

## A Safe Additive Feed Solution for Mycotoxin Contamination

By TOLSA SA

Mycotoxins are toxic secondary metabolites produced by mould and fungi (mainly *Aspergillus* and *Fusarium*) and are found in grain. They occur naturally and can develop in the field (pre-harvest) or in grain storage (post-harvest).

Unfortunately, once mycotoxins develop, they cannot be destroyed without significantly damaging the feed, as they are highly chemically and thermally stable and able to survive through the feed manufacturing process.

Mycotoxin exposure usually occurs when animals ingest feed that is contaminated with mycotoxins. They are then absorbed into the bloodstream and have a toxic effect on the animals at low levels of ppm and ppb, a disease known as mycotoxicosis.



The effects of mycotoxicosis will vary according to the type of mycotoxin and will have a severe effect on the health and yield of animals, and, in severe cases, metabolites may build up in the milk, eggs, and meat.

One of the most common types is deoxynivalenol (DON), a highly toxic mycotoxin belonging to the trichothecenes family. The high levels of DON found in raw materials and feed affects countries throughout the world, and it is also very difficult to treat.

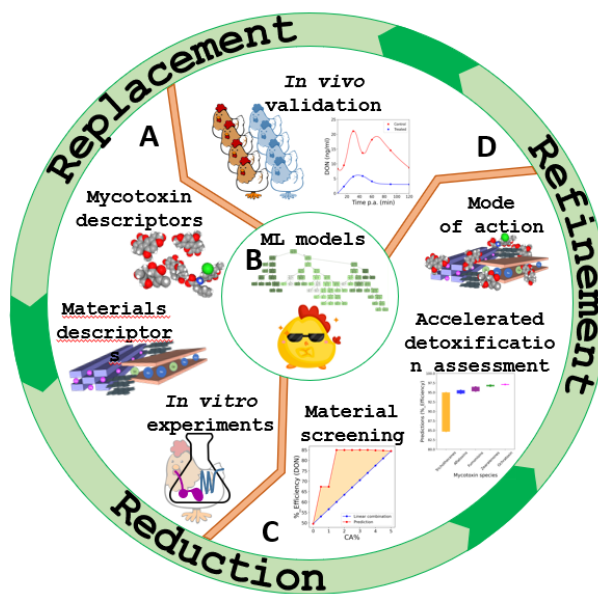
### **What types of toxic diseases are caused by mycotoxins?**

The toxic diseases that can occur depend on the type of mycotoxin, and include hepatotoxicity, genotoxicity, nephrotoxicity, neurotoxicity, reprotoxicity, and immunotoxicity. These can lead to the following production issues:

- Reduction in food consumption and usage of nutrients
- Intestinal disease
- Immunosuppression
- Reduced fertility
- Low yield
- Cancer

## The 3R principle

The three Rs are one of the core tenets of animal protection in research and they are based on the approaches of replacement, reduction, and refinement. This principle was established with the aim of avoiding or replacing the use of animals, reducing the number of animals and minimising their suffering during testing.



In line with this principle and with the objective of minimising environmental impact, the following actions were taken:

- In vivo testing was **replaced** by in vitro and virtual testing with predictive machine learning models.
- Machine learning models help to **refine** material design. Validations via in vivo testing were **reduced** to one experiment for studying the effect of Tolsa's Atox® Nature Silver on oral absorption of deoxynivalenol (DON) in chickens for consumption.

## What is the best strategy for treating mycotoxin problems?

Actively porous materials (binders), such as certain special clays, can absorb mycotoxins and prevent them from entering the animal's blood and leading to mycotoxicosis. Once the binder-mycotoxin complex has been created, this will pass through the gastrointestinal tract and be excreted along with the faeces. The various chemical affinities between binders and mycotoxin molecules complicate the experimental process to find the optimal solution.

The strategy proposed by the European Food Safety Authority (EFSA) recommends using in vitro testing to develop the most optimum formulations. To speed up the process, additional advanced tools, such as predictive artificial intelligence models and machine learning, can increase efficiency to help find the best solution to treat a wide range of

mycotoxins. Once the optimal formulation has been determined, the **3R** principle is followed to reduce the use of animals in research. In vivo testing with biomarkers enables thorough assessment of mycotoxin levels in blood and also helps to quantify the absorbent effect of binders.

### **THREE ACTIVE INGREDIENTS: ONE SOLUTION**

Atox® Nature Silver is a new solution from Tolsa for treating mycotoxins in feed. This innovative tried and tested formula is the result of years of experience and advanced research using machine learning, to develop a broad-range mycotoxin sequestrant for the poultry, swine, and ruminant industries.

Atox® Nature Silver is a combination of three different active ingredients with various physical and chemical properties, designed with three clear objectives:

- To absorb a wider range of mycotoxins, including those with lower polarity.
- To achieve more selective sequestration to ensure that no essential nutrients are absorbed.
- To boost absorption properties so as to avoid desorption of mycotoxins as they pass through the gastrointestinal tract.

The formula is enhanced by the positive synergy between three active components: bentonite, sepiolite, and activated carbon.

### **IN VITRO STUDIES: EXPERIMENT PROCEDURE**

We performed a series of in vitro studies using high-performance liquid chromatography (HPLC) at TRILOGY, a leading American mycotoxin laboratory that the Tolsa technical team has been working closely with for years.

The method involves simulating the gastric and intestinal juices in the stomach at pH 3 (absorption) and the small intestine at pH 6.5 (desorption), in line with the animal's actual gastrointestinal tract. The difference between these methods is the effectiveness (in %), so we can see a real result regarding the efficacy of the products.

#### **Description of the method**

- Absorption tests are performed at different pH levels (acidic and standard).
- We then prepare a buffer solution containing the mycotoxin being studied.
- Afterwards, we add Atox® Nature Silver to this solution and incubate it for 60 minutes at 37°C while stirring continuously.
- Next, we centrifuge the samples.
- Then finally we analyze the mycotoxin levels remaining in the supernatant liquid.

### **Results**

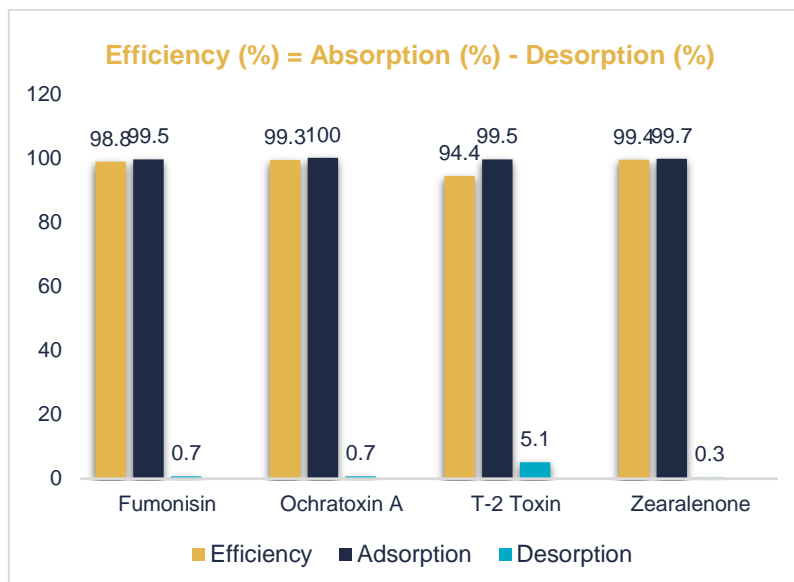
Graphs 1 and 2 show the results obtained using in vitro HPLC methods.

#### **→ Summary of the results obtained in vitro**

*Graph 1 shows a summary of the results obtained in vitro, with efficiency of over 90% for all the mycotoxins studied (FUM, OCRA, T2 and ZEA).*

The experiment involves measuring the sequestration capacity of 2 mg/kg of Atox® Nature Silver with a concentration of 2 ppm for each mycotoxin.

These results show the effectiveness of Atox® Nature Silver, and not just when it comes to the absorption of mycotoxins, but also in terms of maintaining cohesion strength, thus preventing potential desorption of mycotoxins at higher pH levels in the intestinal tract.



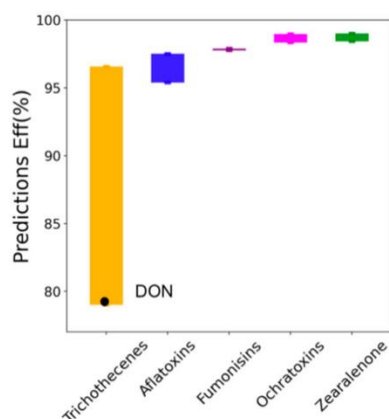
The research and testing carried out is aimed at optimizing and maintaining absorption levels, thus minimizing desorption.

As such, the bond between the mycotoxin and the sequestrant remains stable throughout its journey along the gastrointestinal tract at various pH levels, until it is excreted. This enables us to ensure that the animals only absorb essential nutrients and not mycotoxins.

### Machine learning models

Our machine learning algorithms have been trained using the in vitro test database and can predict the behaviour of any combination of various formulas and mycotoxins. The models were validated through experiments and enabled us to assess the sequestration properties of Atox® Nature Silver against various mycotoxins.

The graph below shows the effectiveness of Atox® Nature Silver (> ca. 80%) when isolating 22 mycotoxins from six different groups.



It also enables us to predict the treatment's effectiveness against the most difficult mycotoxin to absorb, deoxynivalenol (DON), in addition to the most suitable dosage of Atox® Nature Silver that is recommended for administering to animals according to the levels of contamination in their feed.

## IN VIVO STUDY WITH BIOMARKERS

Using in vitro testing and predictions from the models, we were able to demonstrate a minimum effectiveness rating of 94% for Atox® Nature Silver as a sequestrant for all the mycotoxins being tested, aside from (DON), which demonstrated a minimum effectiveness rating of 80%. Based on the latter result, DON was selected to corroborate the advances found through an in vivo trial.

### *Design of the test*

With in vivo testing on chickens for consumption we studied two treatments:

**Control group:** A single bolus with deoxynivalenol was administered orally (0.5 mg/kg animal weight)

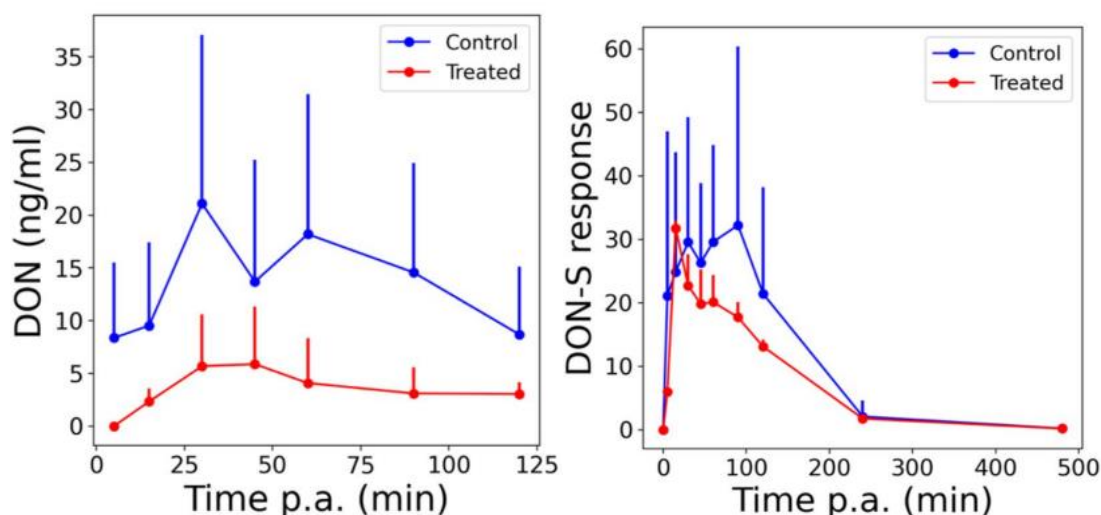
**Treatment group:** A single bolus with deoxynivalenol was administered orally (0.5 mg/kg animal weight) along with Atox® Nature Silver (0.4 g/kg animal weight).

### *Parameters evaluated*

Blood samples were taken at different points of the test and were analyzed by detection of DON and the deoxynivalenol-sulphate biomarker (DON-S).

### *Results*

The graph on the left shows the concentration and standard deviation of DON, after administration, as well as DON in combination with Atox® Nature Silver. The graph on the right represents the mean response and standard deviation of DON-S in blood, after administration, and DON in combination with Atox® Nature Silver in eight chickens for consumption.



The results showed a much lower systemic exposure of DON-S with the treatment group than with the control group, which can be seen on the graph to the right (area below the DON-S response curve). (Details available in Lo Dico, G.; Croubels, S.; Carcelén, V.; Haranczyk, M. Machine Learning-Aided Design of Composite Mycotoxin Detoxifier Material for Animal Feed. *Sci. Rep.* **2022**, 12 (1), 1–11. <https://doi.org/10.1038/s41598-022-08410-x>.)

The following table shows the results of the most notable toxic-kinetic parameters of the DON-S metabolite after oral administration to the eight chickens for consumption.

Parameter TC	Control group	Treatment group	P value
AUC <sub>0-∞</sub> (min response)	4305.39 ± 1684.29	2919.59 ± 1032.94	0.010
C <sub>max</sub> (response)	51.93 ± 21.04	34.39 ± 17.06	0.013
T <sub>max</sub> (min)	64.38 ± 42.71	56.25 ± 38.24	0.637
K <sub>E</sub> (1/min)	0,013 ± 0,001	0,013 ± 0,002	0.976
T <sub>1/2</sub>	53.09 ± 6.09	53.77 ± 9.40	0.877
F relative AUC <sub>0-∞</sub> (%)	N/A	67.81	N/A
90% IC for log (AUC <sub>0-∞</sub> )	N/A	[0.54; 0.88]	N/A

AUC<sub>0-∞</sub>: area under the DON-S response curve

C<sub>max</sub>: maximum DON-S response in blood

T<sub>max</sub>: maximum response time of DON-S

Relative F: relative bioavailability

CI: confidence interval

Furthermore, the maximum DON-S response in blood was significantly lower in the treatment group than in the control group.

As for the bioavailability of DON-S, in the treatment group it was only 67.8%, in comparison to the control group. This shows a significant reduction in systematic exposure to DON during in vivo testing, as a reduction of 20% was demonstrated.

Finally, the 90% confidence interval of the log ratio (AUC<sub>0-∞</sub>) was 54-88% and thus almost falls completely outside the 80-125% range, showing a significant effect on the reduction of systemic DON exposure.

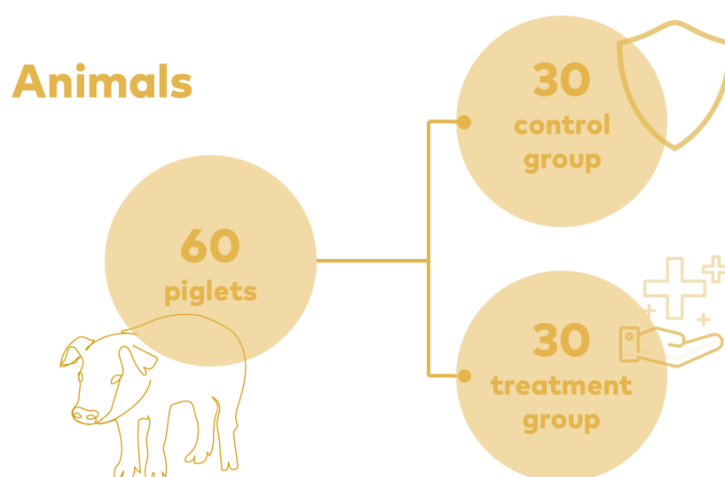
## OTHER IN VIVO STUDIES: EXPERIMENT PROCEDURE

Additionally, once the effectiveness had been determined, we studied the safety and effects of Atox® Nature Silver and whether it sequestered other micronutrients, such as vitamins, trace minerals, and amino acids, which are found in a standard portion of feed.

***The research was carried out on three different species: piglets, beef cattle, and chicken for consumption.***

***In vivo testing was carried out on piglets at three pig farms in various locations across Spain.***

### ***Design of experiment study***



### → **Treatments**

- The experiment group was administered 0.2% of Atox® Nature Silver in their feed.
- The control group was not administered any mycotoxin sequestrant in their feed.

### → **Parameters evaluated**

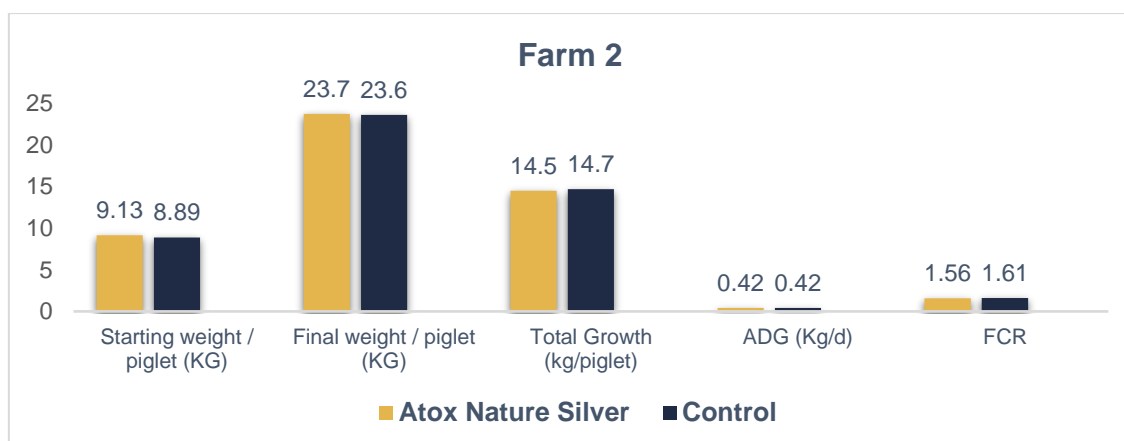
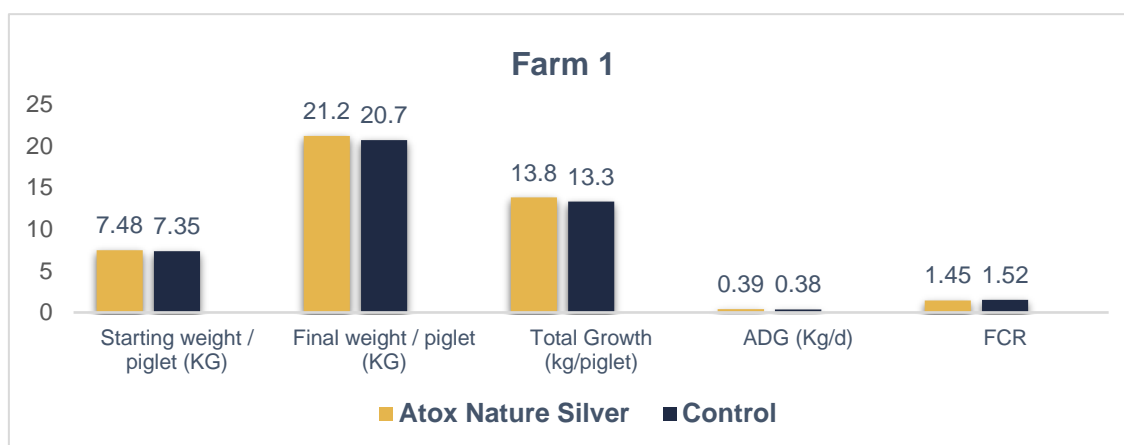
The following production performance parameters were analyzed:

- Growth (BW)
- Average daily gain (ADG)
- Conversion index (FCR) from 0 to 35 days

## Results

→ No statistically significant differences were observed in any of the trial farms so we can conclude that Atox® Nature Silver is safe to use and does not result in the sequestration of any essential nutrients that would affect normal production parameters.

→ We observed a lower conversion index in three farms after using Atox® Nature Silver.







We also carried out in vivo testing at a cattle farm in Spain.

First, we analyzed samples of feed and straw to test for the level of natural contamination caused by the following:

- Fumonisin (FUM)
- Vomitoxin (DON)
- Zearalenone (ZEA)

	Natural contamination of food	
	Straw	Feed
Fumonisin (FUM)	64	123
Vomitoxin (DON)	0.76	0.86
Zearalenone (ZEA)	60	N/A

### **Design of experiment study**

*We studied two treatments in the experiment carried out on beef cattle.*

#### **→ Treatments**

**Control group.** *The feed consumed contained no mycotoxin sequestrant.*

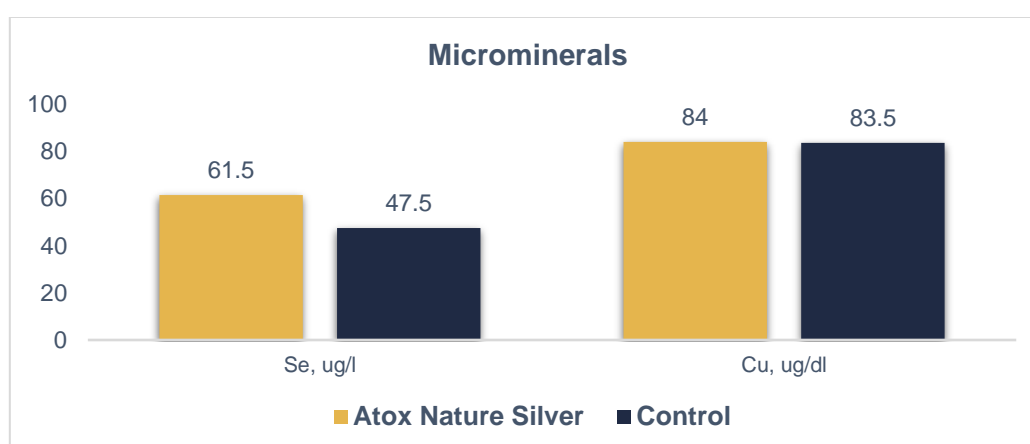
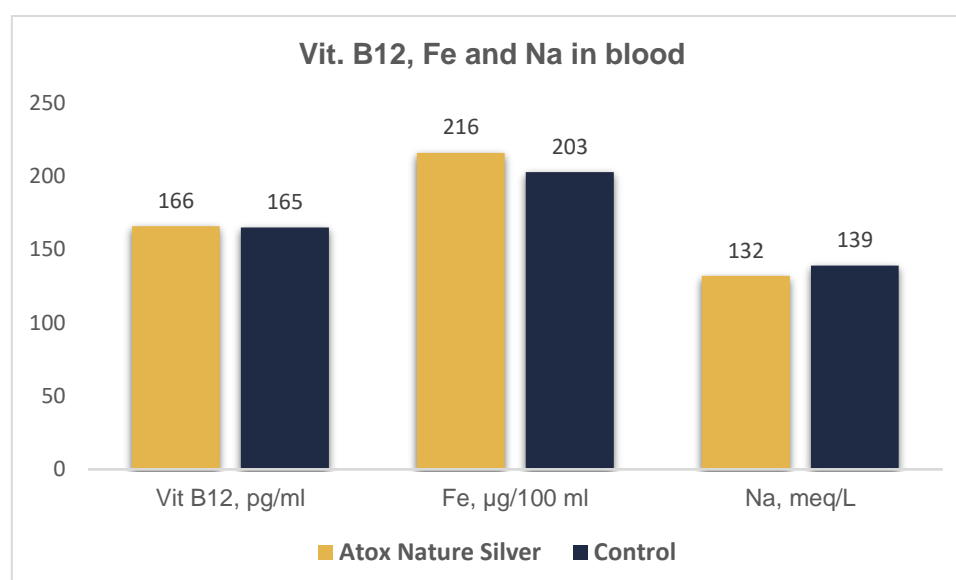
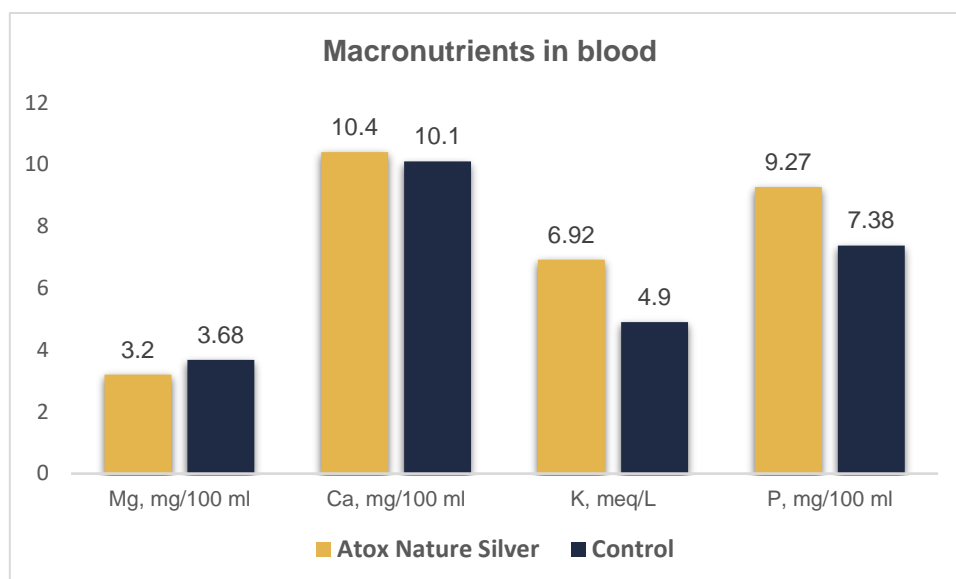
**Treatment group.** *Feed was given with 30 g/animal/day of Atox® Nature Silver over a period of one month.*

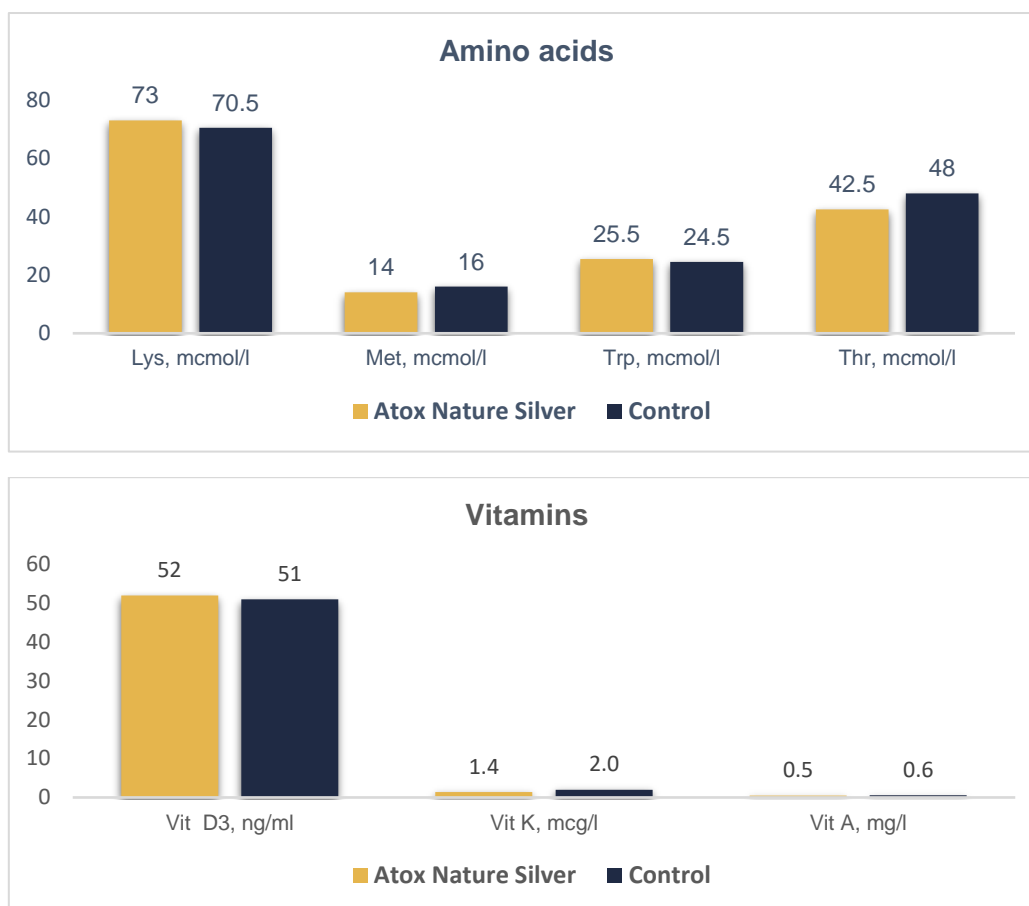
#### **→ Parameters evaluated**

*Blood samples were taken at various points and there was testing for vitamin, mineral, and amino acid levels.*

### **Results**

Once again, we did not observe any significant statistical differences in any of the test treatments, so we can conclude that ATOX® NATURE SILVER (ANS) is safe to use and does not sequester any essential nutrients that affect regular production parameters.





All the studies carried out to date demonstrate that Atox® Nature Silver is effective at sequestering mycotoxins, and they have also ensured it is safe to use, thus offering a natural and fully inorganic solution.

Atox® Nature Silver enables vitamins and minerals to function properly, making it perfect as a solution for both maintenance and shock doses.

*Tolsa, based in Madrid, Spain, has one of the broadest ranges of special clays worldwide, offering additives and industrial products that provide high added value and improve the performance and efficiency of materials. Also, Tolsa is the largest European manufacturer of pet hygiene products, marketed under its own brands as well as private label.*

*The company has more than 32 mining operations to produce the highest quality sepiolite, bentonite, attapulgite, phosphates, peat, and marble, which generate more than 120 million tonnes of raw materials. The acquisition of new deposits is a key priority in order to renew reserves as well as to offer each customer new raw materials to meet their needs.*

*Tolsa focuses on intelligent production that is modern, efficient, flexible, safe, and respectful of the environment. Each process is subjected to stringent quality controls, and the company is certified under ISO 9000 and ISO 14000. Tolsa has a production capacity of more than 1.2 million tonnes and its factories are located near mining*

*operations, or in port areas near our markets. For more information about Tolsa, contact [info@tolsa.com](mailto:info@tolsa.com) or visit [www.tolsa.com](http://www.tolsa.com).*