Impact of fibre degrading feed enzymes on gut microbiome profile in broiler chickens fed corn/corn DDGS or viscous grains (wheat, barley and rye) based diets

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Introduction

- The presence of soluble non-starch polysaccharides (NSP), such as arabinoxylans and β-glucans, in cereal grains can negatively affect nutrient utilization and consequently reduce growth performance in broiler chickens.

- The high content of NSP in viscous cereal grains and cereal co-products limit their use in poultry feed source due to the anti-nutritive effect attributed to an increased digesta viscosity, a condition associated with detrimental effects on digestion and absorption processes as well as increasing incidences of wet and sticky droppings.

- Viscous digesta can stimulate rapid growth of certain pathogenic bacteria in the ileum, which could result in nutrient competition.

- Feed supplementation with NSP-degrading enzymes (NSPases) such as xylanase, β-glucanase, β-mannanase, α-galactosidase and pectinase has become a common practice in poultry diets, and has been shown to decrease intestinal viscosity and enhance nutrient digestibility and performance.

- The gut microbiota, which have a role in the utilization of undigested substrates in the intestinal lumen, may influenced by NSPases due to re-modification of undigested substrates.

- Diversity and activities of gut microbiota are also affected by a number of factors including dietary factors, such as the feed type dietary composition, as well as dietary additives.

- Both the cereal type and the exogenous enzyme supplementation have been shown to influence cecal microbiota in broiler chickens, and may further reduce potentially pathogenic Enterobacteriaceae populations.

- There is limited information on the ileal and cecal microbiota composition in broiler chickens fed corn or wheat-based diets and supplemented with a blend of β-glucanase and xylanase (XB).

- Therefore, our objective was to evaluate the effect of supplementing XB on ileal and cecal microbiota profile using Miseq Illumina sequencing in 21 d old broiler chickens fed diets containing corn or wheat-based diets.

Experimental Design/ Materials and Methods

- A total of 140 d-old male Ross 308 broiler chicks were randomly assigned to 7 replicate cages (5 birds/cage) and fed experimental diets for 21 d.

- The diets were based on either corn or wheat without or with supplemental XB.

- On d 21, ileal and cecal contents were obtained for microbial analysis by illumina sequencing.

- Data were analyzed using QIIME and partial least square discriminant analysis (PLS-DA) was used to identify taxa that were most characteristic of specific dietary treatment groups without or with XB.

- Alpha-diversity of bacterial community was calculated using different richness estimators.

Materials and Methods

Table 1. Ingredient profile and calculated and analyzed composition of experimental diets.

Item	Corn-based diet	Wheat-based diet
	Amount	
Ingredient %		
Corn	45.55	–
Corn DDGS	12.0	–
Hard Wheat (14.5%CP)	–	41.65
Wheat middlings	–	2.00
Barley	–	5.00
Rye	–	5.00
Soybean Meal (44%CP)	32.8	35.4
Soybean Oil	4.55	5.89
L-Lysine HCl	0.37	0.26
DL-methionine	0.30	0.30
L-threonine	0.07	0.07
Salt	0.30	0.38
Limestone	1.48	1.39
Monocalcium Phosphate	1.28	1.36
Vit/Min. Premix[2]	1.00	1.00
Titanium dioxide	0.30	0.30
Calculated composition		
ME, kcal/kg	2868.0	2868.0
CP, %	22.0	22.0
Crude fat, %	7.78	7.30
Starch, %	28.3	31.0
Sugars, %	3.85	5.73
NDF, %	14.2	12.0
NSP, %	14.2	13.6
Dig. Lys, %	1.19	1.19
Dig. Meth, %	0.59	0.56
Dig. Meth + Cys, %	0.87	0.87
Dig. Thr, %	0.73	0.73
Dig. Tryp, %	0.21	0.24
Calcium, %	0.98	0.98
Total Phosphorous, %	0.72	0.71
Available phosphorous, %	0.47	0.47
Sodium, %	0.18	0.18
Analyzed composition		
AMEn, Kcal/kg	3060	3055
CP, %	22.0	22.1
Crude fat, %	8.46	8.04
Starch, %	30.4	29.2
NDF, %	13.3	15.7
ADF, %	5.96	7.35

Library preparation
- PCR amplification of V4 region of 16S rRNA gene
- Clean-up, quality check and quantifying the amplicons

Illumina Sequencing
- 300-cycle paired-end sequencing of the pooled library

Bioinformatics
- Output paired-end reads were merged using PANDASeq assembler and analyzed using QIIME
- Demultiplexing merged reads, quality check and chimera detection and filtering was done in QIIME
- The resulting operational taxonomic units were aligned at 97% similarity threshold to green-genes database
- Alpha- & beta-diversity of microbial communities were determined

Statistics
- Differences at the phylum level were determined using univariate procedure of SAS.
- Multivariate data analysis (PERMANOVA, SIMCA/PLS-DA) was done for detecting differences for beta diversity and at the genus level. $P < 0.05$ was considered significant

Results and Discussion

- In total 8 and 6 phyla (≥ 0.01 %) were observed in the ileum and cecum respectively but no differences were found between XB and non-XB treatments.

- Alpha- and Beta-diversities of microbiota did not differ between XB and non-XB treatments.

- PLS-DA analysis revealed that some taxa were associated with specific treatments at genus level.
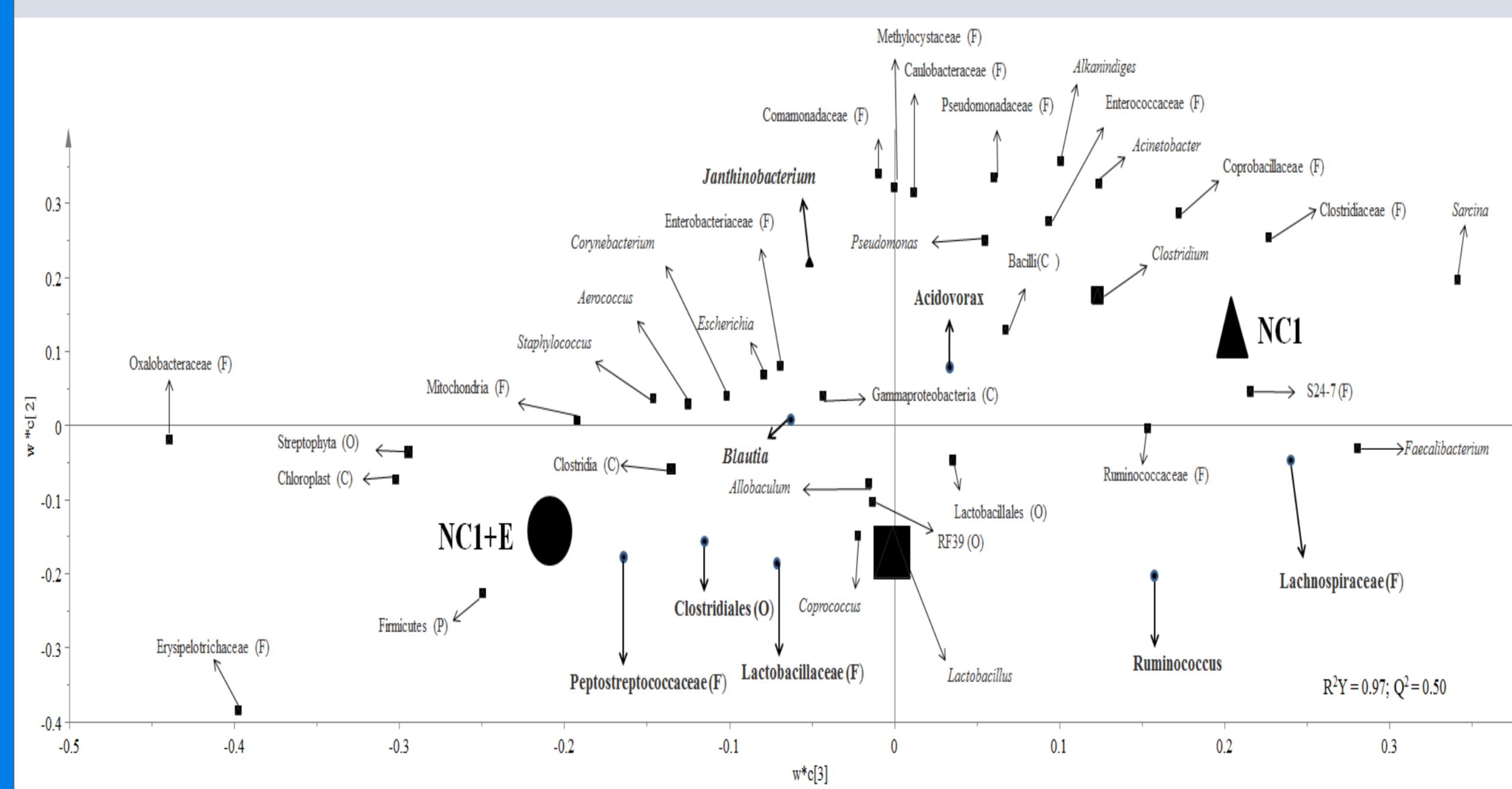

Figure 1: Partial least square discriminant analysis (PLS-DA) loading scatter plot based on the relative abundance of the putative bacterial genera in the **ileal digesta** of 21 d old broiler chickens and their association with **corn-based diet** without (NC1) or with XB (NC1+E) treatments. Genera found to be significantly associated with each treatment are shown in filled circles (NC1+E) and filled triangles (NC1), while non-significant genera are shown in filled squares. Some taxa could only be classified up to family (F), order (O), class (C), or phylum (P) level.
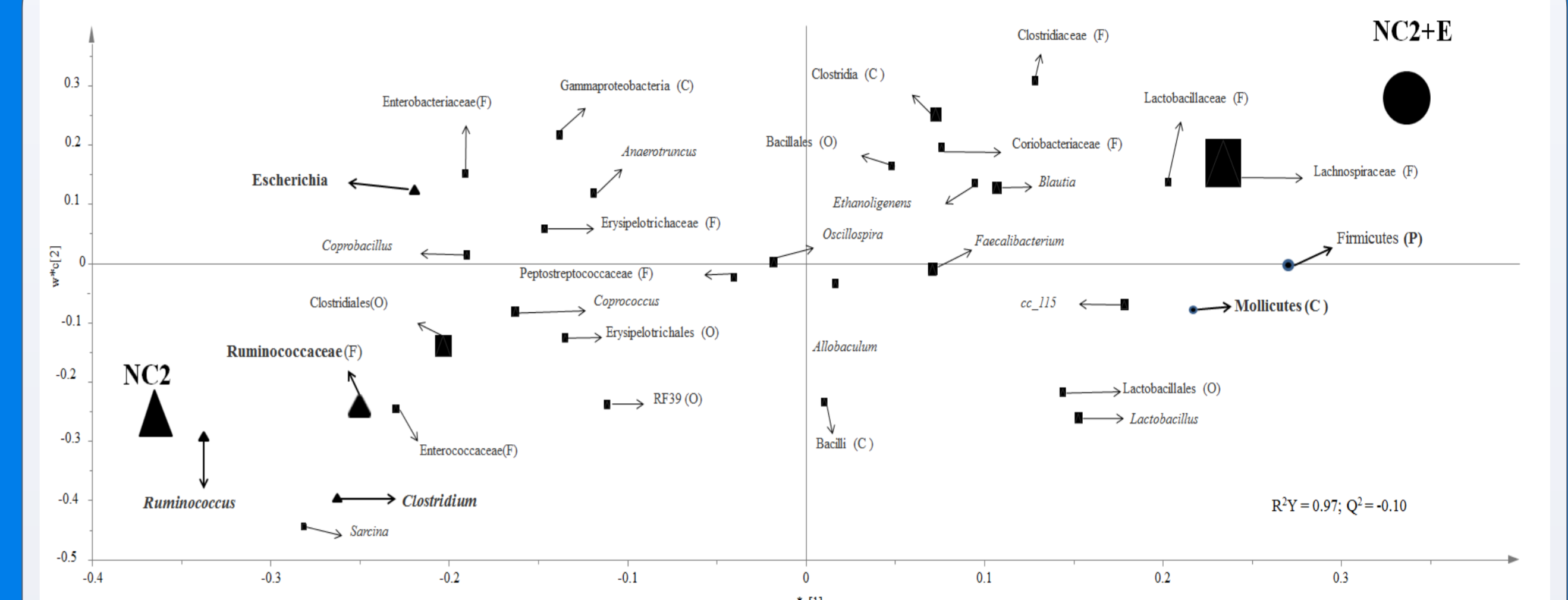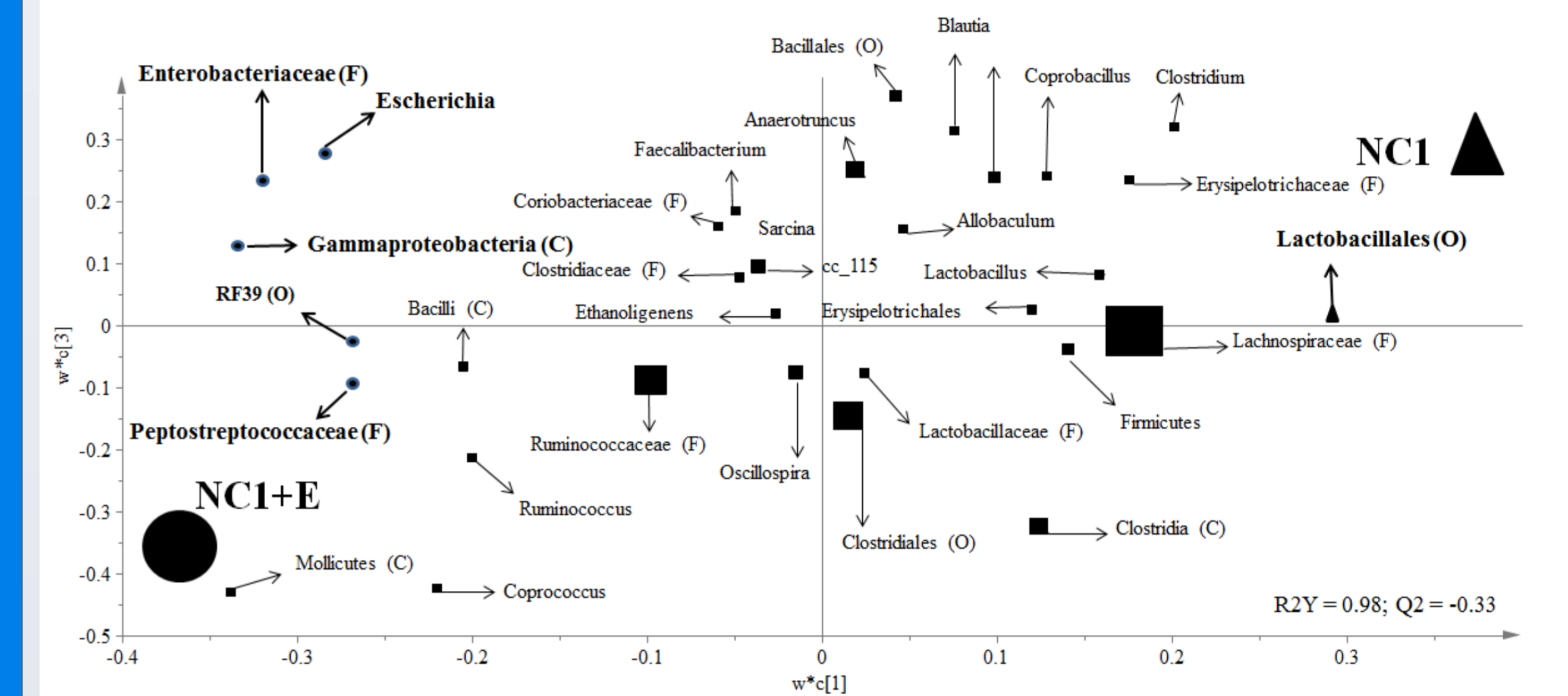

Figure 2. Partial least square discriminant analysis (PLS-DA) loading scatter plot based on the relative abundance of the putative bacterial genera in the **ileal digesta** of 21 d old broiler chickens and their association with **wheat grains-based diet** without (NC2) or with XB (NC2+E) treatments. Genera found to be significantly associated with each treatment are shown in filled circles (NC2+E) and filled triangles (NC2), while non-significant genera are shown in filled squares. Some taxa could only be classified up to family (F), order (O), class (C), or phylum (P) level.
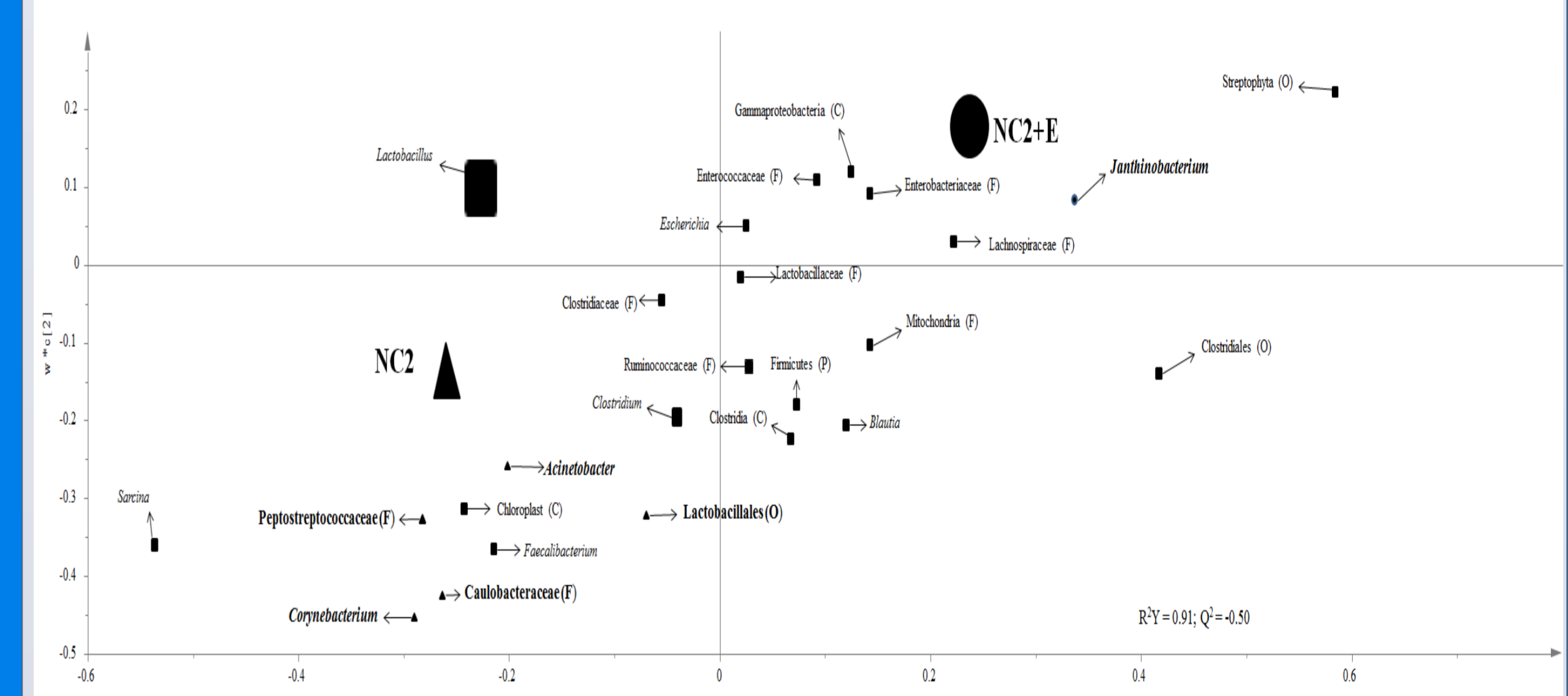

Figure 3. Partial least square discriminant analysis (PLS-DA) loading scatter plot based on the relative abundance of the putative bacterial genera in the **cecum digesta** of 21 d old broiler chickens and their association with **corn-based diet** without (NC1) or with XB (NC1+E) treatments. Genera found to be significantly associated with each treatment are shown in filled circles (NC1+E) and filled triangles (NC1), while non-significant genera are shown in filled squares. Some taxa could only be classified up to family (F), order (O), class (C), or phylum (P) level.

Figure 4. Partial least square discriminant analysis (PLS-DA) loading scatter plot based on the relative abundance of the putative bacterial genera in the **cecum digesta** of 21 d old broiler chickens and their association with **wheat grains –based diet** without (NC2) or with XB (NC2+E) treatments. Genera found to be significantly associated with each treatment are shown in filled circles (NC2+E) and filled triangles (NC2), while non-significant genera are shown in filled squares. Some taxa could only be classified up to family (F), order (O), class (C), or phylum (P) level.

Conclusions

- Microbial community diversity and composition at the phylum level were not influenced by enzyme supplementation, however, significant differences were observed at lower taxonomical levels.

- We observed specific taxa that were associated with corn or wheat-based treatments without or with XB. Some bacteria are ubiquitous and general beneficial components of the gastrointestinal microbiota, however, some of the bacteria have the potential for both positive and negative influence on the host based on the species or the specific strains involved as well as on the local conditions in the gut.

- Our data provided significant insight on ileal and cecal microbial profile associated with the dietary types and XB. Further investigations on the functional roles of these taxa will enhance our understanding on this topic.

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