agrimex NV
agricultural products

# Belfeed

## 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, arabinoxylans, 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 and corn.

**FIGURE 1: STRUCTURAL GROUPING OF CARBOHYDRATES**

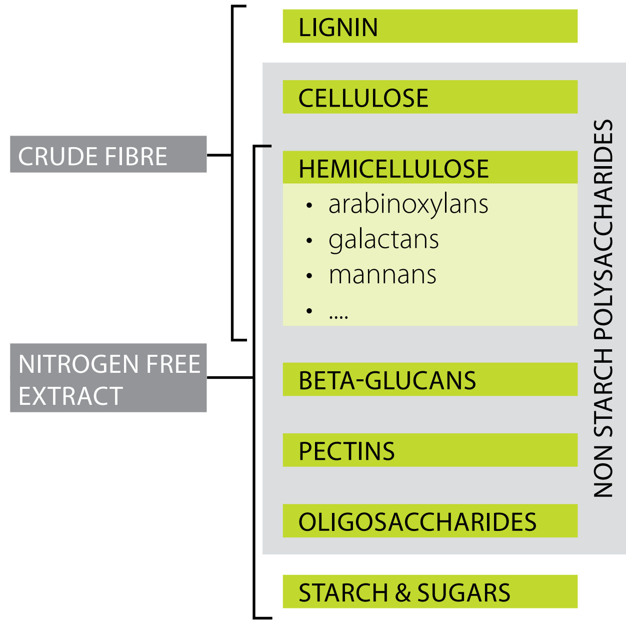
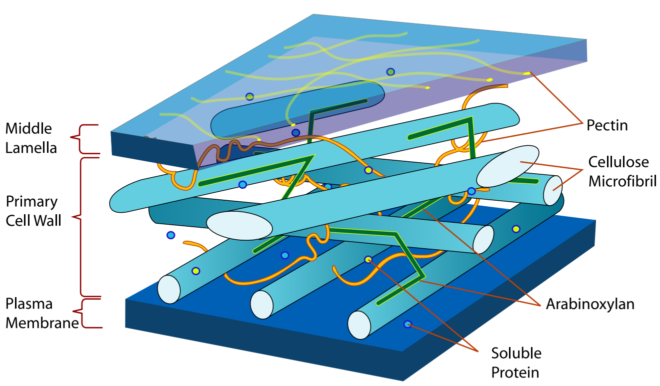



**TABLE 1: NSP CONTENT OF FEED INGREDIENTS (AS % OF DRY MATTER)**

| | 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.

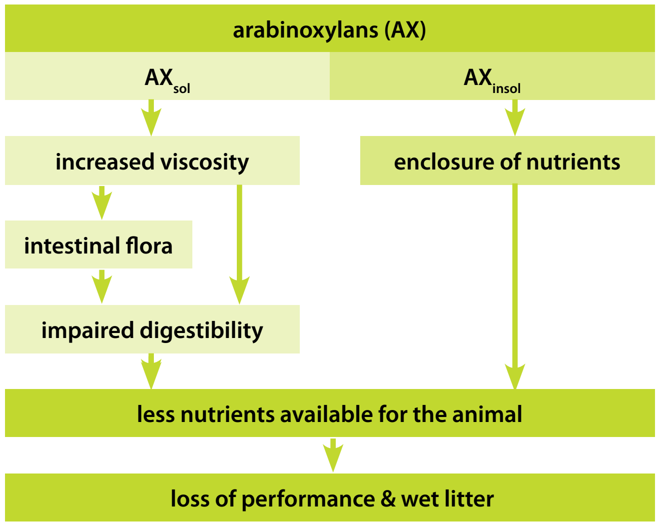
The increased viscosity affects feed digestion and nutrient use in several direct and indirect ways :

- 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)

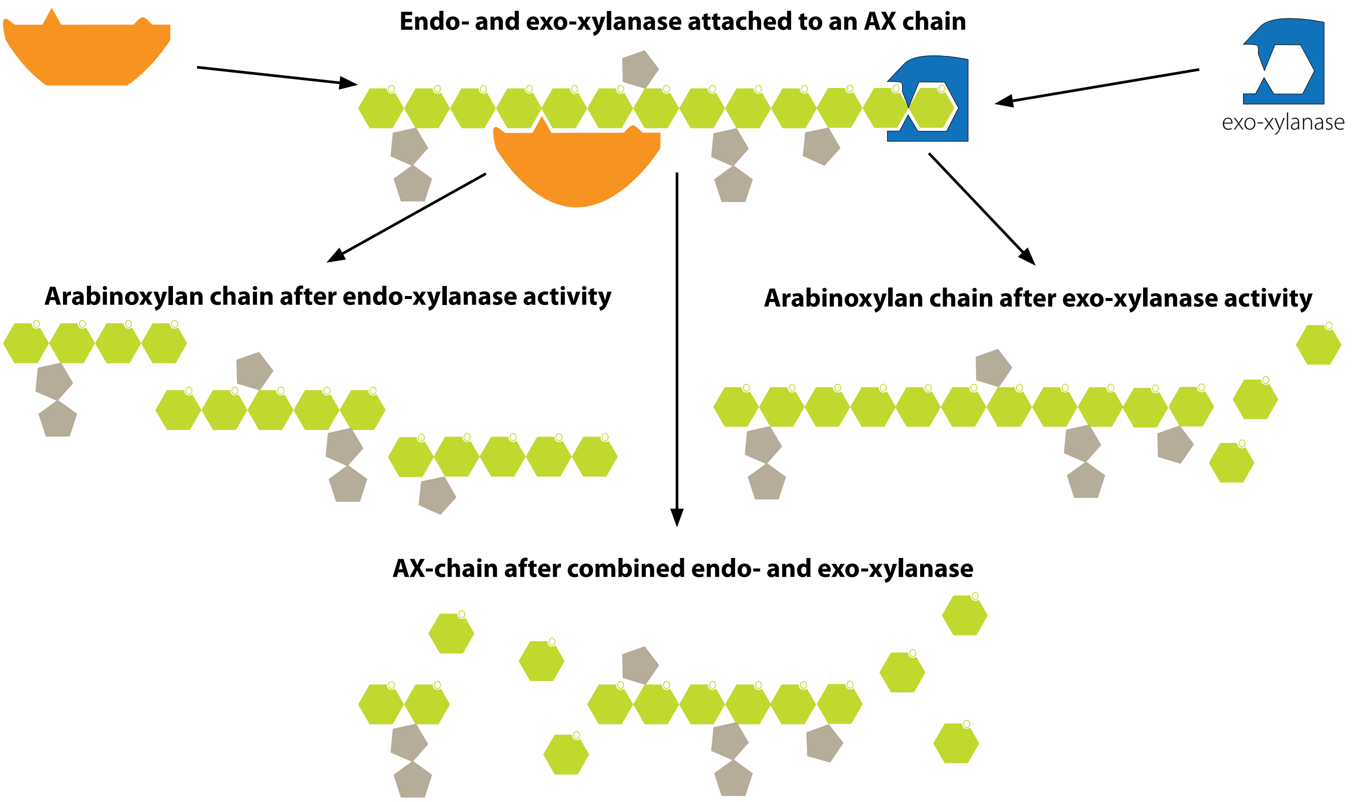
### Exo-xylanases

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 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 Belfeed.

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

**TABLE 2: ORIGIN OF FEED ENZYMES**

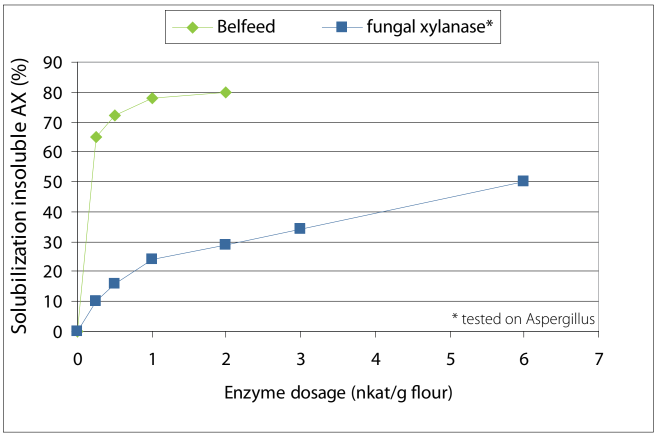
| ENZYME | PRODUCING STRAIN | TYPE |
|---|---|---|
| ***Belfeed*** | ***Bacillus subtilis*** | ***Bacterial*** |
| Allzyme | Aspergillus niger | Fungal |
| Avizyme / Porzyme | Trichoderma longibrachiatum | Fungal |
| Econase | Trichoderma reesei | Fungal |
| Grindazym | Aspergillus niger | Fungal |
| Hostazyme | Trichoderma longibrachiatum | Fungal |
| Natugrain TS | Aspergillus niger | Fungal |
| Ronozyme | Aspergillus oryzae | Fungal |
| Rovabio | Penicillium funiculosum | Fungal |
| Roxazyme | Trichoderma viride<br>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.

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.

**FIGURE 6: ENZYME ACTIVITY ON WATER-INSOLUBLE AX**



# 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

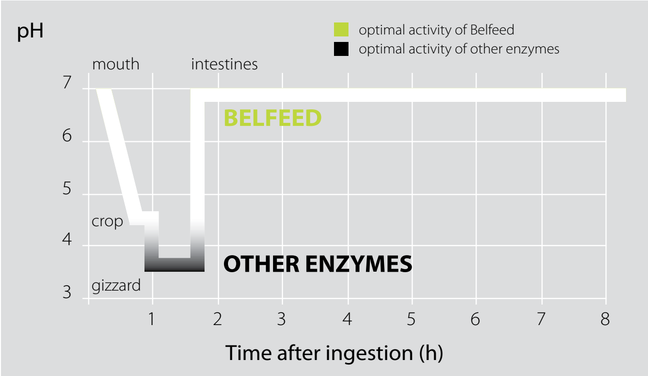
### Fungal xylanases

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).

### Belfeed

Belfeed has a neutral optimal working pH, between 6 and 7. As feed spends the most time in the small intestine, under neutral conditions, Belfeed 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



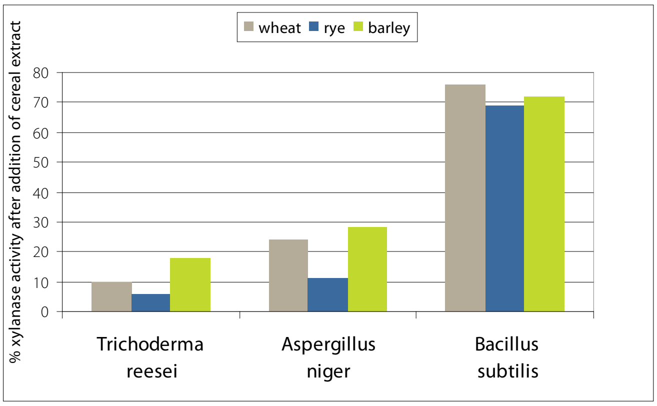

## Sensitivity to xylanase inhibitors

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 Belfeed will only be inhibited by 20-30 % (figure 8).

FIGURE 8: SENSITIVITY TO XYLANASE INHIBITORS



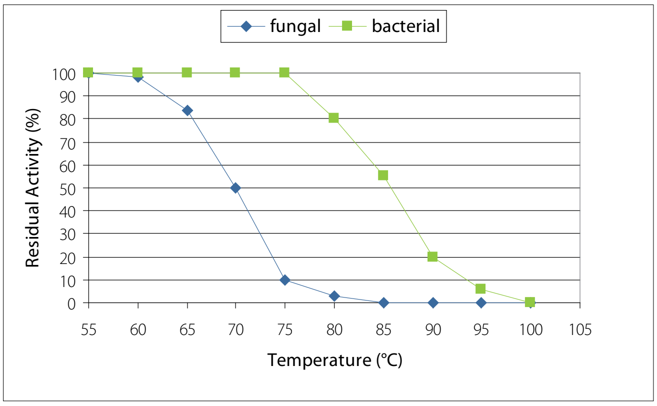

Source: Debyser, 1999

## Thermal stability

Contrary to fungal xylanases, Belfeed 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 Belfeed 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

# Belfeed | one enzyme - a world of benefits

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

**TABLE 3: DIFFERENCES BETWEEN BELFEED AND OTHER ENZYMES**

| | OTHER ENZYMES | BELFEED |
|---|---|---|
| EC registration | Different for each product | Broilers<br>Ducks<br>Layers<br>Turkeys<br>Piglets<br>Pigs |
| Inclusion rate (Belfeed B1100 M) | Different for each product | 100 ppm<br>(diluted products available) |
| Endo-xylanase activity | Different for each product | 9000 U endo-xylanase/g<br>(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 BELFEED IN BROILERS COMPARED TO OTHER ENZYMES***

| | WHEAT | | CORN | |
|---|---|---|---|---|
| | Other enzymes | Belfeed | Other enzymes | Belfeed |
| 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 Belfeed brochure.
** Return on investment depends on the cost price of the feed, prices of meat and general costs (medication, electricity, heating, manure treatment, etc.)

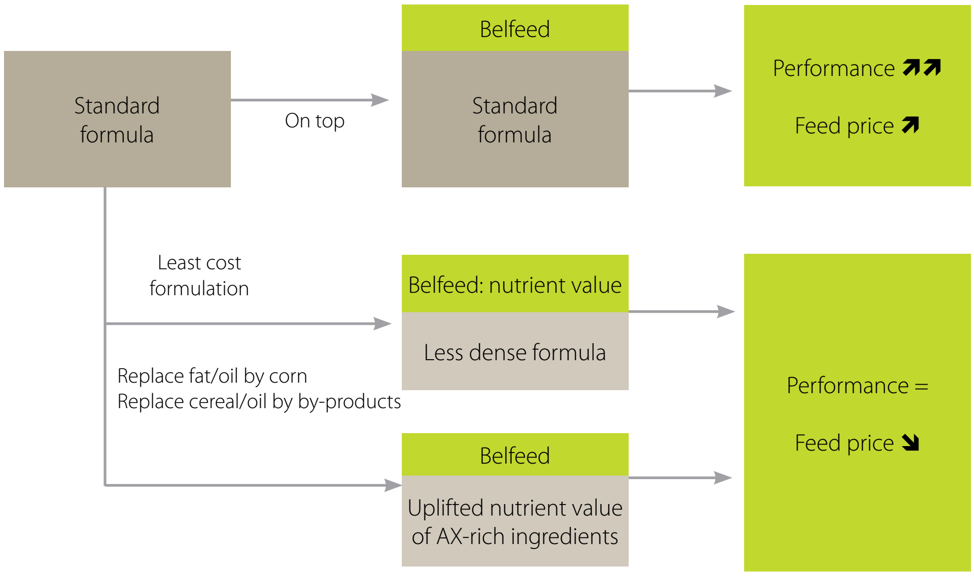
## Performance in pigs

**TABLE 5: AVERAGE IMPROVEMENTS OF BELFEED 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 Belfeed to a negative control, documented in the Belfeed brochure.
** Return on investment depends on the cost price of the feed, prices of meat and general costs (medication, electricity, heating, manure treatment, etc.).

## Belfeed | practical feed formulation




## Belfeed | matrix values

**TABLE 6: MATRIX VALUES FOR BELFEED - 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 | $ME_{broilers\ wheat}$ | 1 680 000 | 7034 |
| Dig. methionine poultry | 105 | | $ME_{broilers\ corn}$ | 1 050 000 | 4396 |
| Dig. meth + cys poultry | 160 | | $ME_{poultry\ wheat}$ | 1 820 000 | 7620 |
| Dig. threonine poultry | 128 | | $ME_{poultry\ corn}$ | 1 100 000 | 4605 |
| Dig. tryptophane poultry | 40 | Layers | $ME_{layers}$ | 700 000 | 2931 |
| | | | $ME_{poultry}$ | 650 000 | 2721 |
| | | 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 | 5862 |
| 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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