nutrex NV

the finishing touch for nutrition

Nutrase Xyla

endo-1,4-β-xylanase

ONE ENZYME - A WORLD OF BENEFITS

arabinoxylans | the principal anti-nutritional factor

Non Starch Polysaccharides (NSP)

A major part of common vegetable feed ingredients consists of carbohydrates, making carbohydrates a crucial factor in animal production.

Besides well digestible nutrients, such as starch and sugars, the carbohydrate fraction of vegetable origin includes indigestible components, such as cellulose, hemicellulose, pectins, beta-glucans and lignin.

All of these poorly digestible components, excluding lignin, are classified in a group referred to as Non Starch Polysaccharides (NSP). The NSP fraction is well known for the anti-nutritional effects it can exert.

Within the group of NSP, hemicellulose itself is a heterogeneous subgroup predominantly made up of xylans, arabinans, galactans, glucans and mannans.

As shown in table 1, arabinoxylan is the principal NSP-fraction in several of the most important feed raw materials, including wheat ánd corn.

FIGURE 1: STRUCTURAL GROUPING OF CARBOHYDRATES

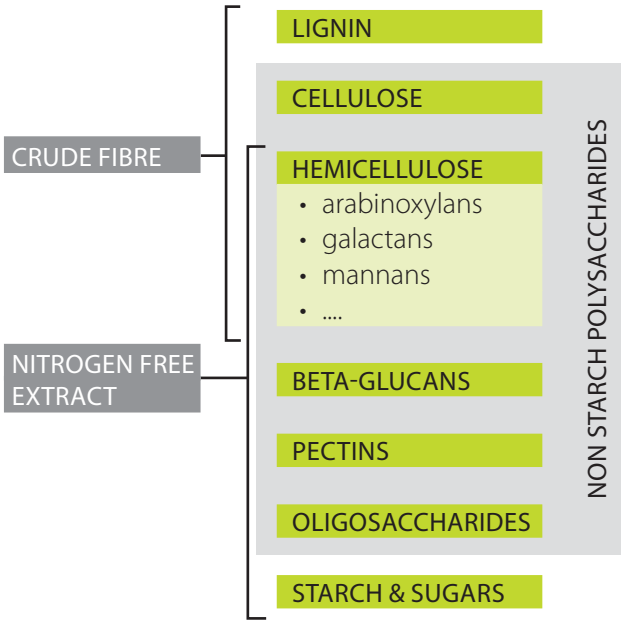
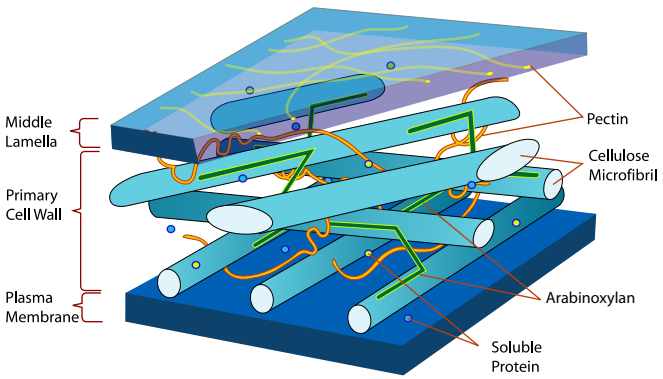


TABLE 1: NSP CONTENT OF FEED INGREDIENTS (AS % OF DRY MATTER)

	AX SOL	AX INSOL	β-GLUCANS	CELLULOSE	MANNOSE	GALACTOSE	NSP	AX/NSP
wheat	1.8	6.3	0.8	2.0	T	0.3	11.4	71 %
rye	3.4	5.5	2.0	1.5	0.3	0.3	13.2	67 %
corn	0.1	5.1	T	2.0	0.2	0.6	8.1	64 %
wheat bran	1.1	20.8	0.4	10.7	0.4	0.8	35.3	62 %
sorghum	0.12	3.8	0.2	2.0	0.1	0.15	6.45	62 %
wheat DDGS	4.9	13.4	2.3	5.8	T	0.9	33.2	55 %
barley	0.8	7.1	4.3	3.9	0.2	0.2	16.7	47 %
corn DDGS	0.4	12.6	T	7.1	0.7	2.1	28.6	45 %
rice bran	0.2	8.3	T	1.2	0.4	1.2	21.8	39 %
rice	T	0.2	0.1	0.3	T	0.1	0.8	25 %
sunflower cake	0.8	5.2	-	12.3	1.2	1.3	31.5	19 %
soy bean meal	0.75	2.25	-	6.2	1.3	4.1	21.7	14 %

T = unquantifiable trace amounts

FIGURE 2: LOCATION OF AX IN PLANT CELL WALL



Arabinoxylan (AX)

AX is found in close association with the plant cell wall (see figure 2), where it acts as a glue linking various building blocks of the plant cell wall and tissue, giving it both structural strength and rigidity.

Its abundance, location within vegetable material and molecular structure cause AX to have a severe, negative impact on feed digestibility; effectively reducing the nutritional value of the raw materials in which it is present.

This makes AX the principal anti-nutritional factor, reducing animal production efficiency.

Arabinoxylan as major anti-nutritional factor

Water-soluble AX (AX_{sol})

The best known anti-nutritional effect of a high AX content in rations for monogastrics, is a considerable increase of the viscosity of the intestinal content, caused by the extraordinary water-binding capacity of water-soluble AX.

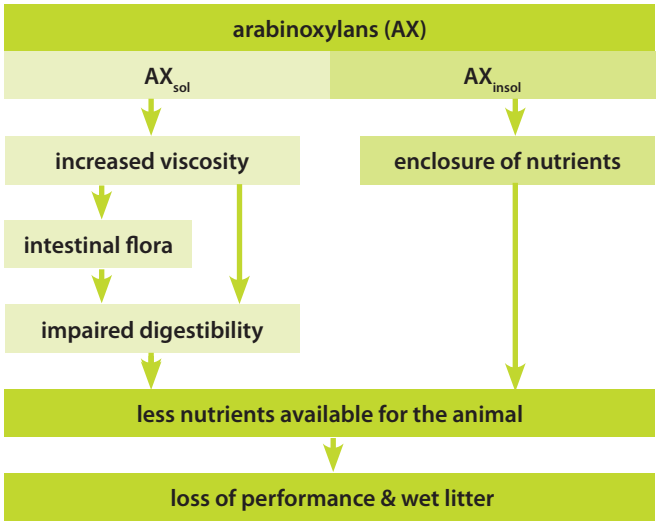
The increased viscosity affects feed digestion and nutrient use in several direct and indirect ways :

- it prevents proper mixing of feed with digestive enzymes and bile salts,
- it slows down nutrient availability and absorption,
- and stimulates fermentation in the hindgut.

Water-insoluble AX (AX_{insol})

The second anti-nutritional property of AX is linked to the water-insoluble AX fraction, which causes nutrient entrapment.

FIGURE 3: ANTI-NUTRITIONAL EFFECTS OF AX



Large quantities of well digestible nutrients such as starch and proteins remain either enclosed in clusters of cell wall material or bound to side chains of the AX. These entrapped nutrients will not be available for digestion and subsequent absorption in the small intestine. Resulting in a waste of nutrients, this anti-nutritional effect is still today severely underestimated.

FIGURE 4: XYLAN BACKBONE WITH ARABINOSE SIDE CHAINS

xylanase | one name - many enzymes and parameters

Whenever using feed ingredients rich in AX, the use of AX-degrading enzymes - known as xylanases or xylanolytic enzymes - to reduce the anti-nutritional impact of AX can provide a considerable gain in animal production efficiency.

The name "xylanase" covers a wide range of enzymes with varying properties. Their function and usefulness in animal production is determined by a variety of parameters, related to the substrate and the enzyme itself.

These include, but are not limited to :

- enzyme mode of action (eg. endo-xylanase, exo-xylanase, or a combination)
- substrate specificity of the enzyme (eg. affinity for $AX_{soluble}$ and/or $AX_{insoluble}$)
- optimal working pH (eg. acidic, neutral, alkaline)
- enzyme sensitivity to xylanase inhibitors
- natural thermal stability

The following pages will focus on various of these parameters and highlight their importance when choosing a xylanase for your type of feed and animal production.

Endo-xylanase versus exo-xylanase

Based on the mode of action, two major classes of xylanases can be distinguished : endo- and exo-xylanase. Depending on the type of enzyme present, their action leads to different results as shown in figure 5.

Endo-xylanases

Endo-xylanases are able to bind anywhere on the xylan backbone of the AX-chain, as long as the enzyme is not physically hindered by side-chains. This results in smaller AX-fragments being formed when it acts on the substrate.

Effects of endo-xylanase activity

- with potential endo-binding loci along the entire AX-chains, it can efficiently break down long AX-polymers into smaller fragments
- small AX-fragments bind less water, resulting in fast and efficient viscocity reduction in the GIT
- efficient release of entrapped nutrients if the xylanase is able to break down the water-insoluble AX-fraction
- no metabolic stress from xylose monomers
- production of prebiotic arabinoxylo-oligosaccharides (AXOS)

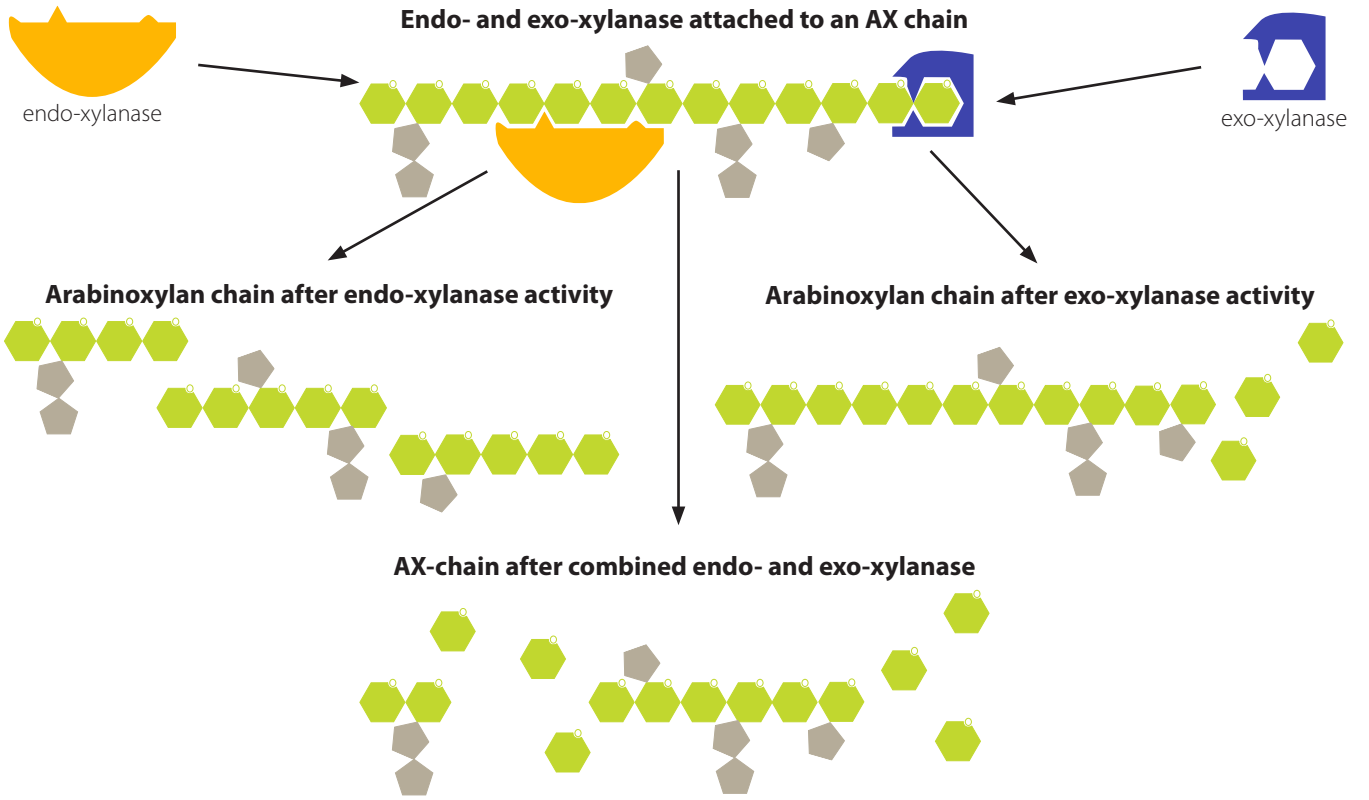
Exo-xylanases

Exo-xylanases can only attach themselves to the reducing sugar end of an AX-chain. Their action results in xylose monomers being split off from a larger AX chain.

Effects of exo-xylanase activity

- the only exo-binding locus is situated at the reducing sugar end of AX-chains. This means only 1 xylanase molecule can "work" on a particular AX-chain at any given time
- inefficient breakdown of AX
- little or no viscosity reduction
- little or no release of nutrients
- production of xylose monomers leads to metabolic stress, as monogastrics cannot use pentose sugars for energy production

FIGURE 5: RESULT OF ENDO-XYLANASE AND/OR EXO-XYLANASE ACTIVITY ON A BRANCHED AX-MOLECULE



Endo-xylanase + exo-xyalanse

A blend of endo- and exo-xylanases results in the intial breakdown into smaller AX fragments, after which the exo-xylanase cuts off xylose monomers from the accessible chain ends.

Effects of a combination of endo- and exo-xylanase

Leads to a more advanced breakdown of AX, decreasing viscosity and releasing of nutrients. However, the combined action produces even more xylose monomers and thus a higher metabolic stress. Xylo-oligosaccharide fragments are further broken down to xylose, thereby losing the benefit of their prebiotic function.

High activity on water-soluble AND water-insoluble AX

From the data in table 1, it is clear that the amount of AX_{insol} is significantly more important than that of AX_{sol} in all major vegetable feed ingredients.

Although the anti-nutritional effect of AX_{insol} often goes unnoticed - it doesn't cause particular production problems, such as wet litter or serious digestive problems - this effect is not to be neglected or underestimated. One should remember that entrapped nutrients are not available to the animal, resulting in sub-optimal yield.

Therefor, regardless of the main cereal used, it is of utmost importance that - in order to obtain the maximum benefit from a xylanase - the xylanase is able to break down both AX types, effectively reducing viscosity ánd liberating nutrients.

This element is particularly important in corn-soy diets and in animals that are less sensitive to viscosity in the GIT.

AX_{sol} and AX_{insol} in corn-soy rations

As corn contains only 0.1 % AX_{sol} versus 5.1 % AX_{insol} (table 1), the anti-nutritional effect of AX_{sol} (viscosity increase) is marginal in corn-soy diets, while the entrapment of nutrients caused by the AX_{insol} fraction is of major importance.

Therefor, only a xylanase able to break down the AX_{insol} fraction will bring a significant benefit in animals fed corn-soy diets.

Animals with low sensitivity to viscosity in the GIT

The anti-nutritional effect of viscosity-inducing raw materials having a high content of AX_{sol}, such as rye or wheat, can be very important for some animals, such as broilers for example.

However, this is not the case for all production animals. Fattening pigs and layers, for example, are not nearly as sensitive to viscosity. In these animals, the anti-nutritional effect of entrapped nutrients, caused by a large AX_{insol} fraction, will be more important than that of an increase in viscosity, caused by AX_{sol}.

Fungal vs. bacterial xylanases

Table 2 gives an overview of a series of widely marketed NSP enzymes - mostly enzyme blends - that all have xylanase as one of their principal activities.

What stands out, is that virtually all major NSP-enzymes are produced by fungal strains, with the exception of Nutrase Xyla.

From figure 6 one can clearly see that Nutrase Xyla is far superior to fungal xylanase with regard to the breakdown and solubilization of the AX_{insol} fraction.

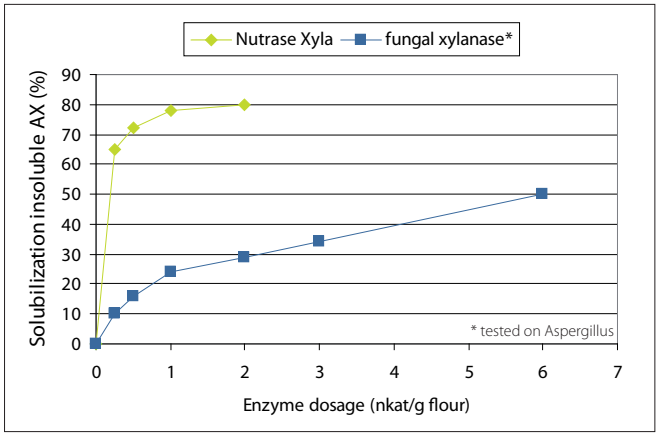
TABLE 2: ORIGIN OF FEED ENZYMES

ENZYME	PRODUCIN G STRAIN	TYPE
Nutrase Xyla	***Bacillus subtilis***	***Bacterial***
Allzyme	Aspergillus niger	Fungal
Avizyme / Porzyme	Trichoderma longibrachiatum	Fungal
Econase	Trichoderma reesei	Fungal
Grindazym	Aspergillus niger	Fungal
Hostazyme	Trichoderma longibrachiatum	Fungal
Natugrain TS	Aspergillus niger	Fungal
Ronozyme	Aspergillus oryzae	Fungal
Rovabio	Penicillium funiculosum	Fungal
Roxazyme	Trichoderma viride Trichoderma longibrachiatum	Fungal

Xylanase for corn-soy diets

With a vast majority of NSP-enzyme products from fungal origin (table 2), demonstrating inferior capabilities for the breakdown of AX_{insol}, this has resulted in a lot of nutritionists around the world being disappointed by the performance of several xylanases in combination with corn-soy diets.

FIGURE 6: ENZYME ACTIVITY ON WATER-INSOLUBLE AX



The above is, still today, a major reason why a lot of feed millers making corn-soy based feed hesitate to use xylanase or any other enzymes, besides phytase, in their feed, regardless of the type of animal production concerned.

environmental factors affecting enzyme activity

Besides parameters linked to the substrate and the type of xylanase, environmental factors can also have an important effect on enzyme efficacy and efficiency.

An enzyme that can work at its maximum potential will have an obvious advantage over enzymes whose potential is limited by unfavorable conditions.

Optimal working pH

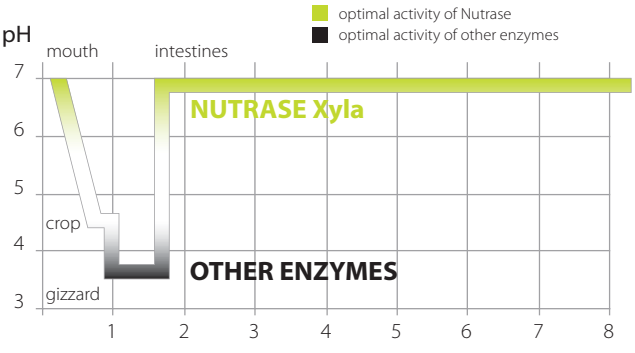
Fungal xylanases

Fungal enzymes have an acidic optimal pH, in the range of 4.5-5, and they loose a large part of their activity under neutral conditions. Given the rather short time feed spends in acidic conditions, in the stomach or in crop and gizzard, fungal enzymes have only a short time span to break down NSP fractions (figure 7).

Nutrase Xyla

Nutrase Xyla has a neutral optimal working pH, between 6 and 7. As feed spends the most time in the small intestine, under neutral conditions, Nutrase Xyla will have a much longer time to exert its activity on the soluble and insoluble AX fractions to release nutrients and reduce viscosity.

FIGURE 7: ENZYME pH PROFILES



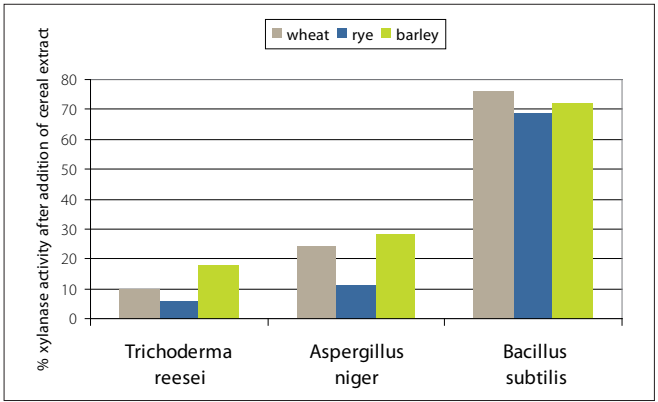
Sensitivity to xylanase inhibitors

Certain cereals, such as rye, barley and wheat, contain a type of proteins that can interact with xylanases and inhibit their action.

Fungal xylanases are much more sensitive than bacterial xylanases to these xylanase inhibitors, named TAXI (Triticum aestivum xylanase inhibitors).

Their activity can be inhibited by as much as 70-95 % in feed using these cereals, whereas the activity of Nutrase Xyla will only be inhibited by 20-30 % (figure 8).

FIGURE 8: SENSITIVITY TO XYLANASE INHIBITORS



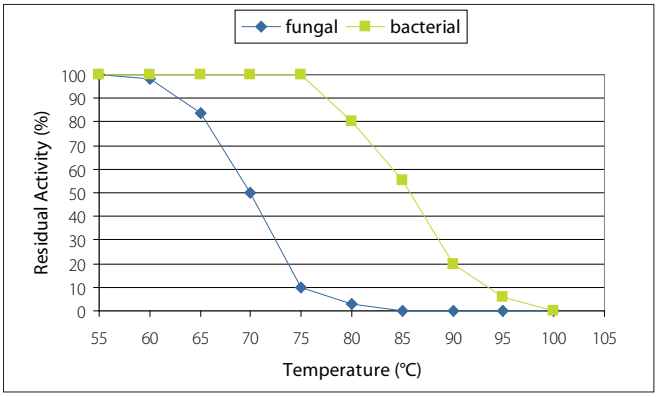
Source: Debyser, 1999

Thermal stability

Contrary to fungal xylanases, Nutrase Xyla has a natural thermal stability, not acquired by coating or other additional processes.

Depending on the exact conditions, enzyme activity loss due to pelleting conditions with Nutrase Xyla will be minimal up to about 85°C, whereas this is much higher for uncoated fungal xylanases.

FIGURE 9: NATURAL ENZYME THERMAL STABILITY



Source: Himmelstein, 1985

Nutrase Xyla | one enzyme - a world of benefits

The below tables give a general overview of the differences between Nutrase Xyla and other NSP enzymes with regard to several properties, as well as product performance during *in vivo* trials.

TABLE 3: DIFFERENCES BETWEEN NUTRASE AND OTHER ENZYMES

	OTHER ENZYMES	NUTRASE XYLA
EC registration	Different for each product	Broilers Ducks Layers Turkeys Piglets Pigs
Inclusion rate	Different for each product	100 ppm (diluted products available)
Endo-xylanase activity	Different for each product	9000 U endo-xylanase/g (Δ A590 - XylaZyme method at pH 6)
Heat stability	75 °C*	up to 85 °C
Storage temperature of liquid enzyme**	8 °C	25 °C
Inhibition of enzyme activity by TAXI***	70 - 95 %	20 - 30 %
Effect on water-insoluble arabinoxylans	20 - 50 %	70 - 80 %
Optimal pH	4.5 - 5	6 - 7

* Non-coated fungal endo-xylanase preparations
** To guarantee at least 6 months stability
*** TAXI are Triticum aestivum xylanase inhibitors, present in cereals (Debyser, 1999)

Performance in broilers

TABLE 4: AVERAGE IMPROVEMENTS OF NUTRASE IN BROILERS COMPARED TO OTHER ENZYMES*

	WHEAT		CORN	
	Other enzymes	Nutrase Xyla	Other enzymes	Nutrase Xyla
Body weight	1.9 %	3.7 %	1.1 %	3.3 %
Feed conversion	2.7 %	3.8 %	0.5 %	2.2 %
Return on investment**	8 - 10	10 - 13	3 - 5	7 - 9
Energy contribution to the feed (kcal/kg) - $ME_{poultry}$	80 - 130	182	25 - 60	90 - 110

* Based on elaborate zootechnical trials comparing commercial dosages to a negative control, documented in the Nutrase brochure.
** Return on investment depends on the cost price of the feed, prices of meat and general costs (medication, electricity, heating, manure treatment, etc...).

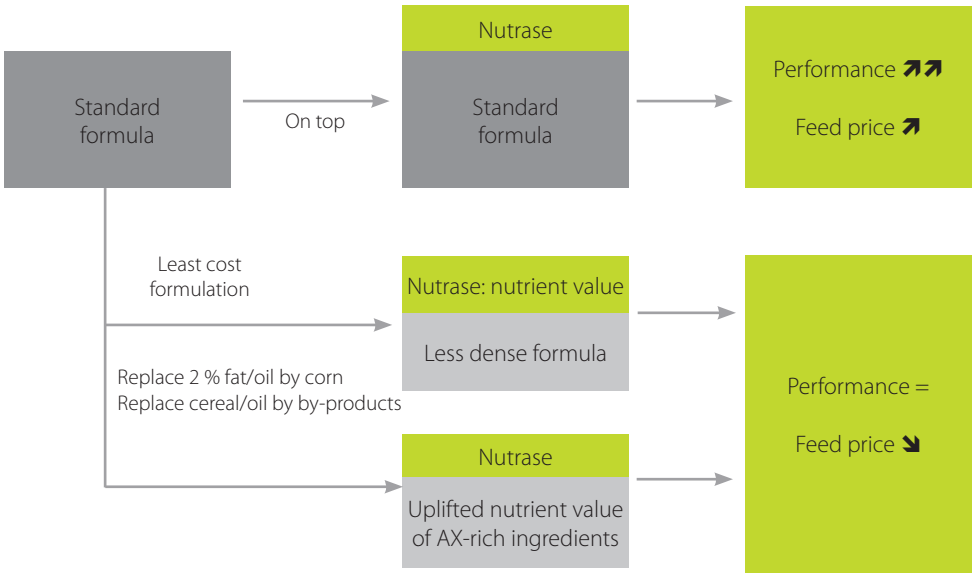
Performance in pigs

TABLE 5: AVERAGE IMPROVEMENTS OF NUTRASE IN PIGS*

ANIMAL SPECIES	PIGLETS	FATTENING PIGS	
Rations	Barley/wheat & corn	Wheat	Corn
Average daily gain	6.2 %	2.6 %	4.4 %
Feed conversion	5.8 %	2.3 %	2.1 %
Return on investment**	15 - 20	6 - 8	6 - 8
Energy contribution to the feed (kcal/kg) - NE_{pigs}	100	60	34

* Based on elaborate zootechnical trials, comparing 100 ppm Nutrase to a negative control, documented in the Nutrase brochure.
** Return on investment depends on the cost price of the feed, prices of meat and general costs (medication, electricity, heating, manure treatment, etc...).

Nutrase Xyla | practical feed formulation



Nutrase Xyla | matrix values

TABLE 6: MATRIX VALUES FOR NUTRASE XYLA - 100 PPM PRODUCT

	%		%
Humidity	10.0	Calcium	0.030
Crude protein	13.5	Phosphorus	0.200
Crude fat	1.5	Sodium	0.004
Starch	65.5	Potassium	0.220
Sugars	1.5	Chlorine	0.070
Starch + sugars	67.0	Magnesium	0.070
Crude minerals	0.7	Salt	0.110
Crude fibre	0.5		
N free extract	70.8		

Amino acids	%	Energy		kcal/kg	MJ/kg
Dig. lysine poultry	220	Broilers	$ME_{poultry\ wheat}$	1 820 000	7620
Dig. methionine poultry	105		$ME_{poultry\ corn}$	1 100 000	4605
Dig. meth + cys poultry	160		$ME_{broilers\ wheat}$	1 680 000	7034
Dig. threonine poultry	128		$ME_{broilers\ corn}$	1 050 000	4396
Dig. tryptophane poultry	40	Layers	$ME_{poultry}$	650 000	2721
			ME_{layers}	700 000	2931
		Turkeys	$ME_{poultry\ wheat}$	1 500 000	6280
			$ME_{poultry\ corn}$	1 050 000	4396

Amino acids	%	Energy		kcal/kg	MJ/kg
Ileal dig. lysine pigs	168	Piglets	ME_{pigs}	1 400 000	586
Ileal dig. methionine pigs	54		NE_{pigs}	1 000 000	4187
Ileal dig. meth + cys pigs	100	Finishing pigs	$ME_{pigs\ wheat}$	800 000	3349
Ileal dig. threonine pigs	100		$ME_{pigs\ corn}$	450 000	1884
Ileal dig. tryptophane pigs	30		$NE_{pigs\ wheat}$	600 000	2512
			$NE_{pigs\ corn}$	338 000	1415

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