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DIPARTIMENTO DI MEDICINA
VETERINARIA E SCIENZE ANIMALI


University of Milan, Department of Veterinary Medicine and Animal Sciences.
Italy, Milan, Via dell'Università 2, Lodi

# DIETARY EFFECTS OF XYLANASE AND FLAXSEEDS ON PERFORMANCE, MEAT QUALITY AND BONE HEALTH IN BROILERS

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# INTRODUCTION

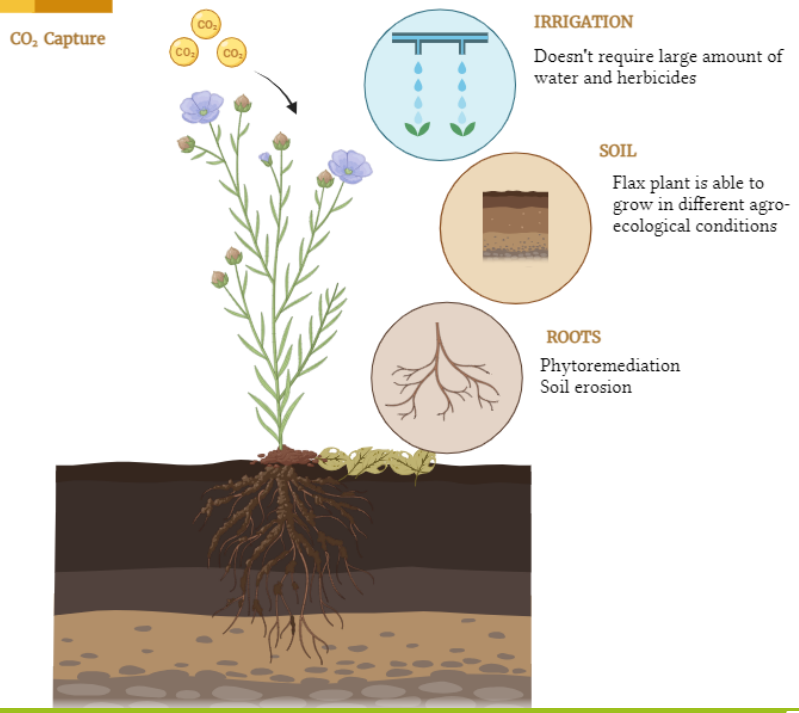
WHAT MUST BE THE NEXT STEPS FOR SCIENTIFIC RESEARCH, TO IMPROVE FEED AND LIVESTOCK SECTOR?

Green Deal


VALORISATION OF MATRICES WITH A LOW ENVIRONMENTAL IMPACT THAT CAN GUARANTEE FOOD/FEED SAFETY

POSITIVE EFFECTS ARE ENSURED BY THE SYNERGETIC ACTION OF INGREDIENTS IN THE ANIMAL'S DIETS

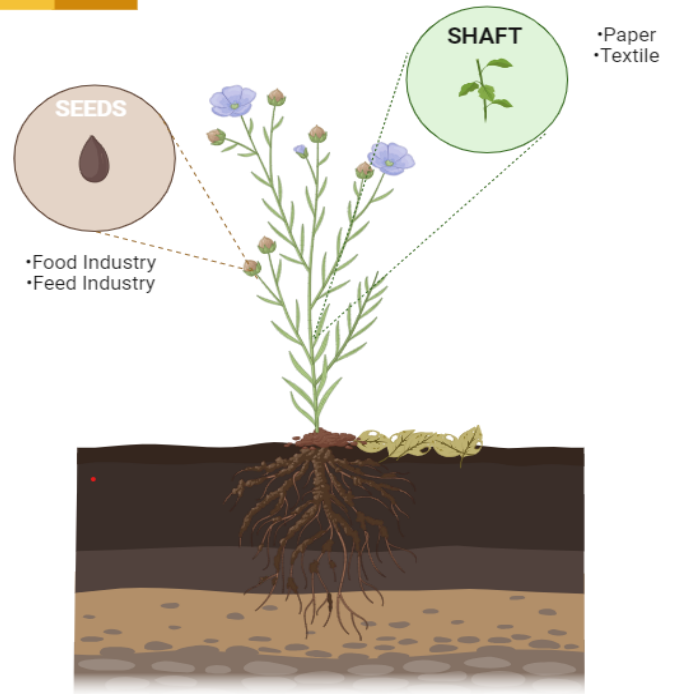
## LINUM USITATISSIMUM L.




ENVIRONMENT

APPLICATIONS

## LINUM USITATISSIMUM L.

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# INTRODUCTION

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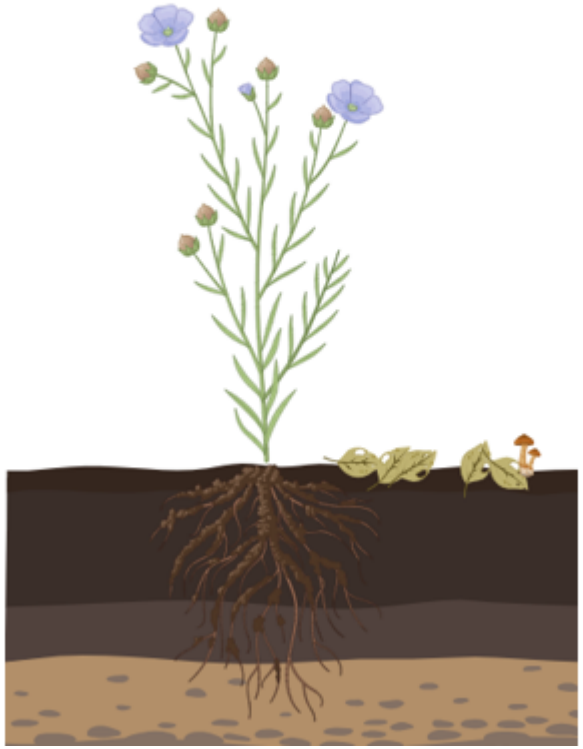
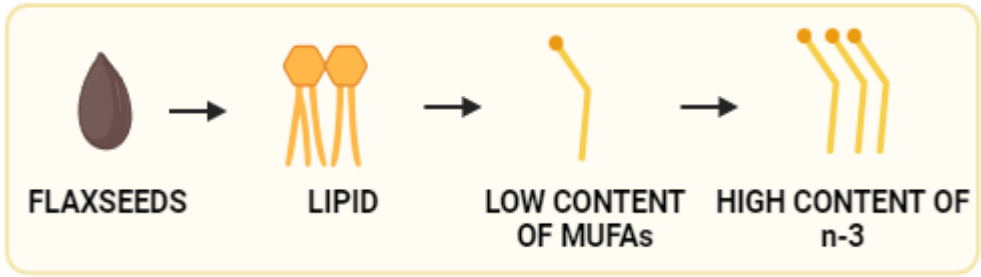


| Sample | DM | CP | EE | NDF | ADF | ADL | ASHES |
|---|---|---|---|---|---|---|---|
| Hempseeds | 94.6 ± 0.12[a] | 23.1 ± 0.57[a] | 27.9 ± 0.75[a] | 44.6 ± 0.21[a] | 33.2 ± 0.31[a] | 14.4 ±0.32[a] | 5.8 ± 0.10[a] |
| Flaxseeds | 91.6 ± 0.14[b] | 23.0 ± 0.45[a] | 36.0 ± 0.07[b] | 41.5 ± 1.27[a] | 19.5 ± 0.35[b] | 9.2 ± 0.41[b] | 2.7 ± 0.41[b] |

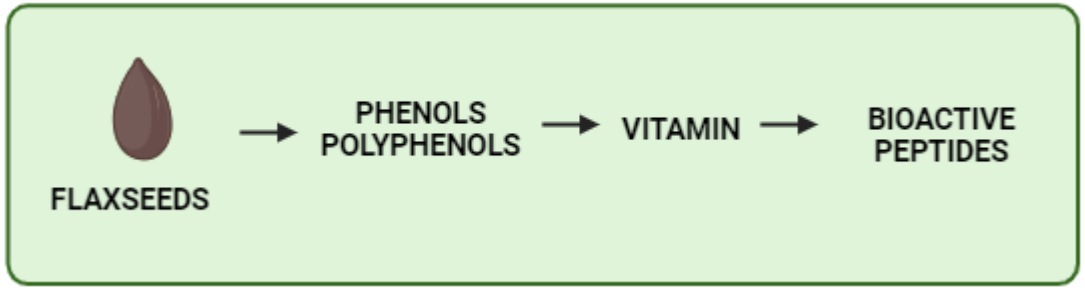
Modified by Lanzoni et al. (2023)

PROTEIN CONTENT


LIPID CONTENT




FUNCTIONAL ACTIVITY

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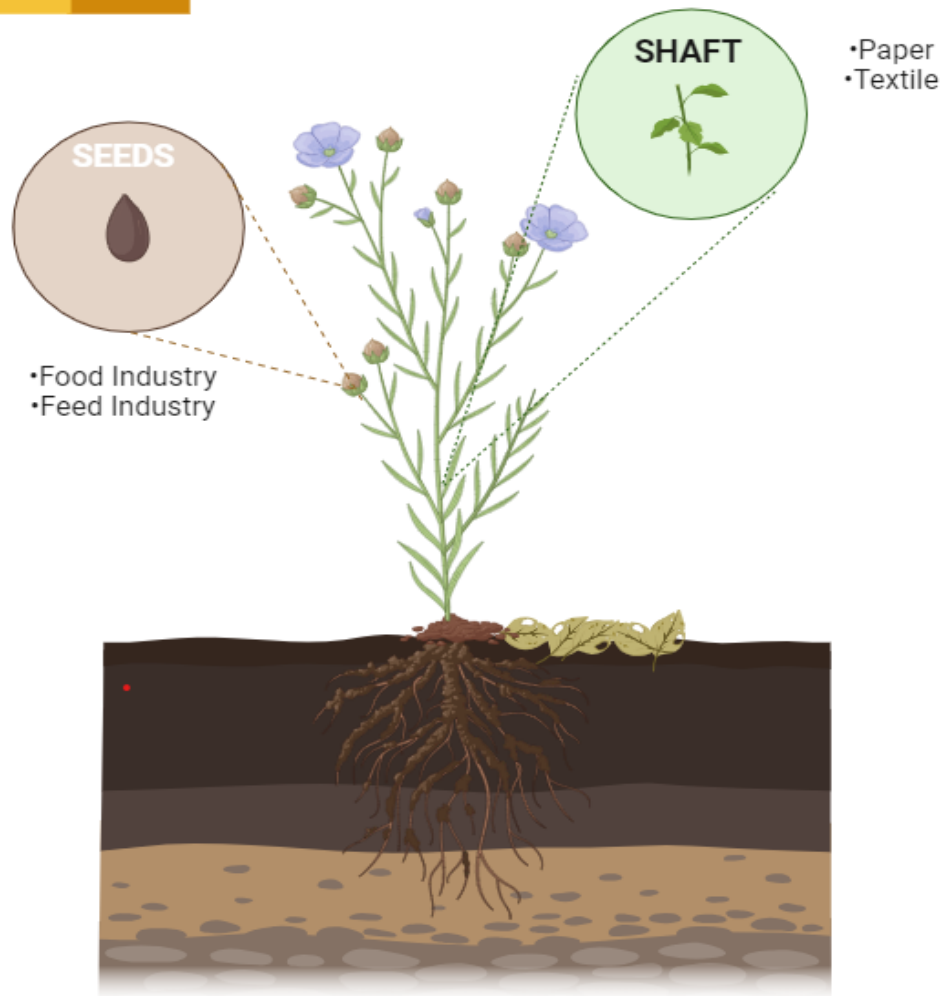
# INTRODUCTION

## LINUM USITATISSIMUM L.




Cultivation depends on the production purposes

LINSEEDS

TEXTILE

BUILDINGS

PAPER

EUROPE

FLAXSEEDS

FOOD

FEED

CANADA (80% of trade)

140-160 £/100 kg

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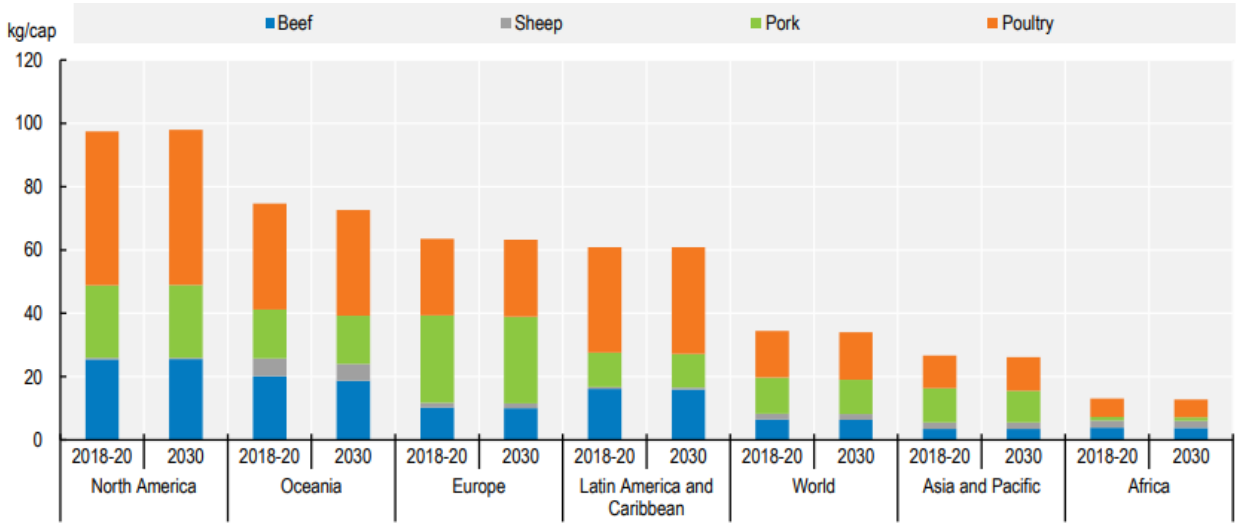
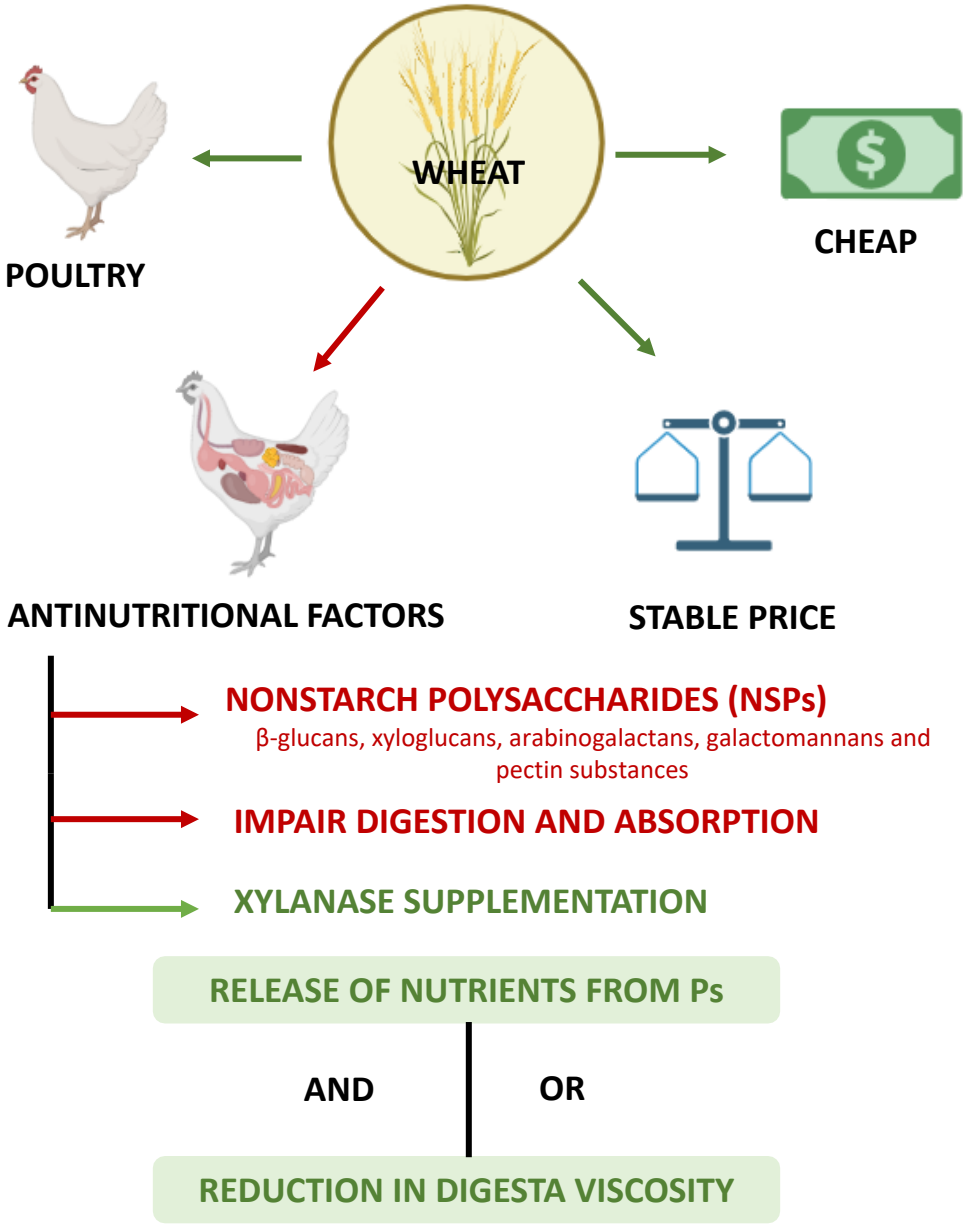
# INTRODUCTION

## MEAT CONSUMPTION PER CAPITA 2020-2030




Meat consumption per capita, expressed in retal weight. Figure reported by OECD/FAO 2021

**CONTINUED RISE FOR POULTRY**

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# AIM

**TO EVALUATE HOW THE COMBINATION OF A WHEAT-BASED DIET WITH THE ADDITION OF FLAXSEEDS AND XYLANASE IS ABLE TO MODULATE NUTRIENT RETENTION, GROWTH PERFORMANCES, BONE HEALTH, OXIDATIVE STABILITY AND MEAT QUALITY.**

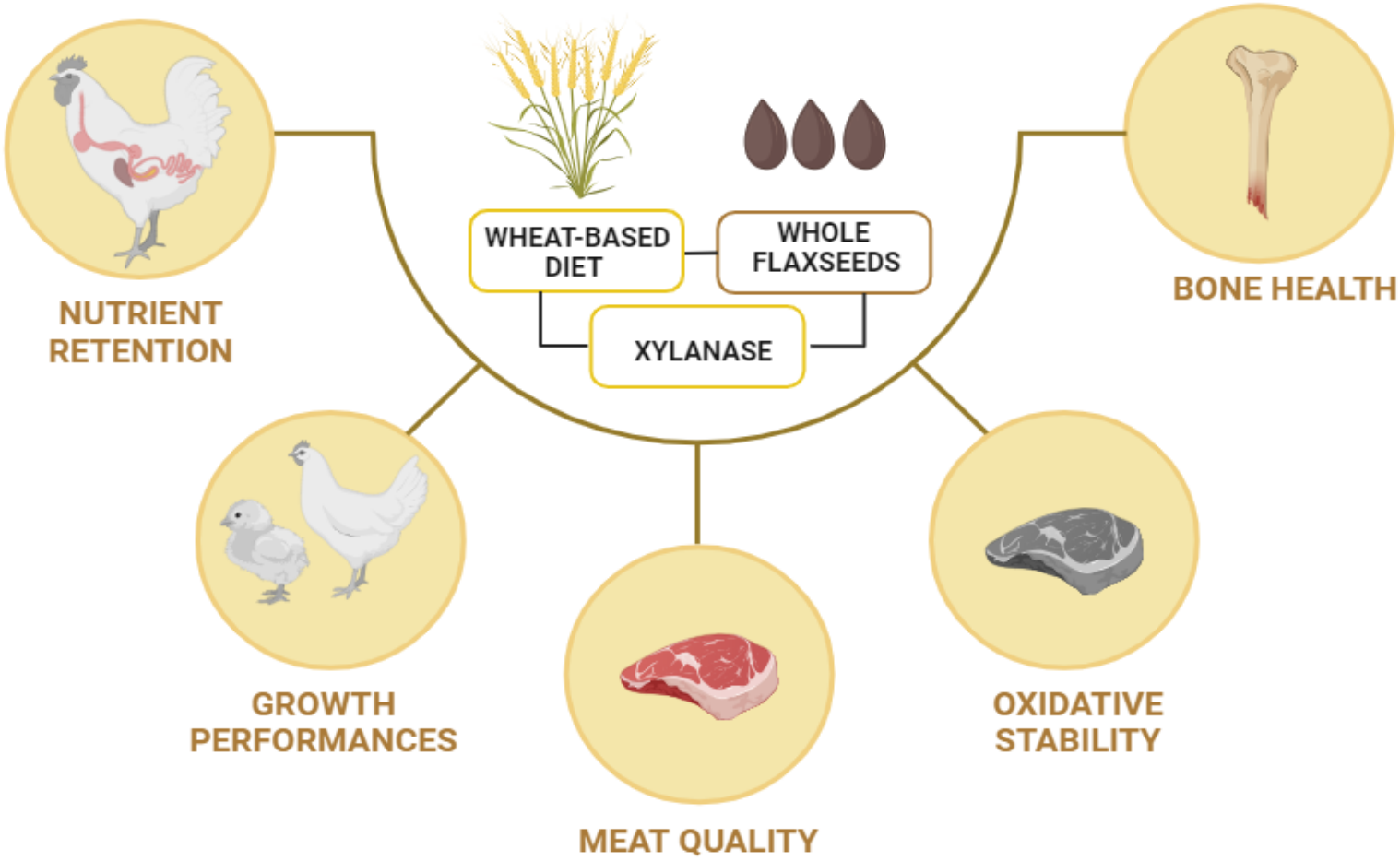
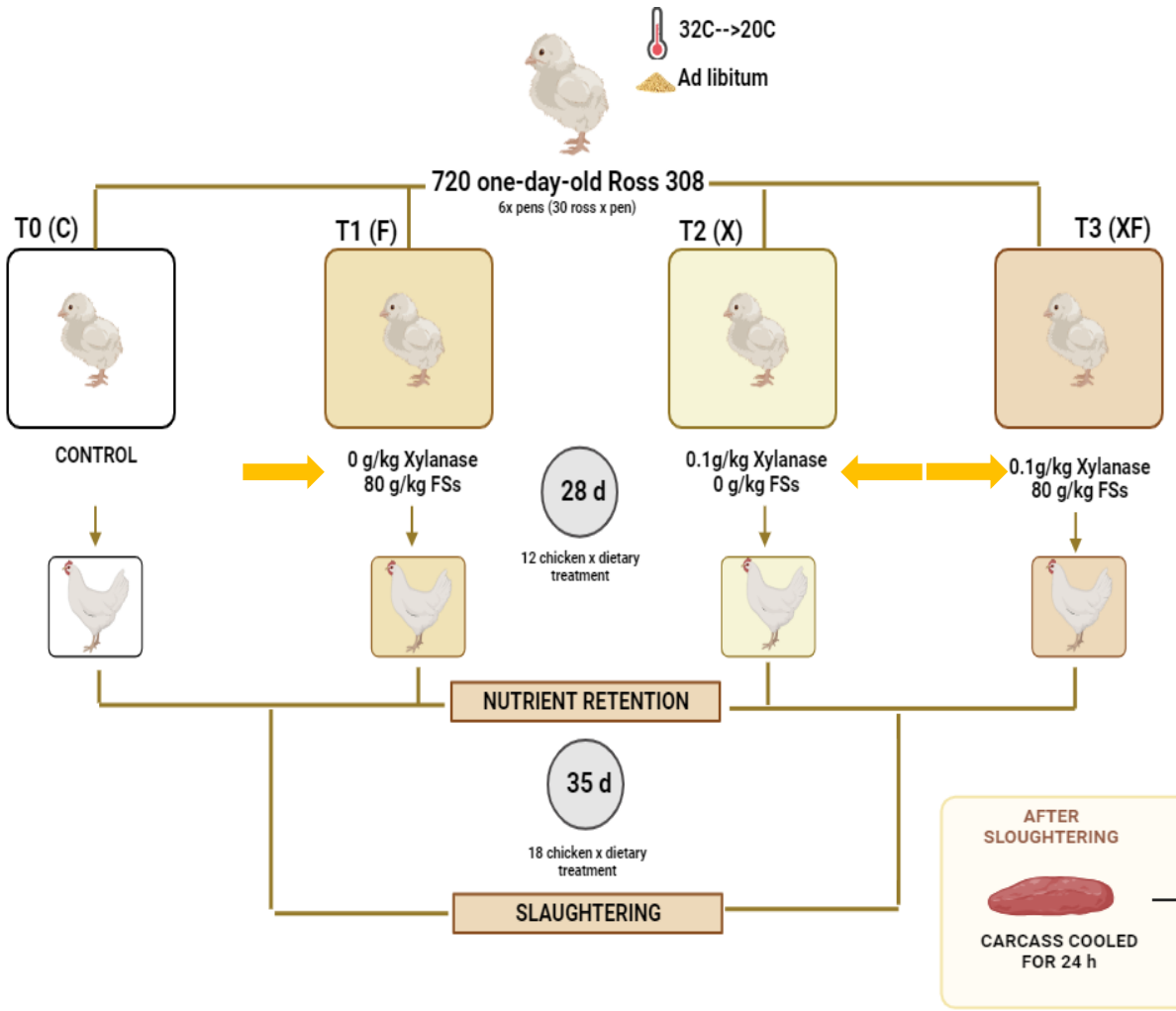
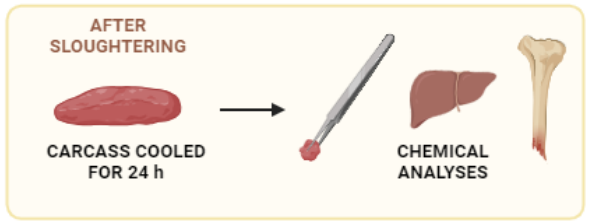



Figure created using Biorender.com

# EXPERIMENTAL DESIGN




| Ingredient (g/kg) | C | FSs |
|---|---|---|
| Wheat | 408.0 | 399.0 |
| Corn | 179.0 | 162.0 |
| Soybean meal | 328.0 | 294.0 |
| Flaxseed | 0.0 | 80.0 |
| Soybean oil | 44.2 | 21.0 |
| Calcium dihydrogen phosphate | 15.0 | 15.2 |
| Limestone | 13.5 | 13.8 |
| Sodium chloride | 2.0 | 2.0 |
| Sodium bicarbonate | 3.0 | 3.0 |
| L-Lysine | 0.8 | 2.1 |
| DL-Methionine | 1.4 | 2.0 |
| L-Threonine | 0.1 | 0.9 |
| Vitamin-mineral premix[2] | 5.0 | |

| Analyzed nutrient content (g/kg) | | |
|---|---|---|
| Dry matter | 892 | 896 |
| Crude protein | 208 | 206 |
| Ether extract | 59.8 | 60.4 |
| Crude fiber | 44.6 | 48.2 |
| $ME_N$ (MJ/kg) by calculation | 11.6 | 11.8 |
| Ca | 9.0 | 9.0 |
| Available P | 4.5 | 4.5 |
| Soluble NSP | 21.5 | 26.5 |
| Insoluble NSP | 82.7 | 85.9 |
| Total NSP | 104.2 | 112.4 |

Figure created using Biorender.com

Xylanase Nutrizyme® XY (Sunson Industry Group CO., LTD, Beijing, China) is a highly concentrated xylanase preparation produced by submerged fermentation of *Trichoderma reesei*. This xylanase is thermostable and active in a wide pH range. The enzyme activity of xylanase was ≥ 15,000 u/g.

# METHODS

## 1 Chemical Analyses

**DIET, BREAST MEAT & FAECES**

AOAC methods (AOAC, 2005)

***Additional info:*** *Calcium concentration by atomic absorption spectrometry Phytate P by capillary isotachophoretic metod*

## 2 Fatty acid Profiles

**DIET, FLAXSEEDS, BREAST MEAT**

**GAS CHROMATOGRAPHY**

Chloroform-methanol extraction of the total lipids

## 3 Oxidative Stability


**FRESH AND STORED MEAT**

→ **Malondialdehyde**

**HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)**

A: water-acetonitrile (95:5)
B: Acetonitrile

## 4 Bone Health

**TIBIA BONES**

***Additional info:*** *Tibia bones were excised from the carcasses and cleaned of all tissue. The strenght and tibia deformation were measured with Instron 3342 apparatus with a 50 kg load cell with a crosshead speed of 50 mm/ min*

Two-way ANOVA with general linear model (GLM) procedure in SAS Software (Statistical Analysis Software, Version 9.3, 2003). The main effects were xylanase content (X), the Flaxseed content (F) and the interaction between these two factors (X *x* F). Differences were considered significant at $P<0.05$. Results are presented as the means ± standard errors of the means

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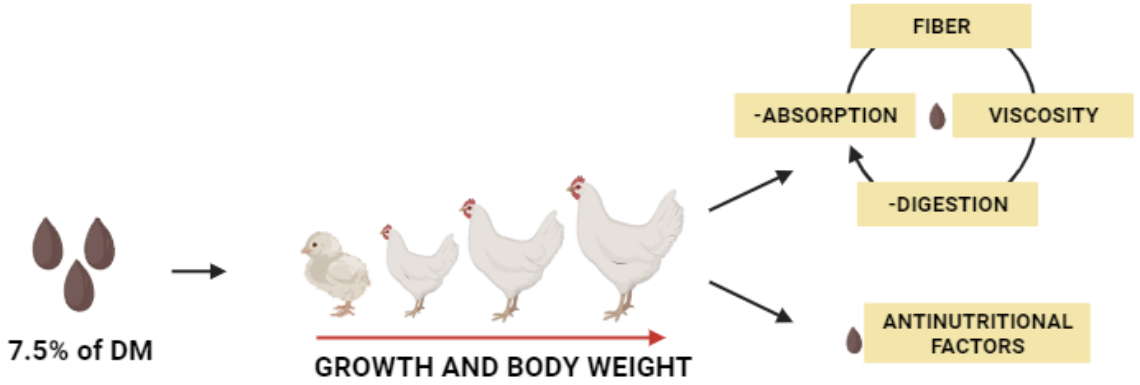
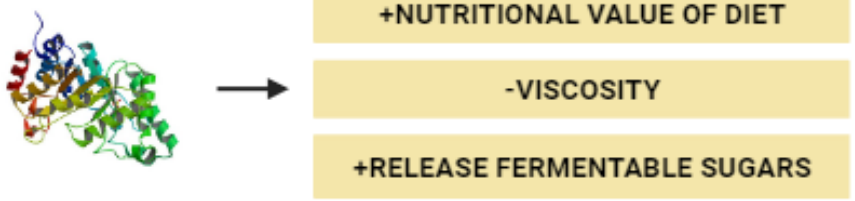
# RESULTS: PERFORMANCES





| Diet | C | F | X | XF | | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | SEM | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| Body weight (Day 0; g) | 37.5 | 36.5 | 36.9 | 35.7 | 0.19 | NS | NS | NS |
| Body weight (Day 14; g) | 370 | 378 | 373 | 387 | 3.7 | NS | NS | NS |
| Body weight (Day 35; g) | 2135 | 2150 | 2140 | 2169 | 19.1 | NS | NS | NS |
| 35 d Feed intake (g/bird per day) | 87.2[a] | 89.1[a] | 80.2[c] | 83.4[b] | 0.32 | <0.001 | NS | 0.050 |
| 35 d FCR (kg/kg) | 1.46[a] | 1.48[a] | 1.33[c] | 1.37[b] | 0.08 | <0.001 | NS | 0.030 |
| Mortality (%) | 3.9 | 2.6 | 1.3 | 2.0 | 0.09 | 0.050 | NS | NS |



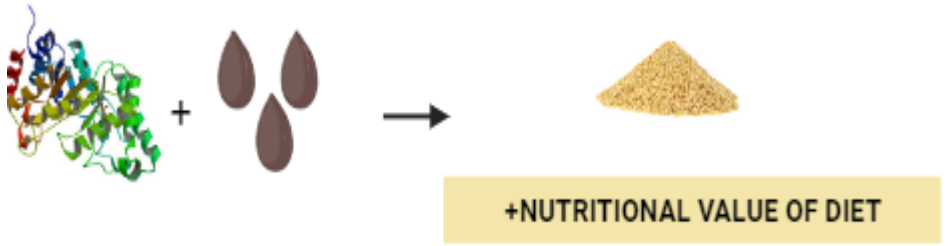



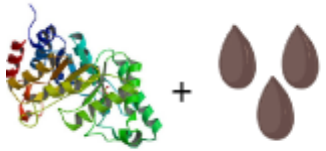

Figure created using Biorender.com

# RESULTS: TOTAL TRACT NUTRIENT RETENTION

| Diet | C | F | X | XF | SEM | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| Dry matter | 50.2 | 39.4 | 56.9 | 54.7 | 2.03 | 0.001 | 0.016 | NS |
| Crude protein | 8.5 | 7.1 | 11.2 | 10.2 | 0.45 | <0.001 | 0.012 | NS |
| Ether extract | 4.69 | 4.25 | 5.49 | 5.33 | 0.177 | 0.005 | NS | NS |
| Crude fiber | - | 0.598 | 0.596 | 0.578 | 0.0073 | NS | NS | NS |
| Nitrogen-free extract | 36.9[a] | 26.1[b] | 37.8[a] | 37.5[a] | 1.49 | 0.004 | 0.008 | 0.011 |
| Organic matter | 49.3 | 38.0 | 55.1 | 53.2 | 2.00 | 0.001 | 0.016 | NS |
| Ash | 0.91[c] | 1.32[b] | 1.82[a] | 1.54[ab] | 0.099 | <0.001 | NS | 0.007 |
| Ca | 1.02 | 0.91 | 1.21 | 1.22 | 0.038 | <0.001 | NS | NS |
| P | 0.199 | 0.192 | 0.247 | 0.215 | 0.0076 | 0.010 | NS | NS |

INCREASED → RETENTION OF ALL TESTED NUTRIENTS

DECREASED → RETENTION OF DRY MATTER, CRUDE PROTEIN, ORGANIC MATTER AND NITROGEN FREE EXTRACT



INCREASED → RETENTION OF ASH; RETENTION OF NITROGEN FREE EXTRACT

Nutrient retention in broilers (g/kg/day), n = 6

The retention of individual nutrients was calculated on the basis of the nutrient content in faeces and feed, feed consumption and faeces production.

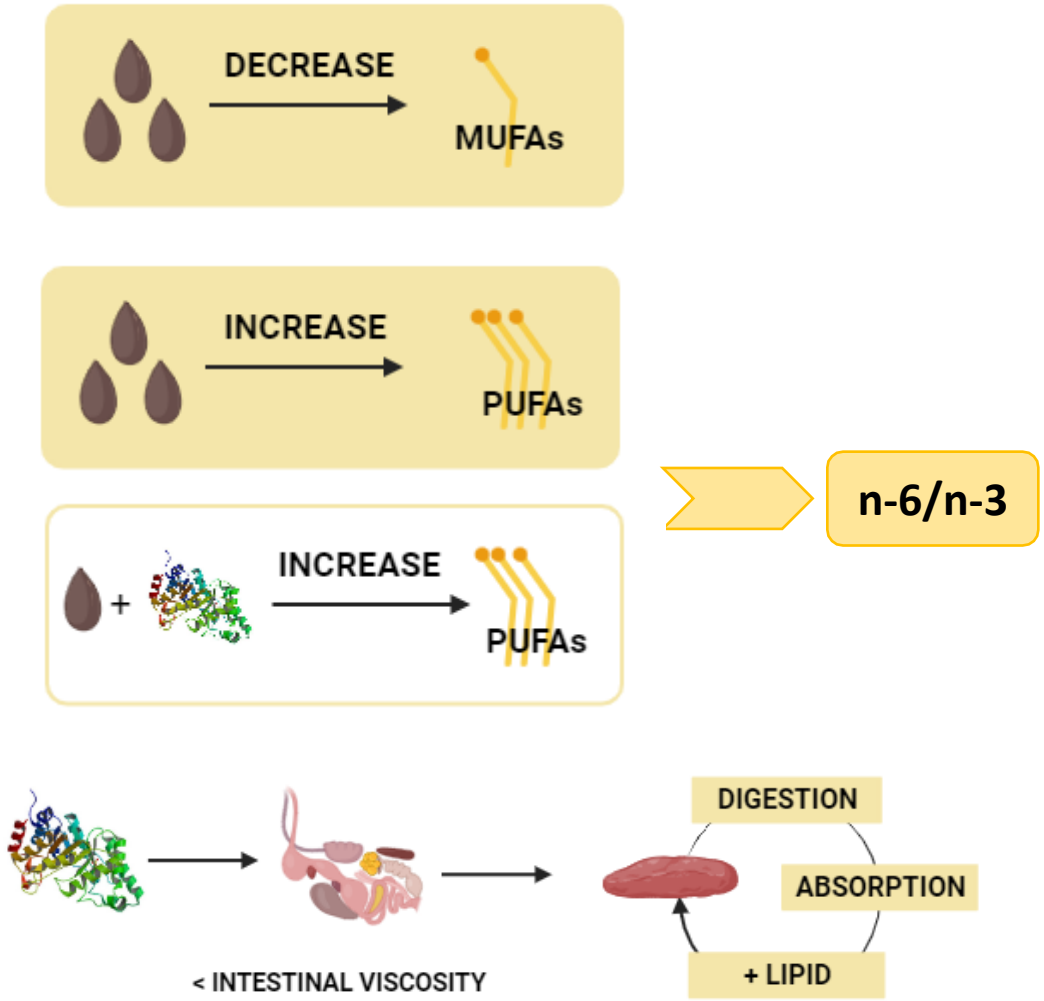
Figure created using Biorender.com

# RESULTS: LIPID PROFILE OF BREAST MEAT

| Diet | C | F | X | XF | SEM | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| SFA | 163 | 135 | 166 | 168 | 5.3 | NS | NS | NS |
| MUFA | 135$^{a}$ | 102$^{b}$ | 138$^{a}$ | 149$^{a}$ | 5.6 | 0.014 | NS | 0.031 |
| PUFA | 210 | 193 | 235 | 274 | 9.2 | 0.002 | NS | NS |
| n3 | 24.3$^{c}$ | 65.5$^{b}$ | 29.6$^{c}$ | 97.2$^{a}$ | 5.70 | <0.001 | <0.001 | 0.006 |
| n6 | 186 | 127 | 205 | 176 | 7.6 | 0.006 | 0.001 | NS |
| n6/n3 | 7.66$^{a}$ | 1.96$^{c}$ | 6.96$^{b}$ | 1.81$^{c}$ | 0.491 | <0.001 | <0.001 | 0.001 |
| AI | 0.334 | 0.313 | 0.312 | 0.291 | 0.0036 | <0.001 | <0.001 | NS |
| TI | 0.685 | 0.432 | 0.633 | 0.363 | 0.0246 | <0.001 | <0.001 | NS |
| h/H | 2.84 | 3.05 | 3.06 | 3.33 | 0.039 | <0.001 | <0.001 | NS |
| PI | 80.5$^{c}$ | 104.2$^{a}$ | 77.4$^{d}$ | 93.3$^{b}$ | 1.98 | <0.001 | <0.001 | 0.001 |

Values expressed in mg/100 g

AI, atherogenic index; TI, thrombogenic index; h/H, hypocholesterolemic/hypercholesterolemic fatty acid ratio; PI, peroxidation index.

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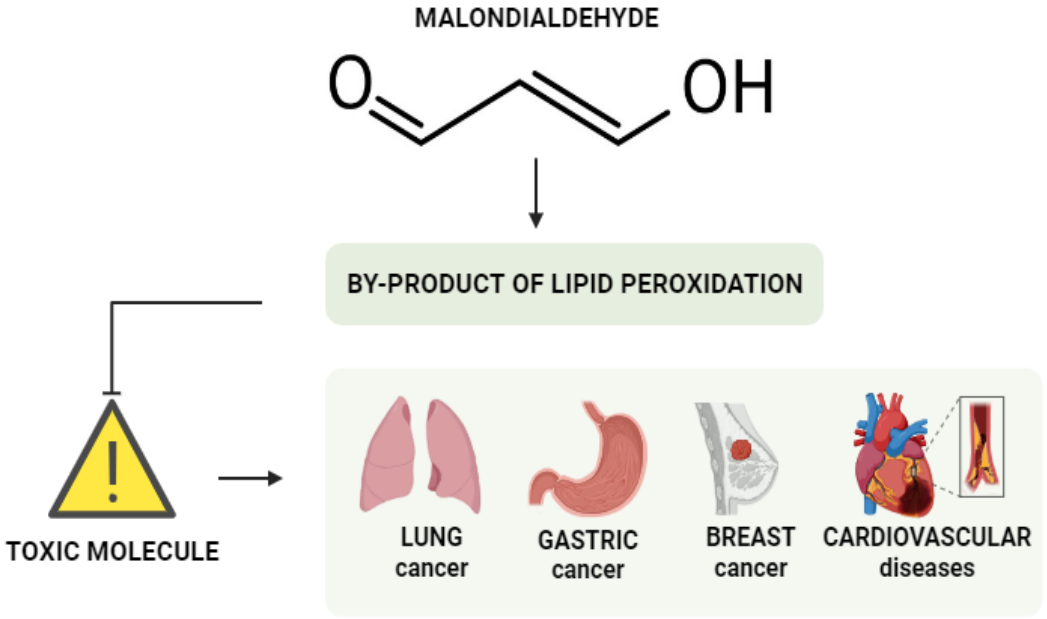
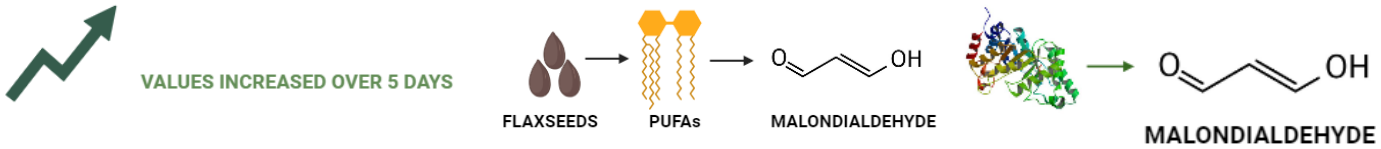
# RESULTS: OXIDATIVE STABILITY



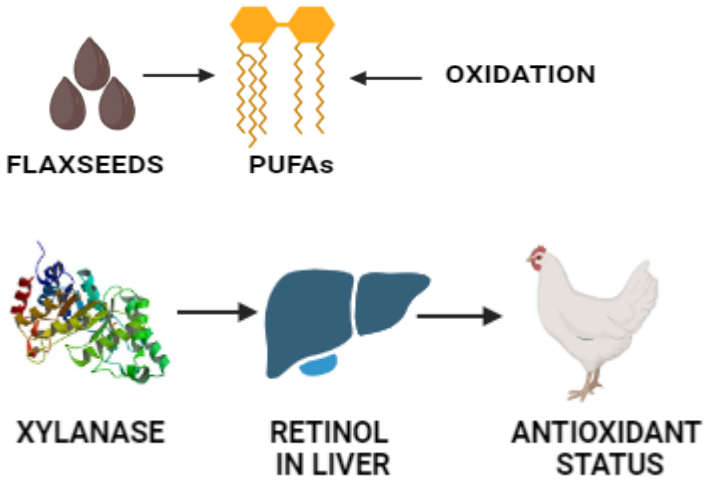

| Diet | C | F | X | XF | | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | SEM | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| MDA, Day 0 | 0.318[a] | 0.295[a] | 0.221[b] | 0.288[a] | 0.0103 | 0.005 | NS | 0.014 |
| MDA, Day 5 | 0.362[a] | 0.348[a] | 0.256[b] | 0.346[a] | 0.0123 | 0.014 | NS | 0.017 |

Values are expressed in mg MDA/kg of meat




| Diet | C | F | X | XF | | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | SEM | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| α-Tocopherol | 11.9 | 12.5 | 11.8 | 12.7 | 0.65 | NS | NS | NS |
| γ-Tocopherol | 1.23 | 1.15 | 1.01 | 0.93 | 0.059 | NS | NS | NS |
| Retinol | 99.8 | 77.6 | 119.8 | 103.5 | 4.63 | 0.007 | 0.021 | NS |

Values are expressed in mg/kg

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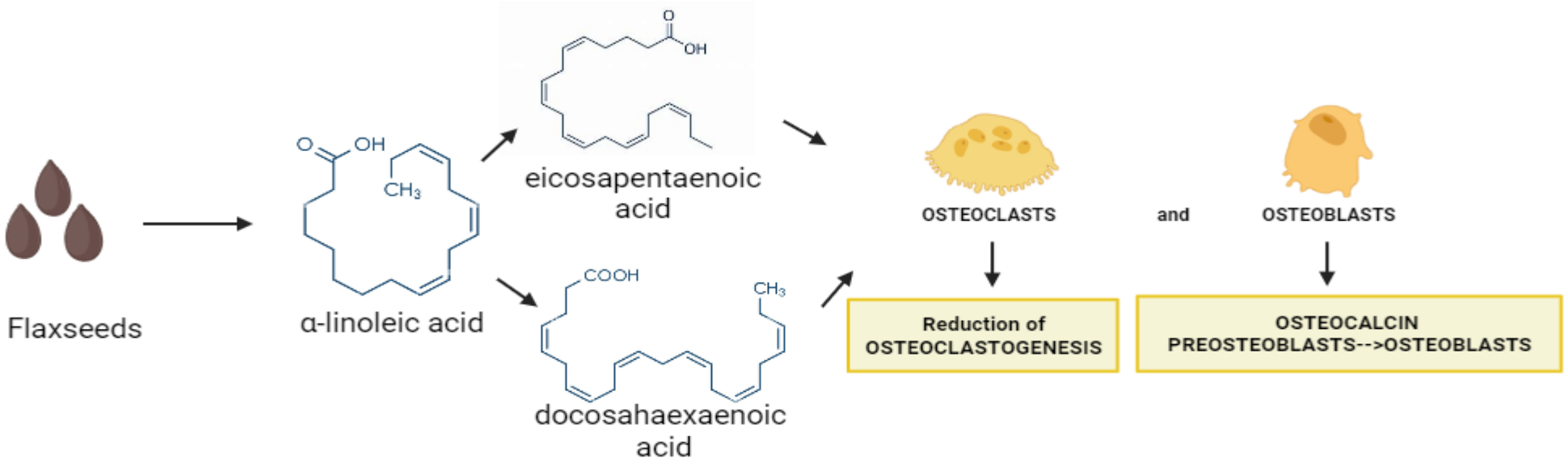
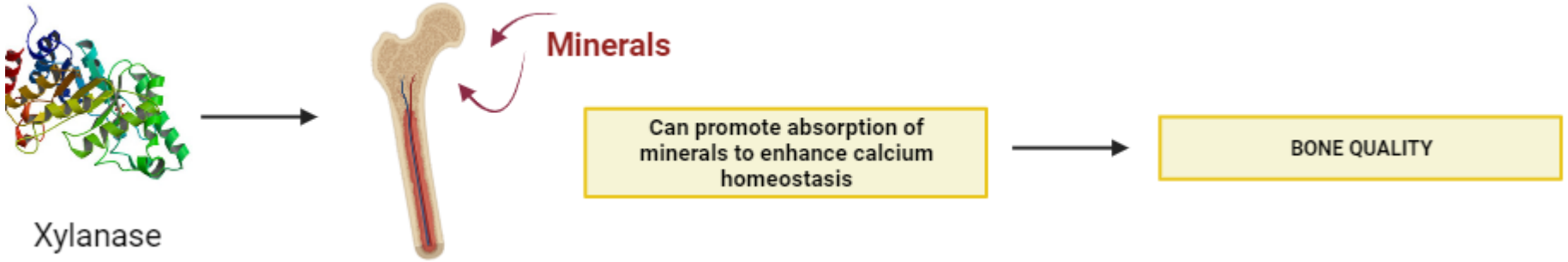
# RESULTS: BONE HEALTH

| Diet | C | F | X | XF | SEM | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| Strength of tibia | 332[b] | 389[a] | 385[a] | 366[ab] | 8.1 | NS | 0.010 | 0.030 |

INCREASED STRENGTH OF TIBIA

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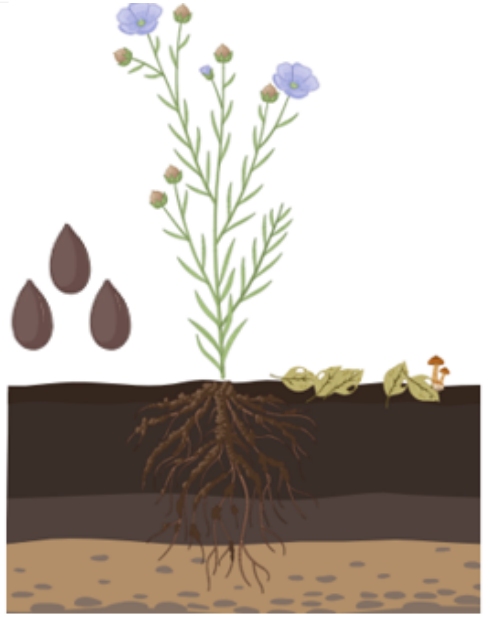
## CONCLUSIONS

XYLANASE + FLAXSEEDS → DIET, GROWTH PERFORMANCES, QUALITY, BONE HEALTH →



### FUTURE PROSPECTS

SET UP OF IN VIVO TRIALS TO IDENTIFY THE BEST CONCENTRATION OF FLAXSEEDS

/ CO-PRODUCTS →

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# THANKS FOR YOUR ATTENTION

**DIETARY EFFECTS OF XYLANASE AND FLAXSEEDS**

**ON PERFORMANCE, MEAT QUALITY AND BONE HEALTH IN BROILERS**

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Italy, Milan, Via dell'Università 2, Lodi

# SUPPLEMENTARY MATERIALS

**Table 2.** Activity of xylanase in mixed feed and liver (mg saccharide/g sample/h), n = 6.

| Diet | C | F | X | XF | | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | SEM | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| Mixed feed | 0.859 | 0.852 | 1.508 | 1.573 | | | | |
| Liver | 0.463$^{b}$ | 0.577$^{b}$ | 0.728$^{b}$ | 1.343$^{a}$ | 0.0781 | <0.001 | 0.001 | 0.021 |

$^{a,b}$Means with different superscripts differ significantly. Abbreviations: SEM, standard error of the mean.

| Diet | C | F | X | XF | SEM | Probability | | |
|---|---|---|---|---|---|---|---|---|
| Xylanase (X; g/kg) | 0 | 0 | 0.1 | 0.1 | | X | F | X*F |
| Flaxseed (F; g/kg) | 0 | 80 | 0 | 80 | | | | |
| 6:0 | 0.025 | 0.022 | 0.037 | 0.044 | 0.0020 | <0.001 | NS | NS |
| 8:0 | 0.115 | 0.113 | 0.082 | 0.081 | 0.0041 | <0.001 | NS | NS |
| 10:0 | 0.114$^{a}$ | 0.070$^{b}$ | 0.078$^{b}$ | 0.074$^{b}$ | 0.0044 | 0.027 | 0.001 | 0.006 |
| 12:0 | 0.261 | 0.236 | 0.142 | 0.148 | 0.0116 | <0.001 | NS | NS |
| 13:0 | 0.069 | 0.057 | 0.072 | 0.062 | 0.0024 | NS | 0.028 | NS |
| 14:0 | 1.96 | 1.69 | 2.16 | 2.27 | 0.087 | 0.023 | NS | NS |
| 14:1-n5 | 0.293 | 0.194 | 0.369 | 0.352 | 0.0182 | <0.001 | NS | NS |
| 15:0 | 0.533 | 0.448 | 0.547 | 0.591 | 0.0232 | NS | NS | NS |
| 16:0 | 108$^{a}$ | 84$^{b}$ | 107$^{a}$ | 114$^{a}$ | 3.9 | 0.049 | NS | 0.036 |
| 16:1-n7 | 8.73 | 6.87 | 11.90 | 10.04 | 0.511 | 0.001 | 0.032 | NS |
| 17:0 | 0.902 | 0.780 | 1.050 | 0.967 | 0.0373 | 0.022 | NS | NS |
| 18:0 | 50.6 | 46.2 | 54.1 | 49.1 | 1.46 | NS | NS | NS |
| 18:1-n9 | 113$^{a}$ | 84$^{b}$ | 113$^{a}$ | 125$^{a}$ | 4.7 | 0.015 | NS | 0.015 |
| 18:1-n7 | 11.6 | 10.1 | 11.6 | 12.1 | 0.40 | NS | NS | NS |
| 18:2-n6 t | 0.093 | 0.078 | 0.163 | 0.183 | 0.0100 | <0.001 | NS | NS |
| 18:2-n6 | 138 | 99 | 162 | 146 | 6.1 | 0.001 | 0.006 | NS |
| 18:3-n6 | 2.76$^{ab}$ | 2.96$^{a}$ | 3.23$^{a}$ | 2.11$^{b}$ | 0.153 | NS | NS | 0.026 |
| 18:3-n3 | 11.1$^{c}$ | 35.1$^{b}$ | 17.4$^{c}$ | 69.5$^{a}$ | 4.33 | <0.001 | <0.001 | <0.001 |
| 18:2 (9.11) | 0.258$^{c}$ | 0.434$^{b}$ | 0.413$^{b}$ | 1.026$^{a}$ | 0.0566 | <0.001 | <0.001 | <0.001 |
| 18:2 (10.12) | 0.043 | 0.044 | 0.055 | 0.060 | 0.0020 | <0.001 | NS | NS |
| 20:0 | 0.394 | 0.381 | 0.558 | 0.497 | 0.0202 | <0.001 | NS | NS |
| 20:1-n9 | 1.19 | 0.82 | 1.45 | 1.22 | 0.058 | 0.001 | 0.002 | NS |
| 20:2-n6 | 5.29$^{a}$ | 3.01$^{c}$ | 5.06$^{ab}$ | 4.26$^{b}$ | 0.214 | NS | <0.001 | 0.019 |
| 21:0 | 0.142 | 0.146 | 0.216 | 0.201 | 0.0083 | <0.001 | NS | NS |
| 20:3-n6 | 4.39 | 3.11 | 3.50 | 3.19 | 0.160 | NS | 0.008 | NS |
| 20:4-n6 | 35.3 | 18.9 | 30.4 | 20.4 | 1.45 | NS | <0.001 | NS |
| 20:3-n3 | 1.13$^{b}$ | 2.57$^{a}$ | 0.76$^{b}$ | 2.80$^{a}$ | 0.170 | NS | <0.001 | 0.032 |
| 20:4-n3 | 0.202$^{b}$ | 0.205$^{b}$ | 0.193$^{b}$ | 0.414$^{a}$ | 0.0192 | <0.001 | <0.001 | <0.001 |
| 22:0 | 0.095 | 0.094 | 0.130 | 0.151 | 0.0058 | <0.001 | NS | NS |
| 20:5-n3 | 1.11 | 6.03 | 1.43 | 6.71 | 0.474 | 0.042 | <0.001 | NS |
| 22:1-n9 | 0.104$^{bc}$ | 0.097$^{c}$ | 0.119$^{b}$ | 0.154$^{a}$ | 0.0057 | <0.001 | NS | 0.019 |
| 23:0 | 0.068 | 0.058 | 0.078 | 0.090 | 0.0037 | 0.003 | NS | NS |
| 24:0 | 0.082 | 0.069 | 0.072 | 0.081 | 0.0031 | NS | NS | NS |
| 22:5-n3 | 6.55 | 15.36 | 6.91 | 12.81 | 0.791 | NS | <0.001 | NS |
| 24:1-n9 | 0.108 | 0.096 | 0.112 | 0.109 | 0.0039 | NS | NS | NS |
| 22:6-n3 | 4.21 | 6.29 | 2.94 | 5.04 | 0.267 | 0.001 | <0.001 | NS |