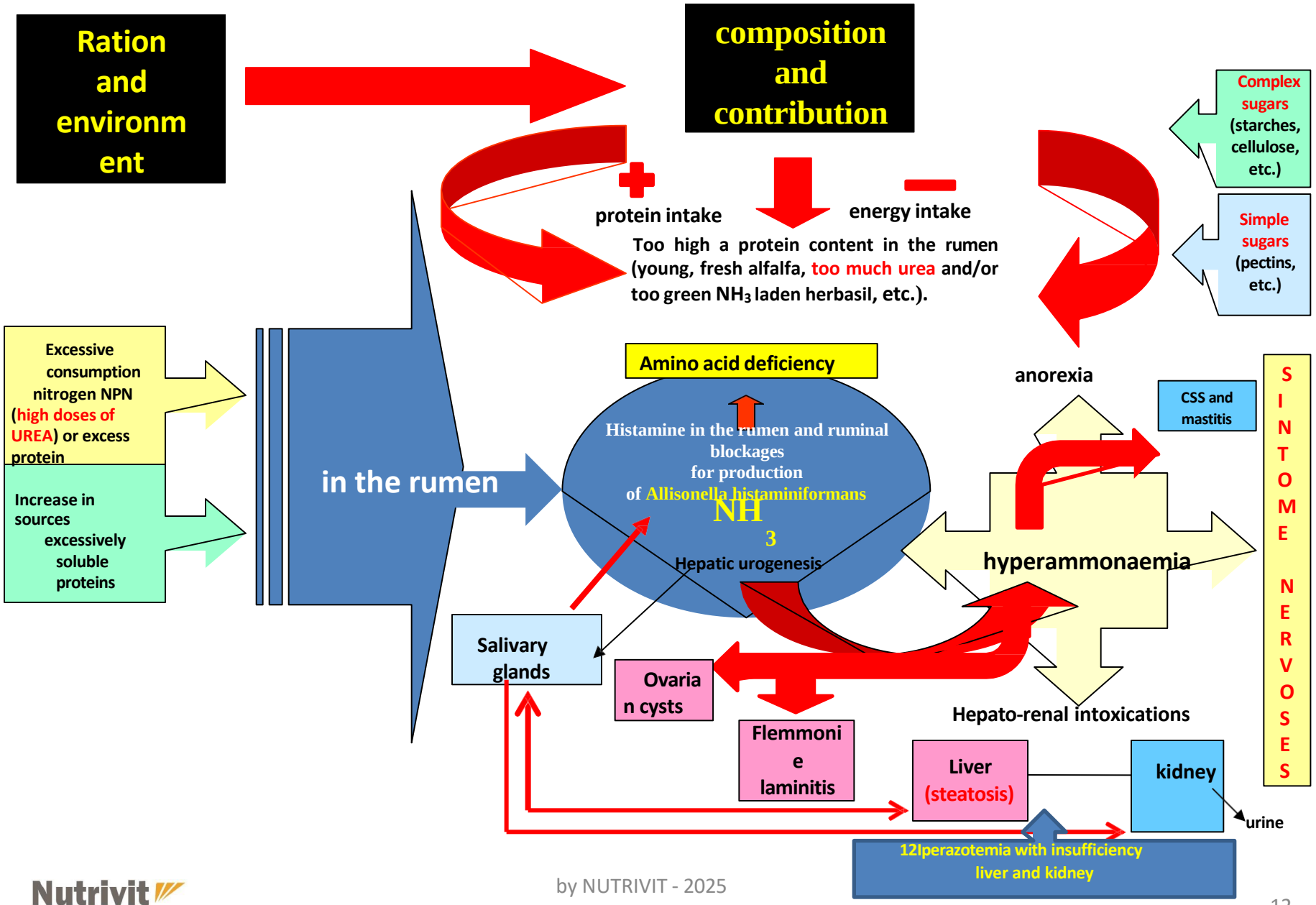
It is known that diet management and the combination of urea with a pool of pre-postbiotic enzymes can **reduce** N **wastage** in both the environmental and ruminal habitats.

It should also be emphasised that improving the processing efficiency in the rumen and the use of appropriately treated NPN simultaneously reduces nitrogen excretion and the emission of **GHG** (**Green** House Gas) into the environment.

Urea cycle in ruminants



Soluble nitrogen intoxication



Impact on animal health

The situation illustrated so far, at ruminal level creates ideal conditions for the development of **Allisonella histaminiformans**, a ubiquitous **histamine-producing** micro-organism of the rumen:

a) **the immediate inflammation of papillae ruminal (increase of cytokines circulating).**

b) **the reduction of AGV assimilation** followed by activation of pathologies in:

1) **breast (increased inflammatory states in the breast originating mastitis)**
2) **feet (interdigital phlegmon ----------> laminitis)**
3) **ovaries (ovarian cysts)**

In some animals, it can also manifest itself in **an acute form** with pathologies:

3) **of the liver (hepatic steatosis)**
4) **of the kidneys (nephritis)**
and in **severe forms**
5) **of the CNS (neuroplegic symptoms** with **ataxia, impaired walking, etc.) and in some more serious cases coma and death of the affected person).**

Acute urea intoxication

Like all non-protein nitrogen compounds (NPN), urea can be used in ruminant ruminant rations with a functioning rumen without any problems as long as certain precautions taken with regard to the dose use, timing of administration and association with certain other products. In the early days, due to *insufficient or poor knowledge of ruminal metabolism*, the use and widespread use of urea in ruminant nutrition **resulted in the death of animals from toxicity.** This led to need to limit, if not prevent, its use in ruminant diets.

Symptoms of urea poisoning

1) The increased organic presence of **NH_3** in internal organs exerts **a caustic action in all organs.**
2) Severe convulsive colic appears.
3) Ataxia: Gait becomes unsteady.
1) Increase of pulsations at jugular (blood pressure).
2) Sudden death follows (maximum 3 hours ingestion) after a violent bellow.

Waste (intoxication) and utilisation urea in ruminant feed

Following extensive research demonstrating its safety and usefulness at correct dosages under particular conditions, urea has become a standard ingredient adopted in ruminant diets.

A vast amount of information and knowledge has been documented concerning the mechanisms of utilisation of urea and other non-protein nitrogen compounds by the ruminal **microbiota**.

Problems of ammonia/urea toxicity can be prevented through proper utilisation of scientific knowledge on feeding urea in the diet of ruminants.

Additives and processing techniques exist today to improve the anabolic use of NPN urea by the rumen microbial population through a remodulation of NPN urea recycling in the rumen.

***(Patent -n°102022000014362* - PCT/IB2023/056515**)

Proper diet management and combination of urea with a pool of acids, enzymes and pre-postbiotics, etc. can reduce NPN wastage in the environmental habitat as much as in the ruminal habitat.

It should also be emphasised that improving the processing efficiency in the rumen and the use of appropriately treated NPN simultaneously reduces nitrogen excretion and the emission of **GHG (Green** House Gas) into the environment.

Speed of hydrolysis at the ruminal level urea

Classical UREA with **rapid NPN** degradation

CH_4N_2O -------> NH_4^+ + NH_3

high percentage

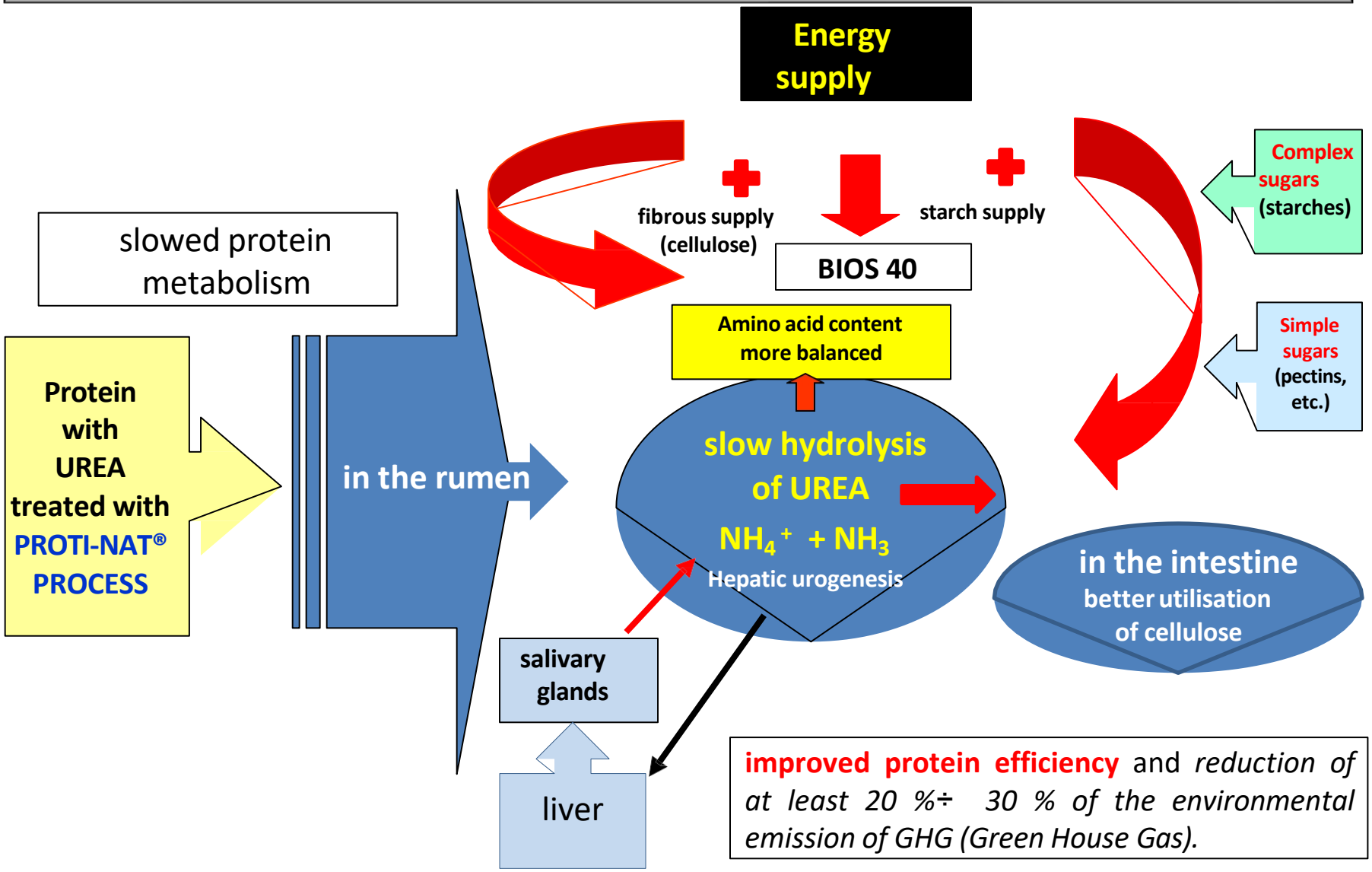
UREA treated with **PROTINAT® PROCESS** with degradation

slow-release NPN

CH_4N_2O (treated with PROTINAT® PROCESS) -------> NH_4^+ + NH_3

is transformed into noble proteins

Metabolic functioning with UREA treated with the PROTI-NAT® PROCESS method (Patent No.° PCT/IB2023056515)



improved protein efficiency and *reduction of at least 20 %÷ 30 % of the environmental emission of GHG (Green House Gas).*

Reflections on the problem

Today, there is much more information and knowledge available on the mechanisms of utilisation of urea and other non-protein nitrogen compounds by the ruminal microbiota, you just need to know how to read and interpret them.

The problems of ammonia and urea toxicity can be almost entirely avoided through the correct use of **scientific, chemical, physical and microbiological** knowledge on the composition of ruminant feed containing slow-release urea.

Today there is a production process of my invention consisting of additives and physico-chemical treatment techniques to improve the anabolic use of UREA by the rumen microbial population thanks to the remodulation of NPN urea recycled in the rumen.
Brevetto IT/102022000014362 PCT/IB2023056515

The creator of this patent knows how feed management and the combination of urea with a pool of plant processors, enzymes, pre-postbiotics and acidifiers, and certain processes, can reduce nitrogen wastage both in the environmental habitat and mainly in the ruminal habitat and thus slow down the rate of nitrogen utilisation in the ruminal microbiota.

It should also be emphasised that the improved processing efficiency in the rumen and the use of **NPNs**, even if not properly processed, simultaneously reduces **nitrogen excretion and the emission of GHG (Green House Gas) into the environment (recent American research - studies from 20 to 30%).**

Comparison different types of UREA treatment patented and on the world market

Long-established patents

a)American patent based mixing UREA with specific buffers (montmorillonite, etc.). Present on the market with poor results **(obsolete)**

b)Another, also American, patent consists of inserting urea into a slowly hydrolysing fat (*by-pass*) by feeding the product to ruminants.

The relatively low success of the products is due to 3 factors:

1) They do not allow large quantities of urea to be fed to ruminants (max. 150 g/head/day);
2) They are not able to replace soya (2/3 Kg.) integrally
3) It has a high distribution cost.

It is used as a nutritional supplement.

Modern patent **PROTINAT® PROCESS**

a)The **PROTI-NAT** patent® **PROCESS** is based on the treatment of urea that is as simple as it is ancient in its mechanism of action: Just think of the hydrolysis of urea that takes place in the soil as fertiliser. Principles of microbiology, biochemistry and physics are used.

b)The product obtained with the Italian Patent, can safely be eliminated from the diet of ruminants with a functioning rumen:

1) Soya and, if desired (or if necessary), other expensive or poorly available vegetarian protein sources;
2) When fully operational, it has no additional costs and remains in the range of normal costs of an extruded and/or expanded feed.

Comparisons

SOY
A

Agricultural UREA

(by Ruminant Academy)

normal premixed additives combined with urea that are readily available on the market for feeding ruminants .

Soybean extraction flour

5:1

costs

The ratio of the production costs of soya and urea to their respective protein production is 1:5, indicating that soya is five times more expensive than urea.

UREA:
Readily available at an industrial price like bicarbonate, etc.

Normal mixing and extrusion machinery

Nutrivit

Production of PROTINAT® PROCESS

The production process includes additives and processing techniques to improve the anabolic utilization of 46% urea (NPN) by the rumen microbial population by remodulating the recycling of transformed urea in the rumen.

***(IT patent no. 102022000014362* - PCT/IB2023-056515)**

normal agricultural urea
46% NPN

soya peelings

Premix PP (additives and grease)

normal mixer and a classic extruder

blended and extruded feed

Processing and mixing of a variable-value protein feed (% protein provided mainly by urea treated with PROTINAT® PROCESS) for use in the feeding of ruminants with functional rumen.

Materials and Methods

Dr. Gabaldo's field trials were to establish whether the innovative intuition had any practical application in ruminants without intoxicating them.

The first practical animal tests on this technology were carried out in 2015 by producing at a trusted feed manufacturer, equipped with an extruder, a feed formula into which a core (Premix PROTINAT®) was inserted at 10%.
This premix had in turn been prepared by a premix manufacturer with whom I am bound by a Confidentiality Agreement, who in addition to the premix added common **46% NPN** (non-protein nitrogen) **urea** at a rate of **50%**.

This **Premix** called '**Premix PROTINAT®**' was itself used at 10% in a feed mixed with a 90% food sub-product, which was very but very fibrous, low in protein and low in energy substances such as 'soya hulls'. The product was also rich in dietary fibre (pectins stimulating ruminal microbial activity) and cellulose, with these average nutritional values on the dried soya hulls market:

- Humidity .....±8.00%,
- Crude protein 12.00%,
- Crude oils and fats 2.00%,
- Crude fibre 30.00%,
- Ash.....6.00% with the addition some barley, minerals and trace elements.

First practical field test

The resulting feed 10 % PROTINAT® PROCESS (consisting 10 % treated urea + Premix PP) + 50 % cereals + 40 % soya hulls.

analytical analysis of the product obtained performed in an accredited laboratory of analysis, the following values were obtained:

	KG	%
MAIZE FLOUR	50,0000	50,0000%
SOYA BUCKETS	40,0000	40,0000%
Premix PROTINAL	10.0000	10.0000%.
Totals	100.0000	100.00%

Analysis	%TQ
PG	23.32 of which 14% from TREATED UREA
LG	3,0 with the PROTINAT PROCESS FG
method	17,70
ASHES	8,33
CA	0,78
NA	0,15
UFL	0,37
UFC	0,36
ADF	17,20
STARCH	34,54
ASHES	3,63

The resulting feed, with a physical appearance similar to that of classic dog kibble, was tested by administering it to dairy cow herds in the semi-wild state (with pasture) in Apulia with an average production of approximately 25 ÷ 28/litres/head/day in the Putignano area and sheep and goats in Sardinia in the Campidano area reared in the semi-wild state (with pasture) to test:

a) **The degree of palatability of the** product since the animals had the product freely available.

b) **The degree of toxicity of the** product, bearing in mind that for every kilo of product, **140 g of urea** would have been administered to the animals, a quantity that is already quite **high and at risk of toxicity**.

c) The amount of product they would have eaten spontaneously and not included in the carts mixers and/or feeds industrial feed where is imposed a quantity

Results

The cattle (dairy cows) under test spontaneously ate the treated feed from **2.0 to 3.5** kg/head/day, thus ingesting **280 to 490 g of treated urea.** Bearing in mind that Sardinian sheep and/or goats weigh around 70 to 80 kg, the sheep even managed to ingest **400 to 500 g/head/day**, thus ingesting **56 to 70 g of treated urea, a dose that if ingested as normal urea by a small ruminant is universally considered very toxic, not to say lethal.**

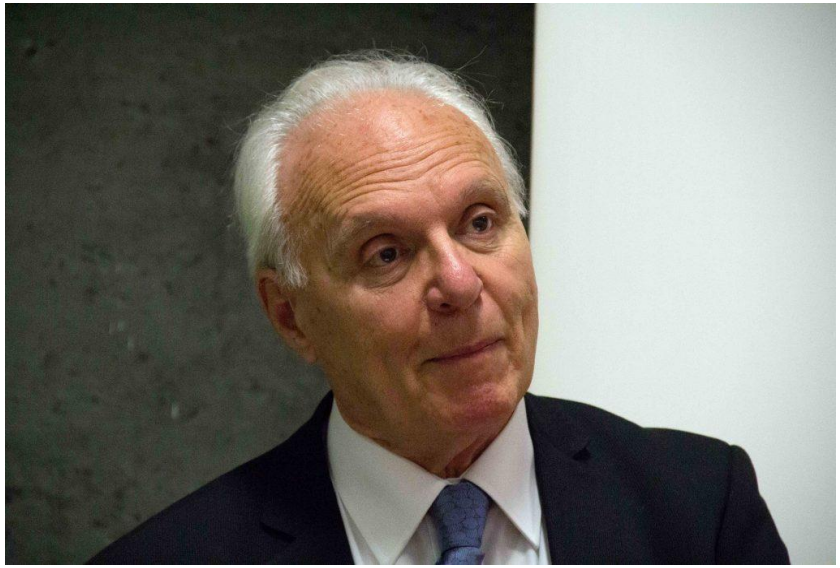
Although we did not have the possibility of dividing into groups using a scientific method (randomisation with treated and control group), milk yields obtained from the treated animals increased significantly in the dairy cows (about a couple of litres) as well as in the sheep and goats (about 1 litre).

Other practical results on animals

Last year, after reflecting on the fluctuating soya market, after protecting my idea, my invention, the process (Patent IT-n° 102022000014 PCT/IB2023/056515), I thought of testing the product scientifically.

I had a concentrated feed called BIOS 40 produced (containing 10% NPN from urea treated with the PROTINAT® PROCESS method, with a protein value of 40% crude protein of which 28% from NPN).

The experiment, agreed upon with the owner of the herd, the stable technician Dr. Marco Bellini and colleagues from the University of Parma, was carried out on cows with a high genetic and productive level (Italian Holstein with an average production of approximately 36 litres/head/day), in particular on breeding heifers with a high genetic profile, in the two months prior to calving. Considering that these are the most delicate subjects in a herd, it is undeniable that in this physiological phase of the cow, any toxicity problems **are immediately manifested, both before and after calving.**



Prof. Antonio Ubaldi

Lecturer at the Faculty of Veterinary Medicine of the University of Parma from 1974 until 2015. He was Lecturer in biochemical and clinical laboratory diagnostics, veterinary toxicology, residue toxicology in foodstuffs and in three postgraduate schools at the Universities of Parma and Milan.

Premise

Initial interest

This project by Dr. Gabaldo interested me a lot because in agriculture and breeding that animals were fed with nitrogen-based products, not from agricultural crops, but from industry, seemed to me an innovative and very interesting thing.

Toxicity Concerns

I had doubts about the toxicity of these nitrogen products, because having never used them it became a very interesting questionnaire.

Historical practices

I knew that farmers sometimes threw urea into the manger. I'm talking about the stables of 50 or 60 years ago, what did they do? When they needed to urinate they went to the barn and threw the urine on the hay in front of the cows who ate with a lot of appetite.

Current innovation

Some time ago Dr. Gabbaldo prepared a very interesting and simple urea derivative and perhaps this is its strength, simplicity, but of excellent effectiveness.

By A. Ubaldi - UNIPR - 2025

Peripartum results

According to the reports of the owner, the veterinarian and the nutritionist following the herd where the test was carried out, it appears that in the five heifers subjected to the administration of 200 g/head/day of treated urea:

a) The cows treated presented themselves for delivery on time and without any problems related to their physiological state.

b) All have gone into natural heat and three of them have already been fertilised.

c) There were no symptoms of ketosis and the BCS was within the expected standard (> 2).

d) Loss appetite within limits.

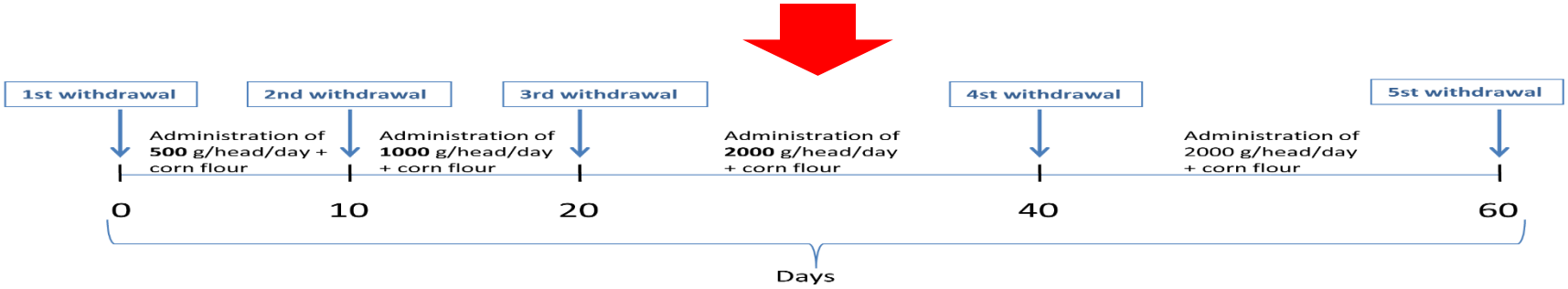
Protocol

Materials and methods

The test was carried out on 2 groups of n° 5 cows each on the farm of Azienda Agricola Cecchin Giovanni e Riccardo - Via Argine Crostolo, 13 - Guastalla (RE) selecting the subjects by the "random" method for both groups (treated "**T**" and control "**C**") on which blood samples of 10 cc. were taken.

The treated heifers were administered **BIOS 40 (containing 10% PG-treated UREA of** 40% mixed 50% with maize flakes) produced by the company Valtramigna Foods s.r.l. of Cazzano di Tramigna (VR), while the control group continued with their usual feed ration. Feeding and sampling followed the following scheme:

Preliminary remarks: In view of the presence of non-protein nitrogen (NPN) in the BIOS 40 product, in the "conditioning" phase for the microbial adaptation of the ruminal population, the product was administered to replace the current ration used, increasing the dosage gradually according to the programme described below:



Procedure for taking and sending samples

The samples were taken at the intervals indicated in the table, by Dr. Gisella Pizzin, Veterinary Doctor, who also took care of their delivery to the 'Laboratory Diagnostics' at the Department of Veterinary Medical Sciences of the University of Parma.

Sample analysis

Urea and liver function values and the required toxicity index were measured in the blood samples

University, and co-chaired by Prof. Antonio Ubaldi.

Personal considerations

Duration of the experiment

Over the course of three months, with the help of a colleague from the University of Parma, I personally made a number of samples in the breeding of all the animals subjected to experimentation and then, all together in the laboratory, we would evaluate any change in the experimental data.

Biochemical-clinical parameters

At this point it was necessary to establish what biochemical-clinical parameters could or should be carried out on the serum of these treated and untreated animals, since, according to the traditional canons, these animals were divided into two groups.

Experimental groups

A first group to which a normal and habitual diet was administered, we called the control group (second protocol). The other group, on the other hand, was fed with the addition of the new product in which the treated urea was inserted, initially at a dose of 200 grams/head/day and was called the experimental group.

Amazing results

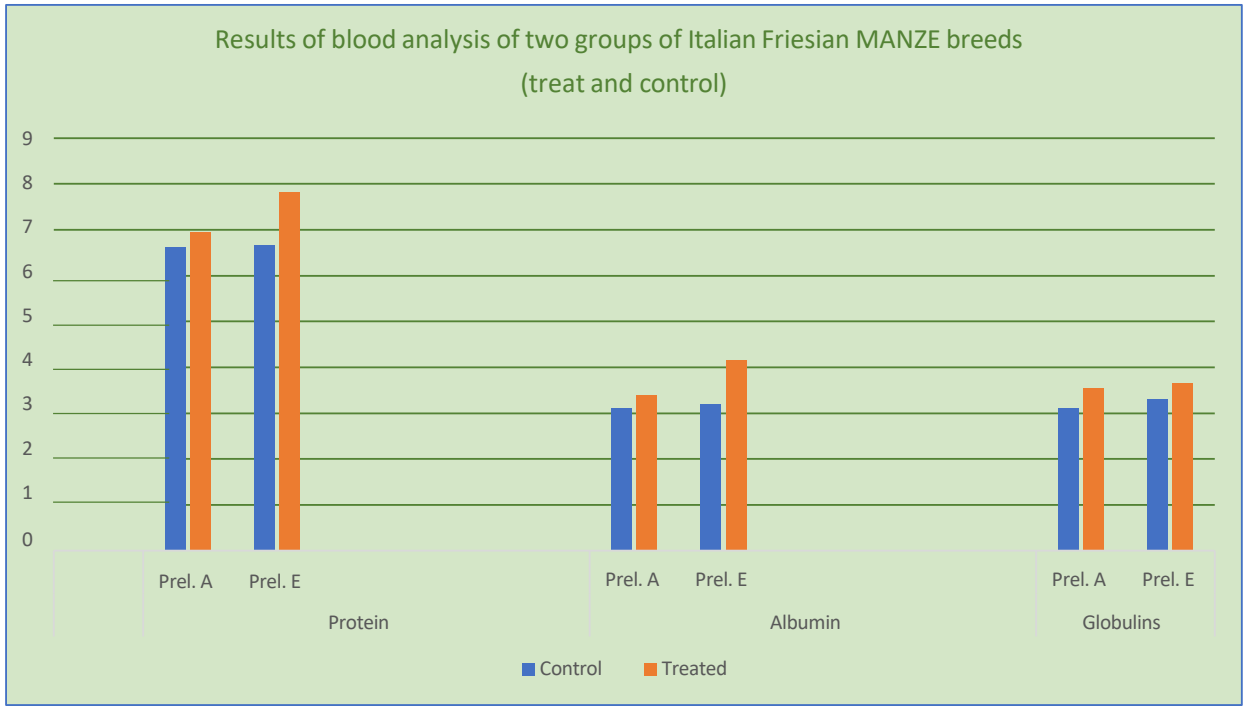
This dose, in my opinion, was stratospheric in itself, but according to the protocol, dr. Gabaldo, increased the dose and I simply followed the data from the laboratory and I was surprised because the treated animals had small changes, small alterations in some blood parameters, but it was an infinitesimal thing in the sense that it could be observed with refined instruments but they did not give any results at a clinical pathological level.

Results

Results of blood analysis of two groups of MANZE (treated and control) of Italian Friesian breed

	Total protein		Albumina		Globuline		Urea		Ammonium		Glucose		Total lipids	
Treat	A	E	A	E	A	E	A	E	A	E	A	E	A	E
5434	6,82	7,99	3,42	4,39	3,40	3,60	28,81	30,12	0,04	0,09	60,43	66,73	347	396
5437	7,12	8,01	3,50	4,42	3,62	3,59	31,67	36,80	0,05	0,15	56,50	62,18	393	402
9442	6,88	7,93	3,23	4,00	3,65	3,93	30,21	38,17	0,06	0,07	60,88	67,00	349	411
9457	6,84	7,12	3,20	4,08	3,64	3,04	36,68	39,00	0,05	0,06	54,52	61,22	378	389
9459	7,09	8,06	3,03	3,88	3,46	4,18	30,22	32,41	0.05	0,05	61,11	72,18	388	433
media	6,95	7,82	3,40	4,15	3,55	3,67	31,52	35,30	0,05	0,06	58,69	65,86	371	407
Control														
5417	6,65	6,03	3,11	3,01	3,54	3,02	25,33	26,81	0,03	0,03	58,61	60,08	341	351
5423	6,42	7,11	3,08	3,04	3,34	4,07	29,78	31,17	0,04	0,03	54,55	53,13	336	348
5432	7,02	6,87	3,09	3,06	3,03	3,01	31,36	30,16	0,03	0,04	60,06	57,67	381	401
5433	6,93	7,00	3,15	3,85	2,79	3,15	30,11	29,34	0,03	0,03	61,11	60,46	354	361
9430	6,06	6,35	3,10	3,07	2,90	3,28	31,76	30,44	0,04	0,05	60,00	50,88	319	345
media	6,62	6,67	3,11	3,20	3,11	3,31	29,69	29,58	0,03	0,04	58,57	58,23	346,2	361,2
Reference values o	6-9 g/dl		3-4 g/dl		3-4 g/dl		10-45 mg/dl		0.04-0.06 mg/dl		40-70 mg/dl		300-450 mg/dl	

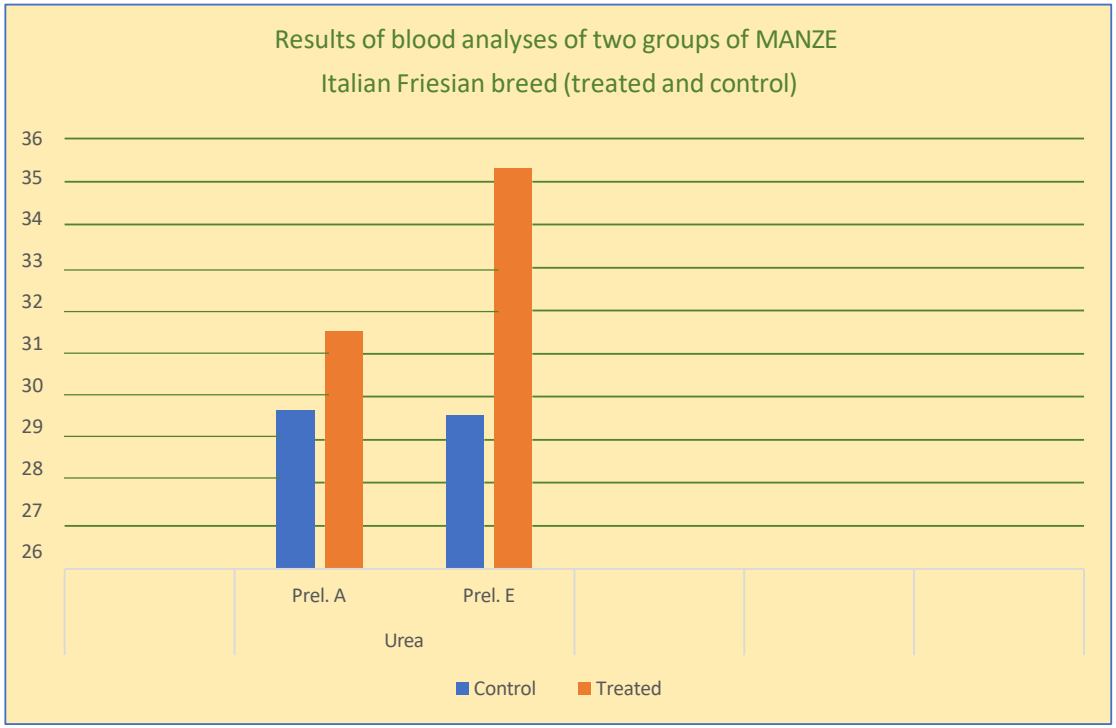
Blood samples taken in the year 2024: first sample A) 15 January, fifth sample E) 18 March



		Protein					Albumina					Globuline	
		Sampling A	Sampling E				Sampling A	Sampling E				Sampling A	Sampling E
Control		6,62	6,67		Control		3,11	3,20		Control		3,11	3,31
Treat		6,95	7,82		Treat		3,40	4,15		Treat		3,55	3,67

Reference values:	6-9 g/dl	3-4 g/dl	3-4 g/dl

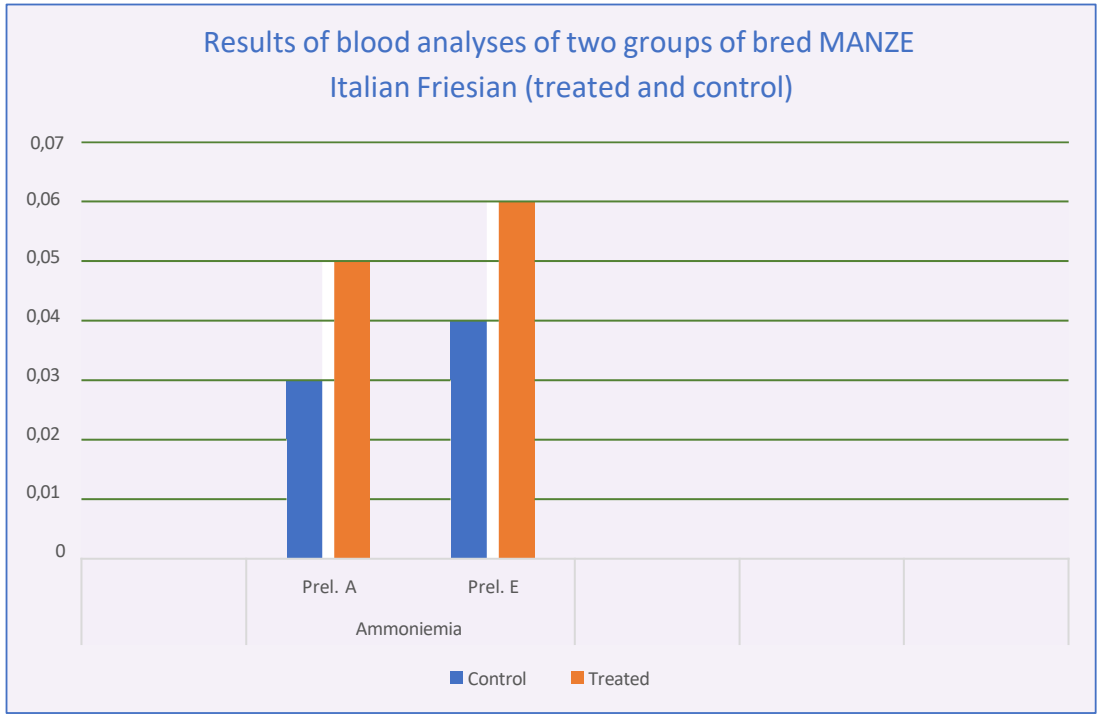
Time sequence of blood samples				
A	B	C	D	E
15/01/2024	25/01/2024	05/02/2024	01/03/2024	18/03/2024



	Urea	
	Sampling A	Sampling E
Control	29,69	29,58
Treat	31,52	35,30

Reference values: 10-45 mg/dl

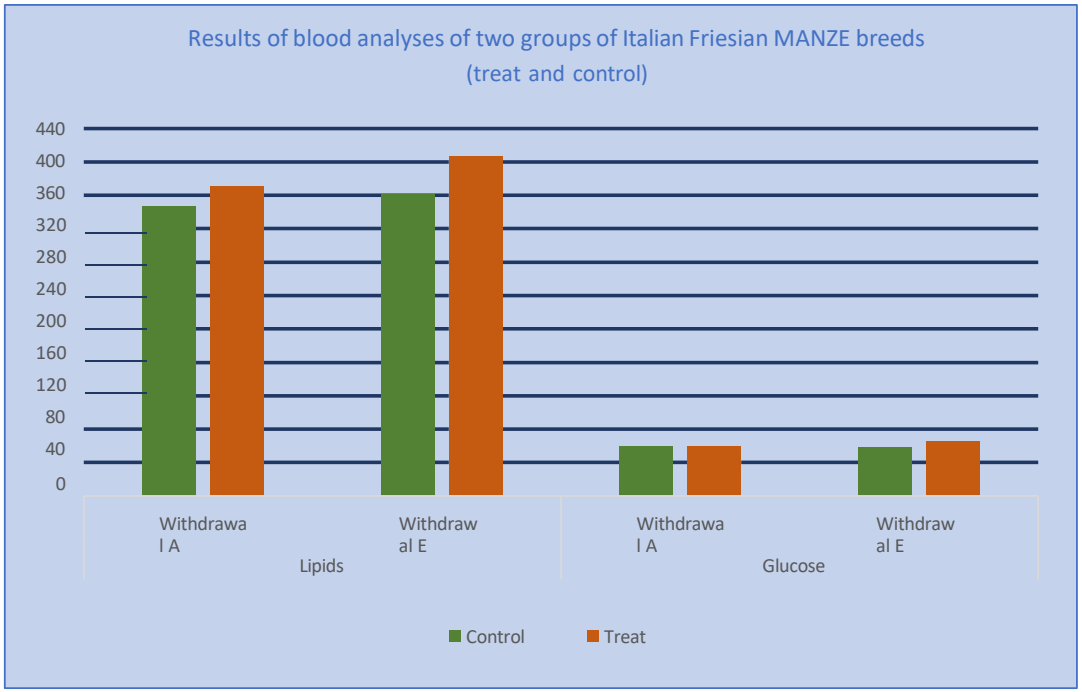
Time sequence of blood samples				
A	B	C	D	E
15/01/2024	25/01/2024	05/02/2024	01/03/2024	18/03/2024



		Ammonemia	
		Sampling A	Sampling E
Control		0,03	0,04
Treat		0,05	0,06

Reference values: 0.04-0.06 mg/dl

Time sequence of blood samples				
A	B	C	D	E
15/01/2024	25/01/2024	05/02/2024	01/03/2024	18/03/2024

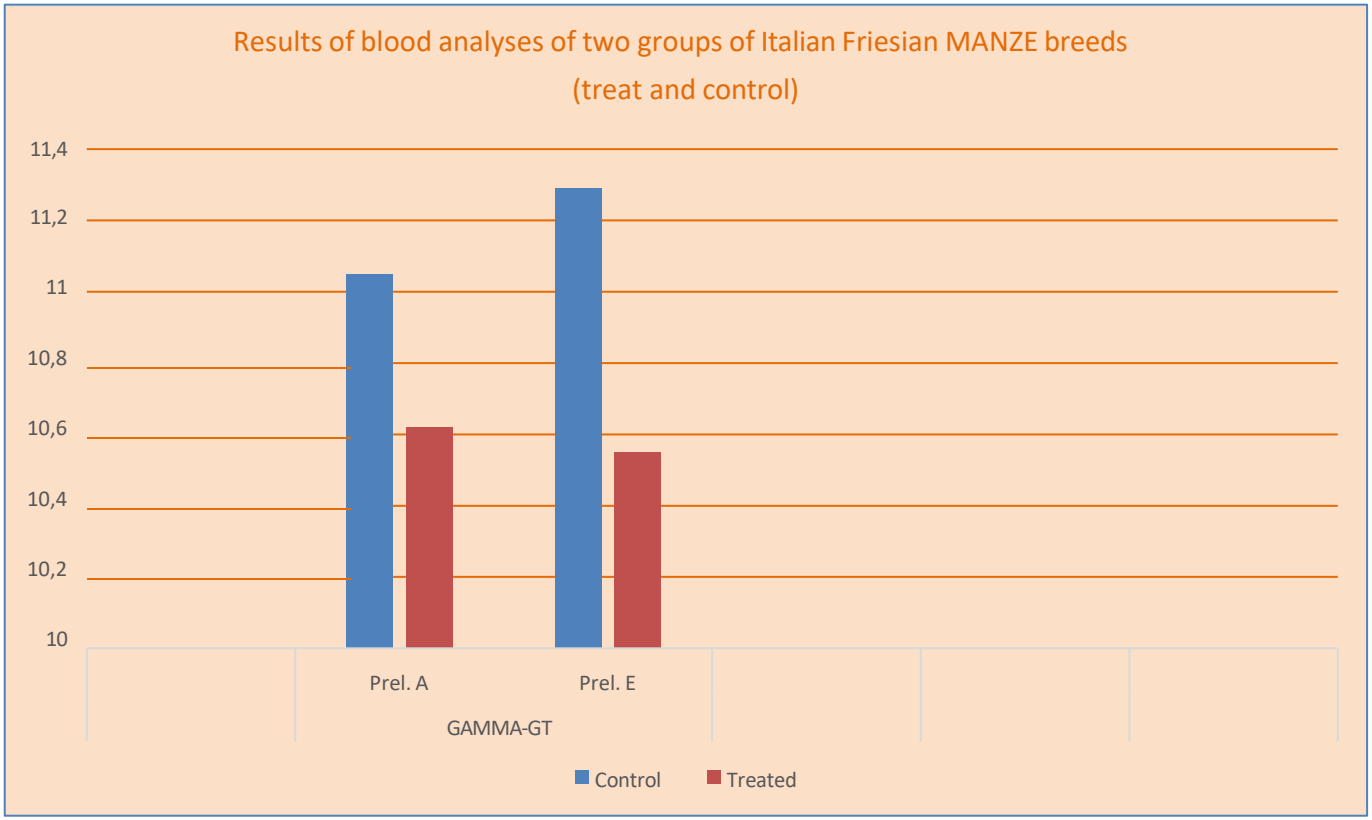


		Lipids	
		Sampling A	Sampling E
Control		346,2	361,2
Treat		371	407

		Glucose	
		Sampling A	Sampling E
Control		58,57	58,23
Treat		58,69	65,86

Reference values:	300-450 mg/dl	40-70 mg/dl

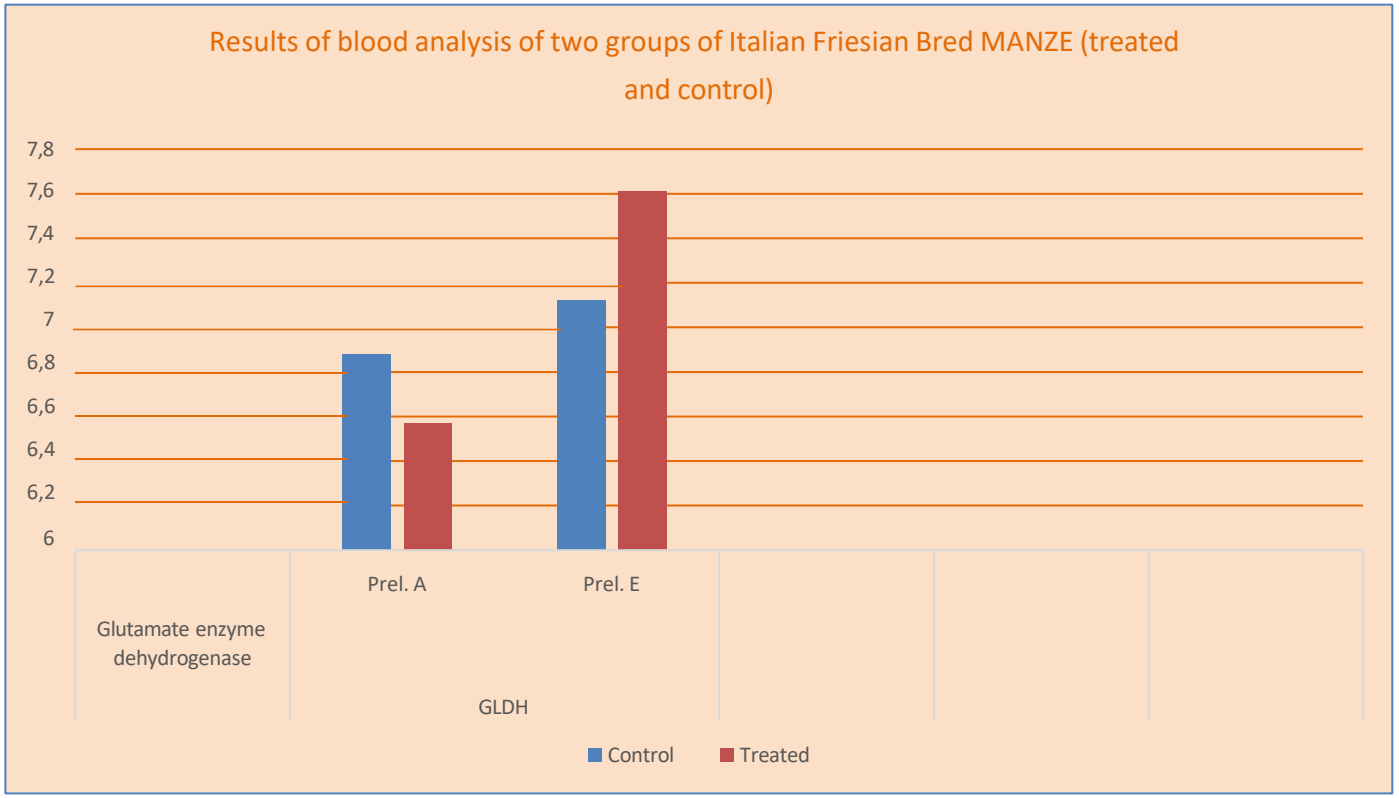
Time sequence of blood samples				
A	B	C	D	E
15/01/2024	25/01/2024	05/02/2024	01/03/2024	18/03/2024



		GAMMA-GT	
		Sampling A	Sampling E
Control		11,05	11,29
Treat		10,62	10,55

Reference values: < 18 U/L

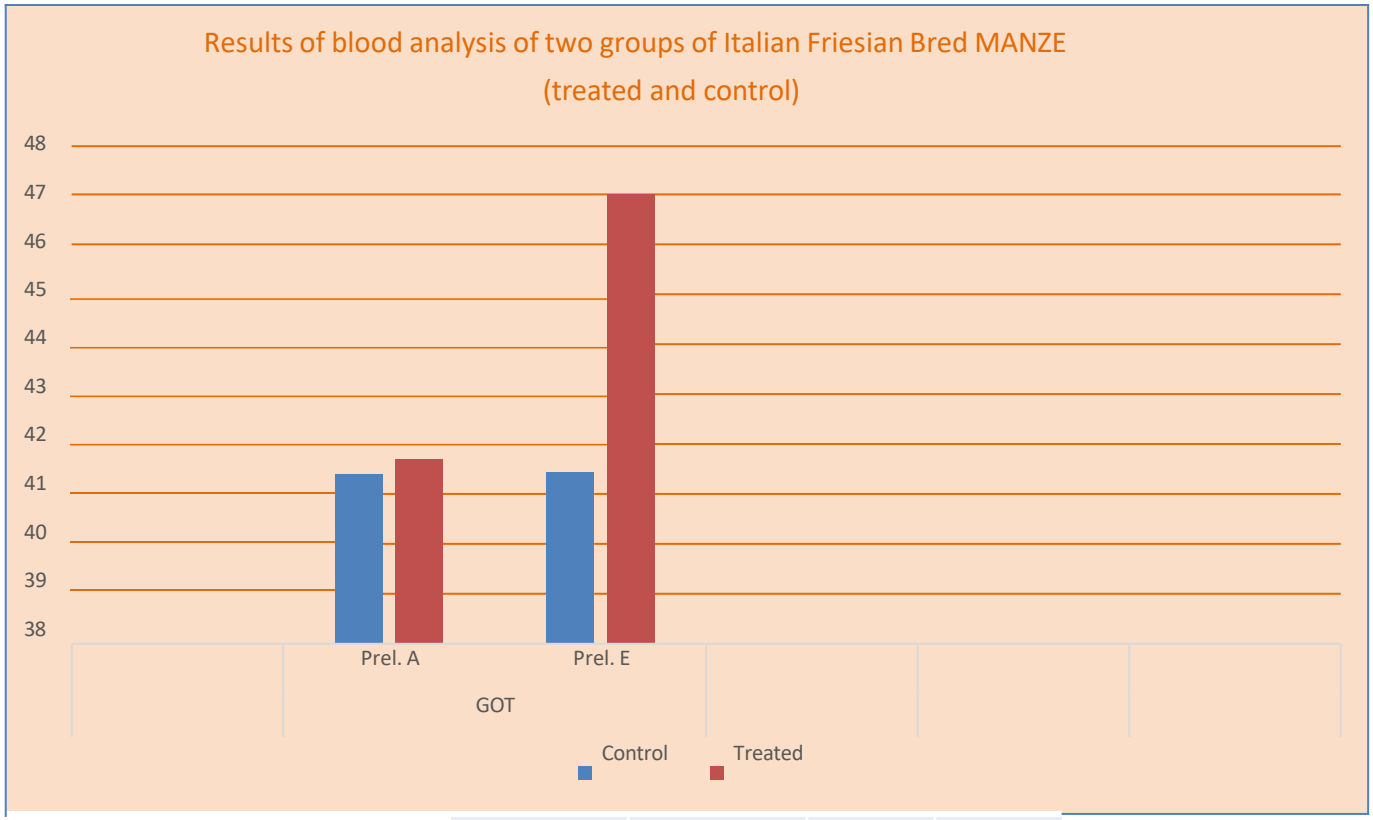
Time sequence of blood samples				
A	B	C	D	E
15/01/2024	25/01/2024	05/02/2024	01/03/2024	18/03/2024



		GLDH	
	Enzyme glutamate dehydrogenase		
		Sampling A	Sampling E
Control		6,88	7,12
Treat		6,57	7,61

Reference values: < 10 U/L

Time sequence of blood samples				
A	B	C	D	E
15/01/2024	25/01/2024	05/02/2024	01/03/2024	18/03/2024

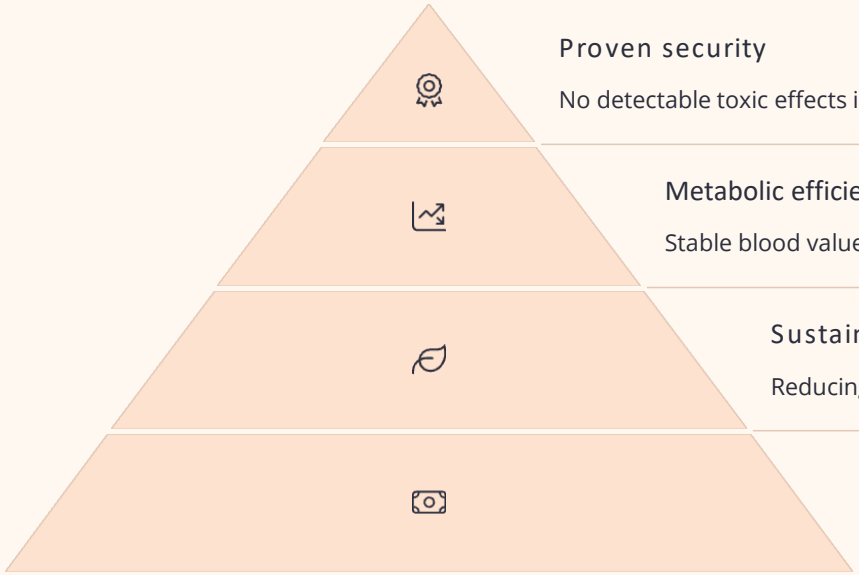


		GOT	
		Sampling A	Sampling E
Control		41,40	41,30
Treat		41,70	47,00

Reference values: < 80 U/L

Time sequence of blood samples				
A	B	C	D	E
15/01/2024	25/01/2024	05/02/2024	01/03/2024	18/03/2024

Conclusions on the effectiveness of the PROTI-NAT® PROCESS



Proven security

No detectable toxic effects in treated animals

Metabolic efficiency

Stable blood values within the reference ranges

Sustainability

Reducing dependence on soy and other traditional protein sources

Cheapness

Reduced feed costs for farmers

The use of urea treated with the PROTI-NAT® PROCESS has proven to be safe and effective even in animals in delicate physiological phases such as gestation. The results indicate that this approach may represent a viable alternative to traditional protein sources, with significant economic and environmental benefits.

By A. Ubaldi - UNIPR - 2025

Future prospects and applications

Advanced search

Further studies on different breeds and production phases

Industrial production

Process scalability for commercial applications

Environmental sustainability

Reducing the environmental impact of cattle farming

Global applications

Adaptation to different farming conditions around the world

The PROTI-NAT® PROCESS represents a significant innovation in the field of ruminant feeding, offering a safe and cost-effective solution for protein supplementation. Future prospects include process optimisation, expansion of applications to other ruminant species, and assessment of the long-term impact on animal health and product quality.

By A. Ubaldi - UNIPR - 2025

Dr. Giulio Gabaldo - DVM- PhD
Inventor of the technology and owner of the Patent - n° 102022000014362) PROTINAT® PROCESS

What BIOS 40 and BIOS 70 are and what they are for

1° and 2° commercial product for testing in Italy

Total and/or partial replacement of soybean meal in rations of adult ruminants with a functioning rumen.

-BIOS **40** is a product consisting of normal urea for zootechnical use, supported by special additives (pre-biotics, post-biotics of the latest generation, sugars, montmorillonite, sulphur, etc.), with a little bit whole flax seed, all heat-treated with PROTINAT® PROCESS (Patent IT-n° 102022000014 PCT/IB2023/056515). The process enables a partial detoxification and a less risky use of urea and allows it to be administered in at least twice the normal amount.

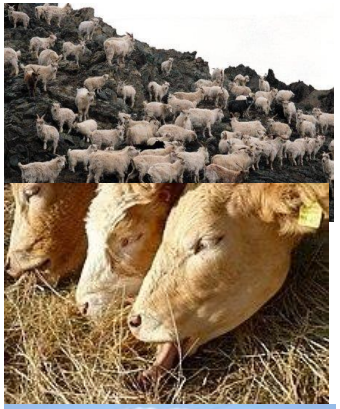
-BIOS **70** allows good protein supply in ruminant rations fed with forage and increased utilisation of 'fibrous food sources' with low feed value such as straw, forages with very high NDF. (Neutral Fibre Detergent).

How are they used?

They should be used in the diets directly in the feed troughs or also freely available in the pastures or included in the feed formulation of adult ruminants with a functioning rumen, at a ratio:

- **BIOS 40** 0.3÷ 0.5%
- **BIOS 70** 0.15÷ 25% per 100 kg live weight**.**

Start gradually with a low dosage of at least ¼ of the recommended dose, gradually increasing over 2 to 3 weeks to the maximum dose indicated.



by NUTRIVIT - 2025

Nutrivit

PREMIX PP





FEED PREMIX FOR RUMINANTS WITH FUNCTIONING RUMEN RESERVED EXCLUSIVELY FOR THE PRODUCTION OF FEED DERIVED FROM THE PROTI-NAT® PROCESS BY APPROVED FEED MANUFACTURERS..

Componenti for Kg: Vitamins-Pro-Vitamin Substances with a similar effect that are chemically well defined:
3a314 Niacin …… 10.000,00 mg.
Emulsifiers, Sanitizers, Thickeners and Gelling Agents:
E 330 Citric acid …… 2.000,000 mg.
1m558i Montmorillonite

Support based on hydrogenated vegetable fats, a micronized mix at low temperature of medicinal aperitif plants:
Seaweed, Glycyrriza glabra, Pimpinella anisum, Tarassacum erythrospermum, Urtica, Cynara scolymus, Silybum marianum, Solidago virgaurea, Berberis vulgaris, Equisetum arvense, Crataegus monogyna, Raphanus sativus var. Niger, Fumaria officinalis, Peumus boldus, Ribes nigrum, Orthosiphon, Magnesium (chloride, sulfate and hydroxide.

USE AND DOSAGE
To be used EXCLUSIVELY for the production of PROTI-NAT® – PROCESS (Línea BIOS) products at a rate of 5 – 10% based on the availability of raw materials and the indications of the Nutrivit Technical Service - TE.CO.S. s.r.l.

Made in France by COFATHIM
Rue d'Epinal - 70210 Vauvillers
with authorization n° α-FR70526001

Net weight at origin in packaging of
Kg. 25,0
production batch No.

RETENTION PERIOD
Product 24 (twenty-four) months from the date of minimum durability
Best before

Nutrivit

Bios 70

COMPLEMENTARY FEED FOR RUMINANTS WITH FUNCTIONING RUMEN

COMPOSITION

Soybean husks, fractionated hydrogenated vegetable fats, calcium carbonate, calcium sulphate, sugar cane molasses, barley grains (germinated and fermented), sodium chloride.

Analytical constituents:

Crude protein	*70.00 %*
Oils and fats	*6.10 %*
Raw cellulose	*21.00 %*
Crude ash	*17.88 %*
Calcium	*0.96 %*
Phosphorus	*0.12 %*
Magnesium	*0.86 %*
Sodium	*0.13 %*

Insoluble ash in HCL< 3,3%

Additives per kg.

Urea and its derivatives: 3d1 Urea ..... 200,00 g
(Urea treated with the PROTI-NAT® PROCESS method
(Patent n° IT n°102022000014362 – PCT/IB2023/ 056515)
Binder, anti-caking and coagulating agents:
1m558i Montmorillonite/Bentonite ..... *2.200,00 mg.*

INSTRUCTIONS FOR USE

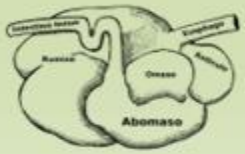
Feed and/or rations of adult ruminants with functioning rumen (dairy cows, sheep, goats, buffaloes, camels, etc.) as a partial and/or total replacement for protein meal at the rate of 0.15 ÷ 0.25 % of live weight.

WARNINGS

Use the product only in adult ruminants with a functioning rumen.
Start with a low dosage of 1/4 and gradually increase over 2 to 3 weeks to the suggested dose.

OTHER INSTRUCTIONS

Non-protein nitrogen corresponds to 56% of the total protein. Maximum urea should only be given as part of diets high in easily digestible carbohydrates and low in soluble nitrogen. A maximum of 30% of total nitrogen in the daily ration should come from urea-N.



Product with authorization
N° 024 VR 00048
VALTRAMIGNA FOODS s.r.l.
Via Molini, 5 – Z.A.I. – 37030 Cazzano di Tramigna (VR)
Tel.: +390457820503 mail: info@valtramignafoods.it
Technical Service: tecnico@valtramignafoods.it

EXPIRATION
Product: 36 (thirty-six) months from the date of minimum durability.
Best before

Distributed by Nutrivit *a proprietary brand TE.CO.S. s.r.l. – P. VAT: 01850760230 – REA VR200270*

Nutrivit

Bios 40

COMPLEMENTARY FEED FOR ADULT RUMINANTS WITH FUNCTIONING RUMEN

COMPOSITION

Soybean husks, whole flaxseed, calcium carbonate, calcium sulfate, sugarcane molasses, barley (germinated and fermented), sodium chloride.

ANALYTICAL CONSTITUENTS:

Crude protein	*40,00%*
**Oils and fats*	*4,81%,*
Raw cellulose	*24,50%,*
Crude ash	*6,19%,*
Calcium	*1,70 %,*
Phosphorus	*0,17%*
Magnesium	*0,14%*
Sodium	*0,24%*
Ceneri insolubili in HCL	*< 3,3 %,*

*(of which 28% provided by non-protein nitrogen).

Non-protein nitrogen should not exceed 40% of protein ration totals.

The product contains cereals and/or their flours and/or sugars.

ADDITIVES PER KG.

Urea and its derivatives: 3d1 Urea ..... 100,00 g
(Urea treated with the PROTI-NAT® PROCESS method
(Patent n° IT n°102022000014362 – PCT/IB2023/ 056515)
Binder, anti-caking and coagulating agents:
1m558i Montmorillonite/Bentonite ..... *2.200,00 mg.*

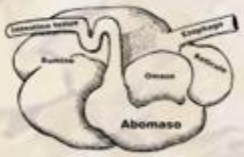
INSTRUCTIONS FOR USE:

To be administered angimi and/or in the rations of adult ruminants with functioning rumen (dairy cows, sheep and goats, buffaloes, camels, etc.) in partial and/or total replacement of protein meal at the rate of 0.5 ÷ 0.7% of live weight.

AVVERTENZE:

Use the product only in adult ruminants with a functioning rumen.

Start gradually with a low dosage of 1/4 and gradually increase over 2 to 3 weeks to the suggested dose.



N° Manufacturer Authorization
IT α000060VR
VALTRAMIGNA FOODS srl
Via Molini, 5 – Z.A.I. – 37030 Cazzano di Tramigna (VR)
Tel.: +390457820503 Mail: info@valtramignafoods.it
Technical Service: tecnico@valtramignafoods.it

Peso netto all'origine in sacconi
Da **Kg 500 - 1000**

Lotto N°

PERIODO DI CONSERVAZIONE:
Prodotto 36 (trentasei) mesi dalla data di conservazione minima
Da consumarsi preferibilmente entro il

Dr. Marco Bellini
Alimentarist who followed the test in the experimental cows

Breeding

The farm

- S.A.U. Hectares 33
- Crop rotation :

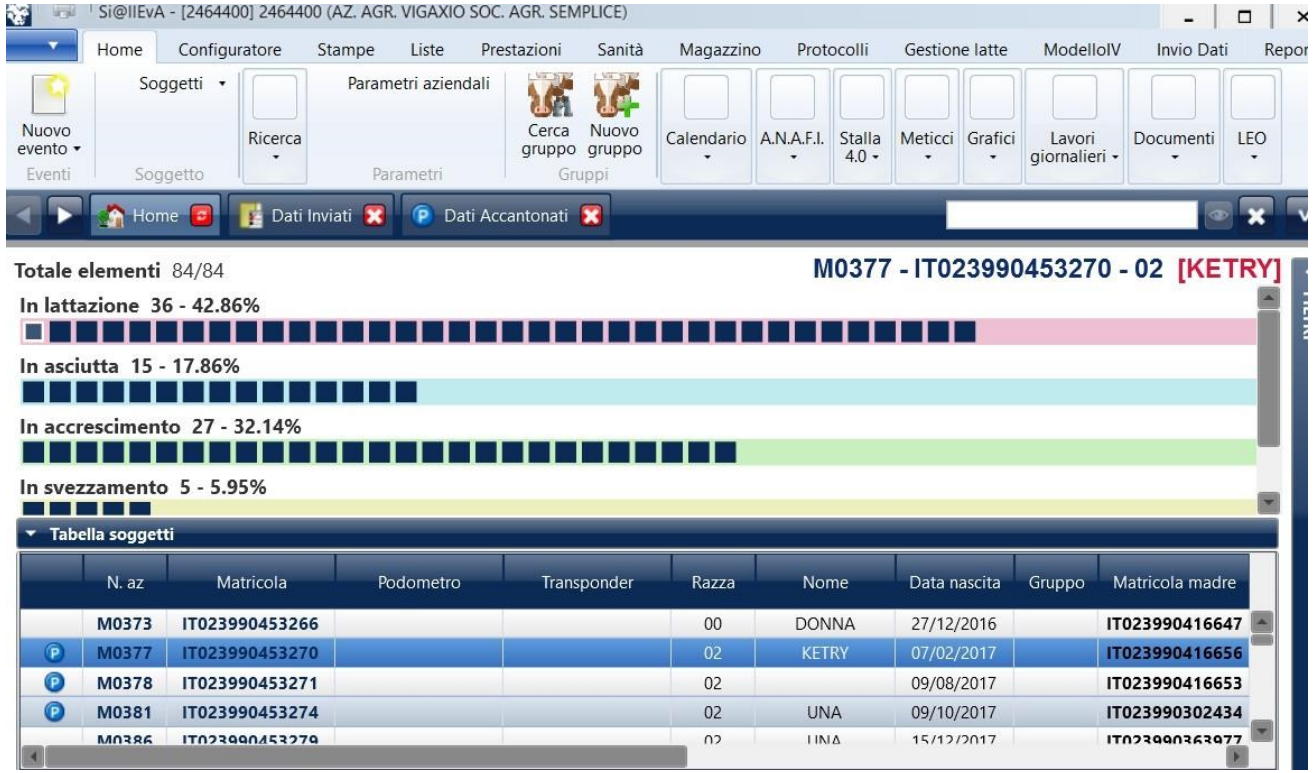
First sowing

- silage Ha 9
- alfalfa Ha 10
- whole corn mash Ha 3
- grass mixture Ha 11

Second sowing

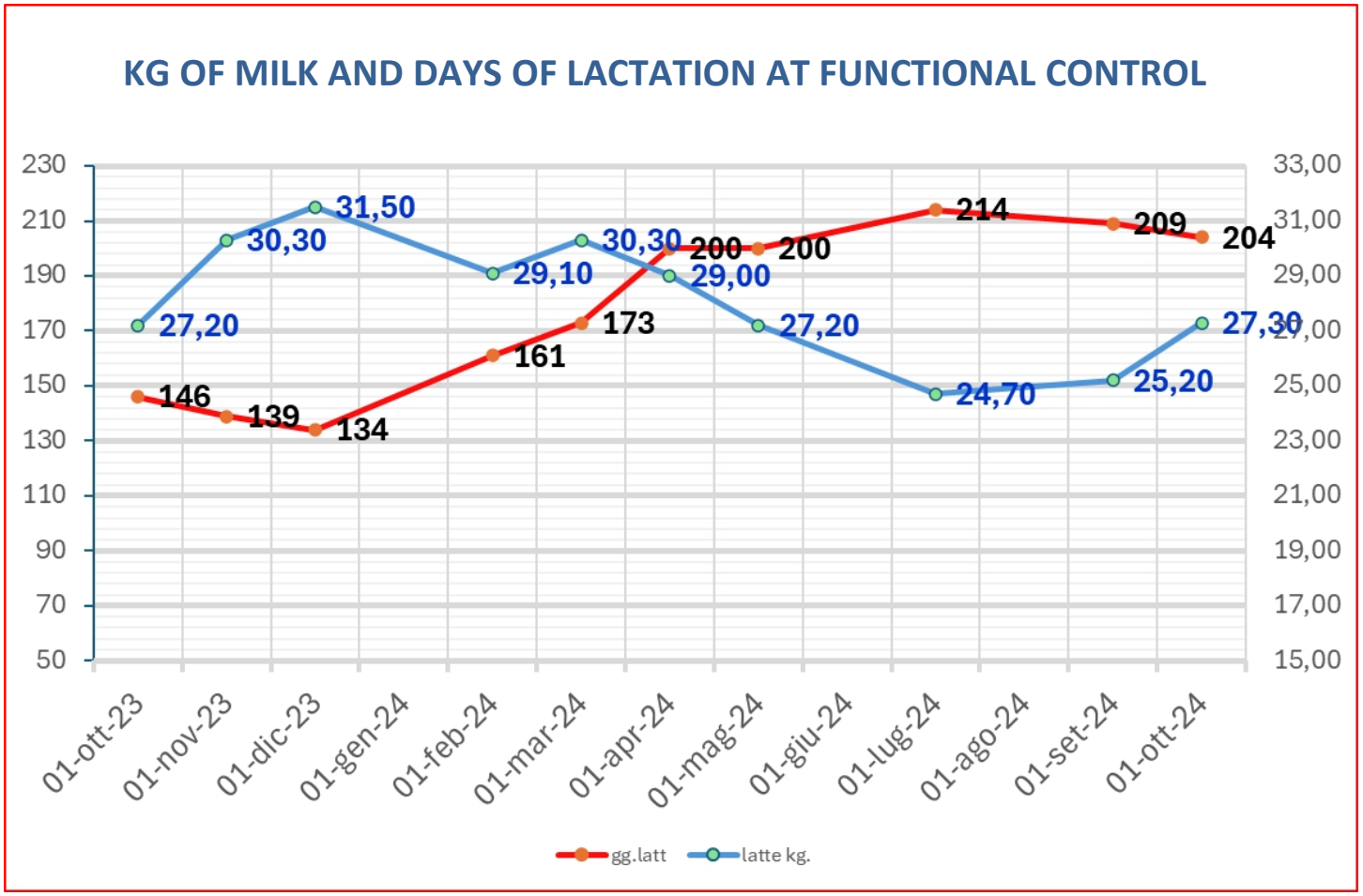
- Fodder sorghum after silage.
- Millet and wholemeal mash after mixing grasses.

The company's management indices were derived from the processing of data collected through the functional checks and processed with AIA's Si@lleva software



The herd

Heads present	83
In lactation	36
Dry	15
Rematch	32


KG OF MILK AND DAYS OF LACTATION AT FUNCTIONAL CONTROL
230
210
190
170
150
130
110
90
70
50
33,00
31,00
29,00
27,00
25,00
23,00
21,00
19,00
17,00
15,00
146
139
134
161
173
200
200
214
209
204
27,20
30,30
31,50
29,10
30,30
29,00
27,20
24,70
25,20
27,30
01-ott-23
01-nov-23
01-dic-23
01-gen-24
01-feb-24
01-mar-24
01-apr-24
01-mag-24
01-giu-24
01-lug-24
01-ago-24
01-set-24
01-ott-24
gg.latt
latte kg.

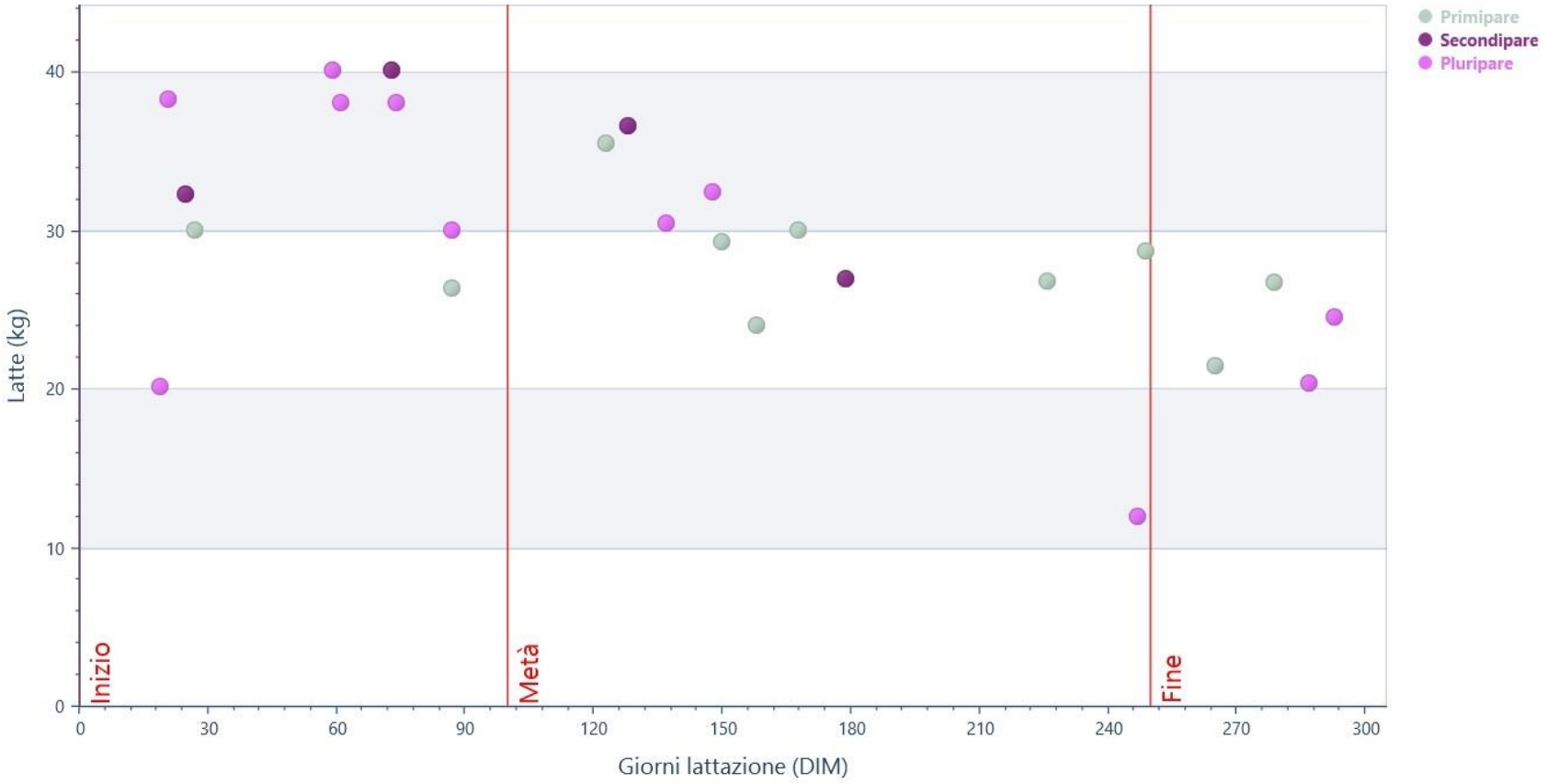
Comparison of Rations

alimento	razione partenza	razione sperimentale
	kg	kg
miscuglio graminacee insilato	9,50	7,00
trinciato di mais	8,00	8,00
mais farina	5,30	8,00
fieno silo di medica 3,5	3,50	5,50
farina di soia proteica	2,00	-
fieno di medica	1,80	1,80
crusca	0,80	0,80
mais pastone integrale	5,00	-
trebbie insilate	13,00	13,00
miscela zuccheri	0,25	0,25
integratore minerale	0,40	0,40
bios 40	-	2,50
SS	24,00	25,00
PG	16,55	16,80
ADF	22,72	23,40
NDF	35,50	33,50
NFC	37,00	38,10
AMIDO	26,00	27,00
EE	4,12	4,30
CA	1,12	1,12
P	0,38	0,38
NA	0,22	0,22
K	1,40	1,40

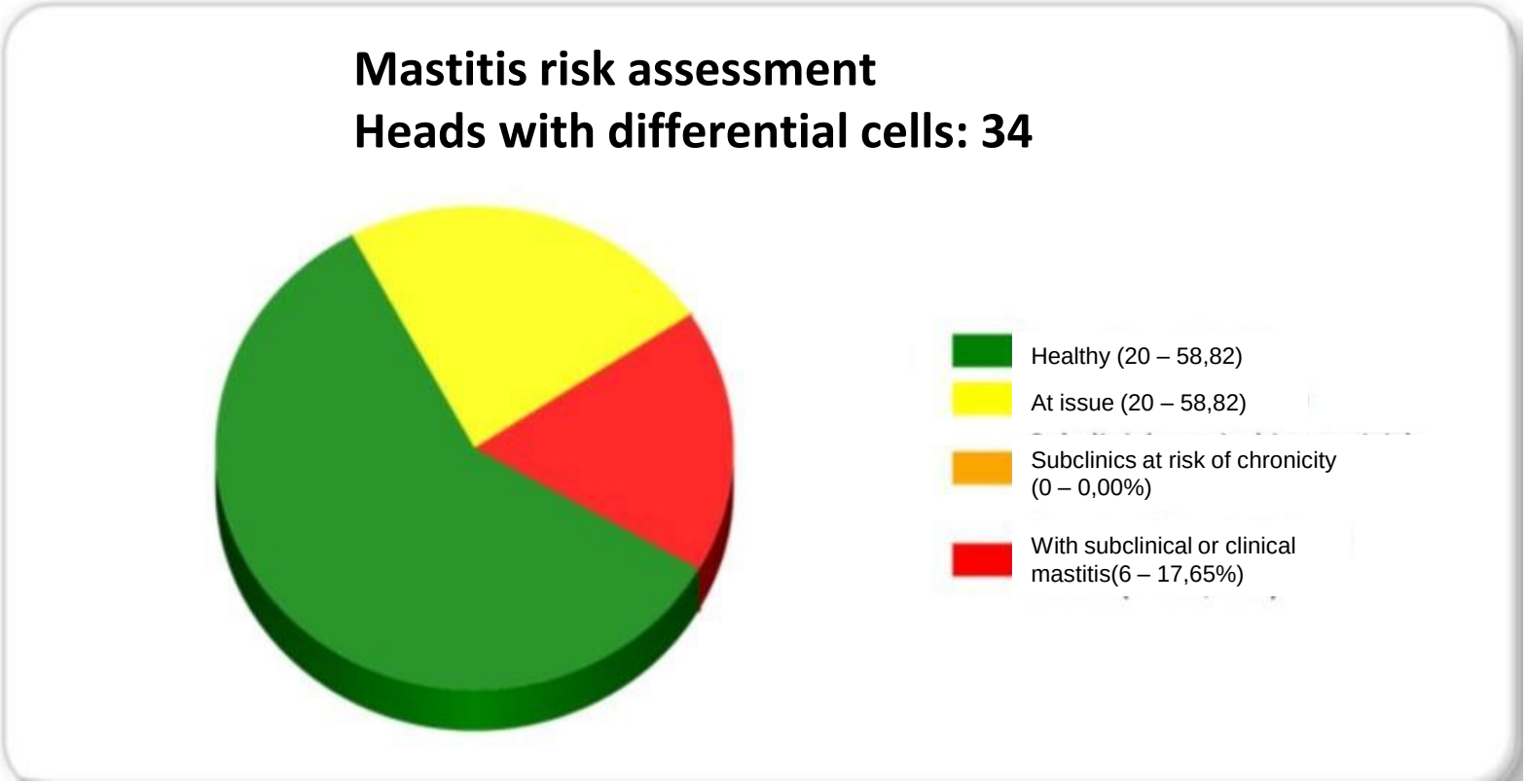
Stable data
during the experimental ration

parameters	annual average	experimental phase
Average lactation length gg	176	172
Average production of controlled animals Kg	28,3	28,60
Average EVM Kg	9504	10.404
Average days to peak lactation	82	81
Fat % w/w	4,83	4,70
Protein % w/w	3,46	3,46
Somatic cells	409	248
Urea	23	24
Gravid heads %	50	56
Average dry time gg	60	60

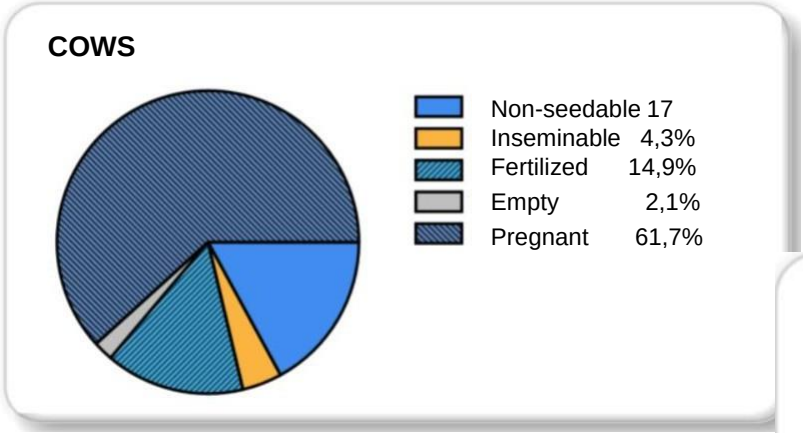
LACTATION CURVES

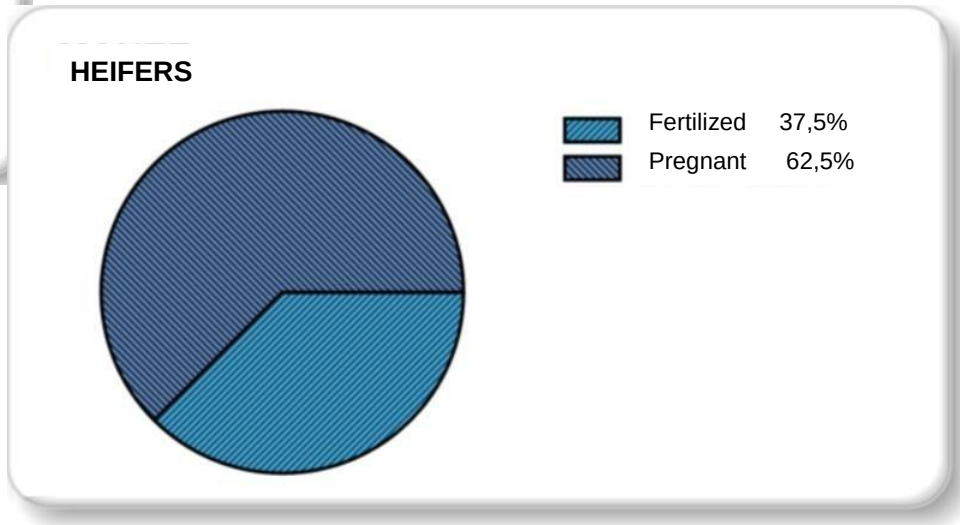


Percentage distribution of somatic differential cells



Reproductive management summary





Fertility index summary

ANALISI FERILITA'							
data finestra ciclo estrale	INSEMINABILI	INSEMINATE	HDR %	GRAVIDANZE DISPONIBILI	GRAVIDANZE ACCERTATE	CR %	PR %
18/09/2023	11	7	64	11	1	14	9
09/10/2023	10	7	70	9	5	71	56
30/10/2023	10	7	70	9	1	14	11
20/11/2023	10	6	60	10	1	17	10
11/12/2023	13	9	69	11	3	33	27
01/01/2023	16	12	75	14	8	67	57
22/01/2023	15	10	67	13	5	50	39
12/02/2023	14	8	57	12	6	75	50
04/03/2023	10	7	70	10	2	29	20
25/03/2023	10	6	60	10	4	67	40
15/04/2023	7	5	71	7	2	40	29
06/05/2023	7	4	57	7	3	75	43
27/05/2023	5	5	100	5	1	20	20
17/06/2023	7	5	71	7	3	60	43
08/07/2023	6	6	100	6	0	0	0
29/07/2023	8	5	63	8	0	0	0
18/08/2023	9	3	33	9	0	0	0
09/09/2023	9	0	0	9	0	0	0
TOTALI	**177**	**112**	**64,28**	**167**	**45**	**45,14**	**32,429**
tempo volontario attesa 70 giorni							

Process protection
by international patent

(PROTINAT® PROCESS (EN Pat. n°102022000014362 - PCT/IB2023/056515)

Ministero dello Sviluppo Economico

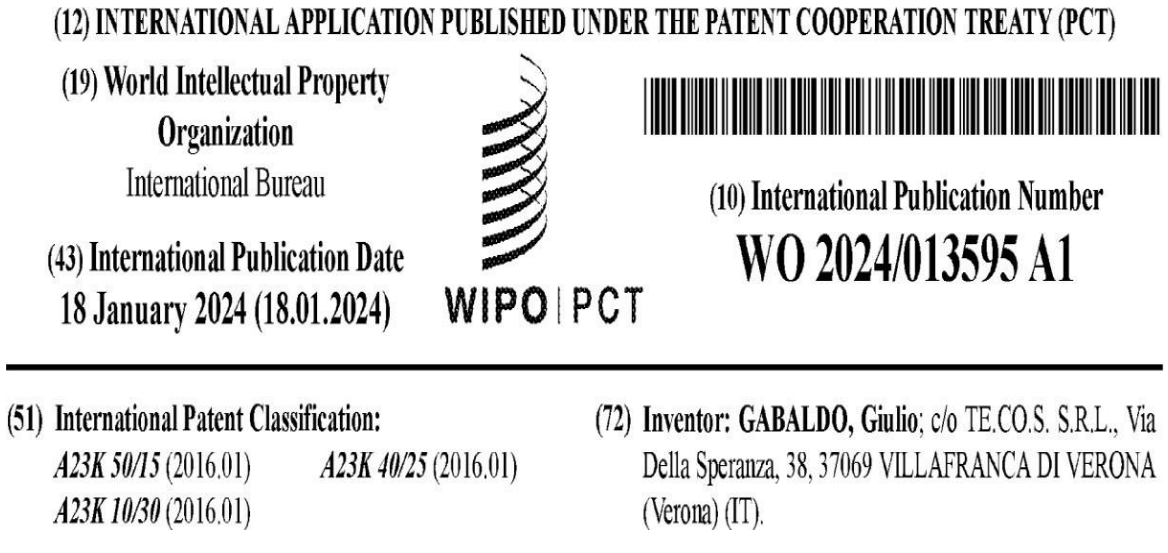
Ricevuta di presentazione

per

Brevetto per invenzione industriale

Domanda numero: 102022000014632

Data di presentazione: 12/07/2022



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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Thank you for your attention

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