Session 20.
Functional feed additives in poultry nutrition
Room: Modicana - Palazzo Affari 3rd Floor
Sunday 1 September

NaturStome

Naturstome

Replacing Vitamin E in Broiler Diets with Polyphenolic-Rich Greek Vinification By-Products

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ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

H.F.R.I. Hellenic Foundation for Research & Innovation

Greece 2.0 NATIONAL RECOVERY AND RESILIENCE PLAN

Funded by the European Union NextGenerationEU

Introduction

89 Mtons
biomass waste
= 170 Mtons
CO_2 eq.

- **Grape** is the world's largest fruit crop, with an annual production exceeding 67 Mtons.

- **Mediterranean** basin area possess 62% of global wine production which results in the production of high **by-products** amounts (≈20% of the entire grapes' biomass) in winery industry.

- Wine by-products are **rich in polyphenols and flavonoids** like procyanidin B1 and B2, gallic acid, caftaric acid, catechin, quercetin, and resveratrol.

- Many studies demonstrating the powerful antioxidant properties of these polyphenols and their ability to act as potent free radical scavengers.

Specific need

01

The use of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxytoluene (BHT) has been linked with possible toxicity (liver damage and carcinogenesis in animal studies).

02

Other studies demonstrate that the dietary supplementation with synthetic vitamin E significantly increased the risk of prostate cancer among healthy men.

03

The bacteria selection mechanisms towards antibiotic resistance led to their ban as GP in livestock, thus there is an intense searching for alternative natural solutions to promote growth performance and enhance host health.

Stage of Conceptualization

1

Sustainable poultry production thought upcycling biowastes.

2

Constructing biorefineries
-
Prototyping of natural feed additives.

3

Polyphenols act as ROS Scavengers reducing the dependence on synthetic Vit. E.

4

B-glucans can serve as prebiotics improving the gut health.

Previous results

Previously, we individually evaluated the response of three winery by-products: ground grape pomace (**GGP**), wine lees extract rich in yeast cell walls (**WYC**), and grape stem extracts rich in polyphenols (**PE**) in broilers chicken diets (supplementary to vitamin E).

Limitations:

- The level of grape pomace having substantial amount of antioxidants was not appropriate to supplemented in broilers diet due to high content of fibers.
- The wine lees exposed positive outcomes possibly due to b-glucans but was poor in terms of antioxidant compounds.
- The stems were an unsustainable source of antioxidants due to persistence extractions protocols and low yield.

Thus, the current project aims to investigate the effect of the partial or complete substitution of synthetic Vitamin E by exploiting different biorefineries of winery by-products in broilers diet.

?

Natural
Vs
Synthetic

Biorefinery of Polyphenols

Extraction of polyphenols

Grape pomace from native Vitis vinifera variety 'Assyrtiko', grown in the island of Santorini, were dried naturally in a dark room.

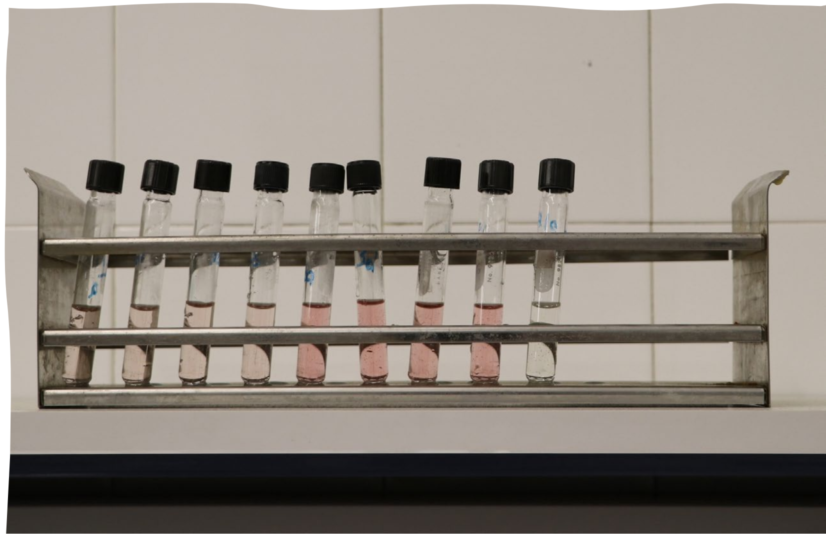
Grape pomace were ground in a hammermill and the powder was consequently extracted in an Ultra Sonic bath.

Wine lees biorefinery

Beta glucan determination process on wine lees final product

B-Glucans content
5.10%
8.90%
7.00%
23.00%

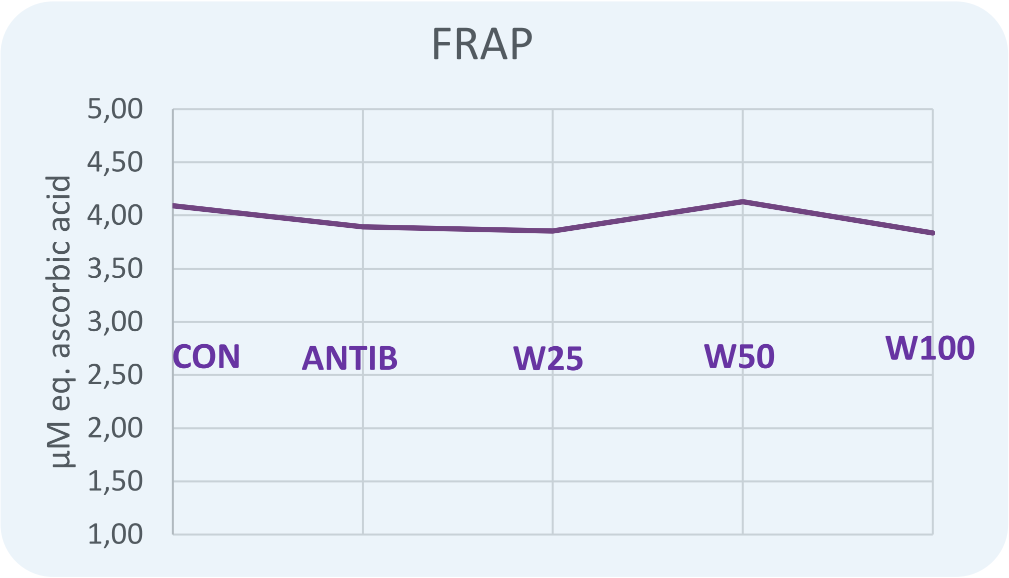
Crete island long fermentation of wines higher b-glucans content

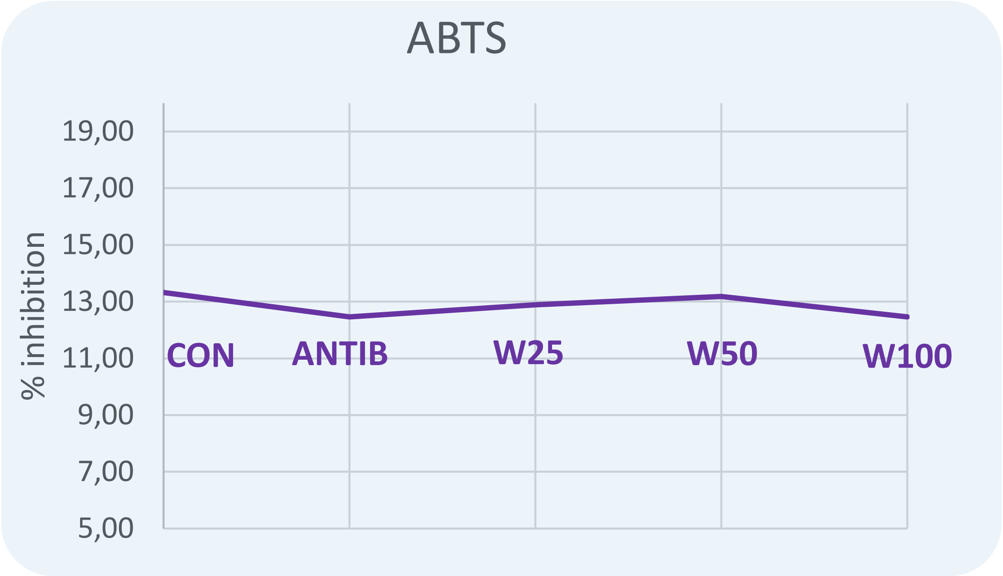


Balancing Feed Antioxidants

Total Polyphenols Content as Gallic Acid Eq. to standardized grape pomace extract

Determine inclusion level





Experimental design

- **200** Ross 308 broilers, 1 day old
- **5 experimental treatments** (4 pens/group),
- **35 days**, 3 feeding phases: 0-10 starter, 11-24 growing, 25-35 finishing

CON

Basal diet (maize and soyabean meal) based on VitE requirements, Control

ANTIB

Antibiotic (55mg/kg zinc bacitracin)

25, 50, and 100% substitution of synthetic VitE

W25

25 mg total polyphenols/kg feed, and 0.14% wine lees (150mg b-glucans)

W50

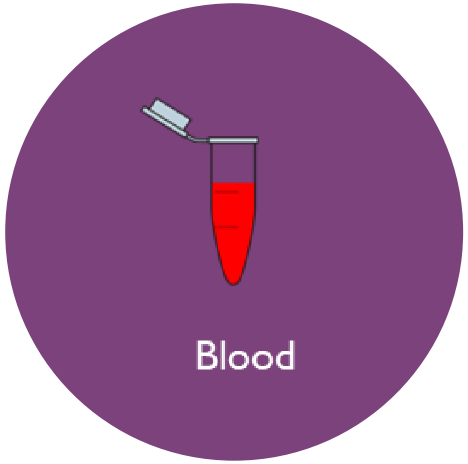
50 mg total polyphenols/kg feed, and 0.14% wine lees (150mg b-glucans)

W100

100 mg total polyphenols/kg feed, and 0.14% wine lees (150mg b-glucans)

Experimental analysis

At the age of 35 days, 40 broilers (8 per treatment and 2 per replicate pen) were randomly selected and sacrificed



Polyphenols encapsulation, extraction of wine lees and beta glucan determination process

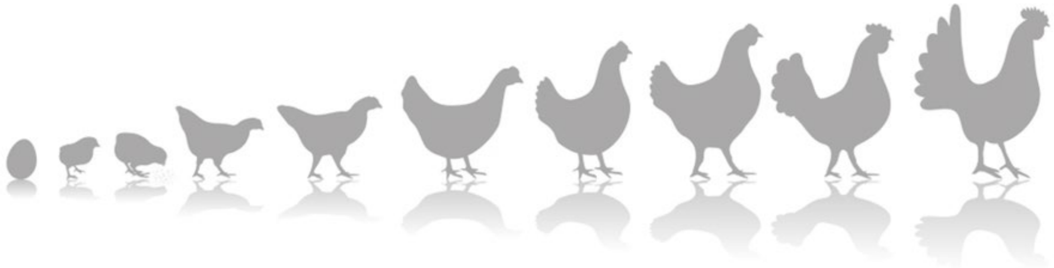
Blood biochemical analysis and oxidative status (enzymes, total antioxidant activity, oxidative stress biomarkers)

Breast and thigh muscle (carcass quality parameters, MDA, total antioxidant activity, fatty acids determination)

Immune-oxidative genes expression (RNA extraction in liver, spleen, bursa of Fabricius)

Results

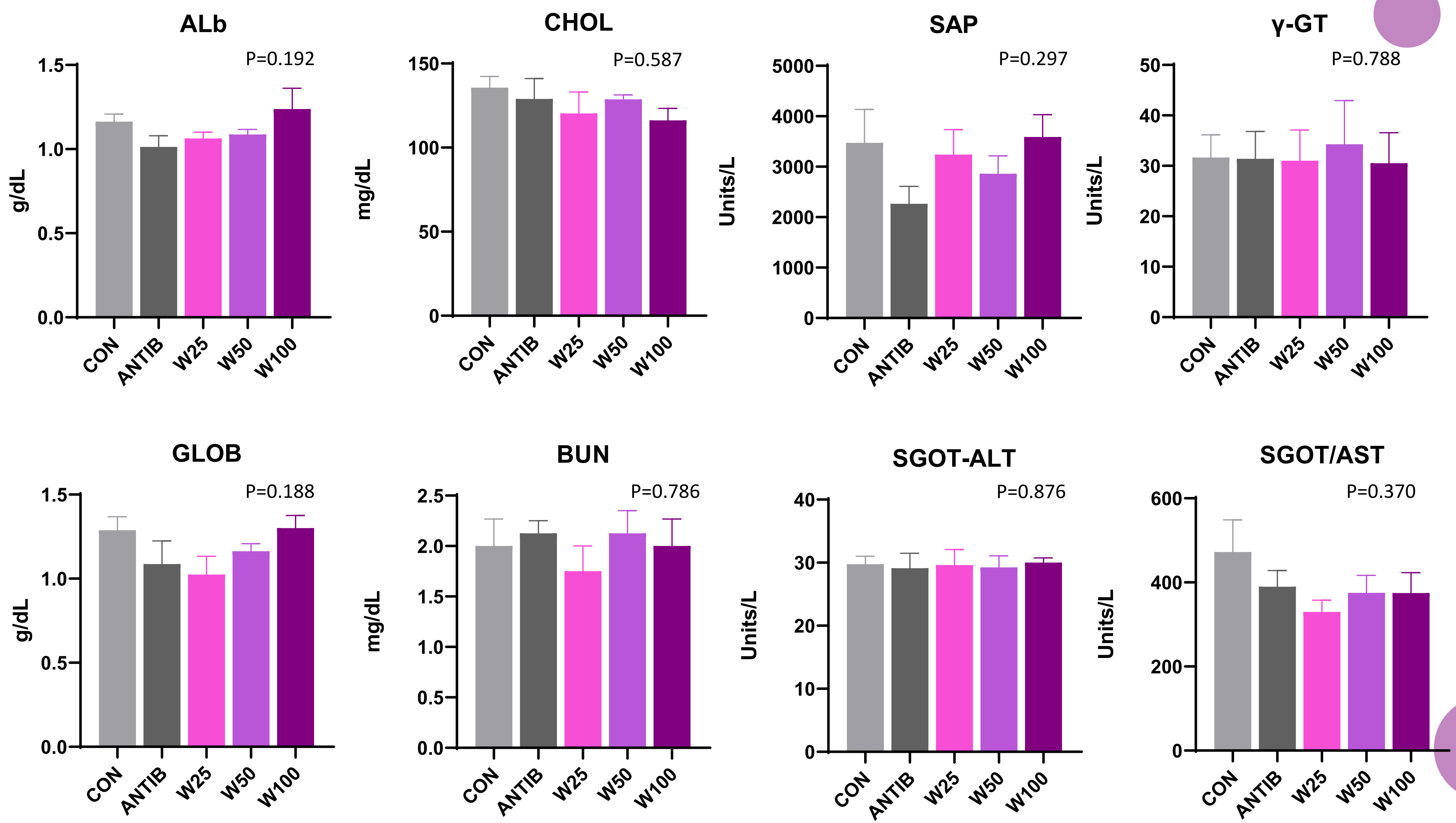
Productivity traits



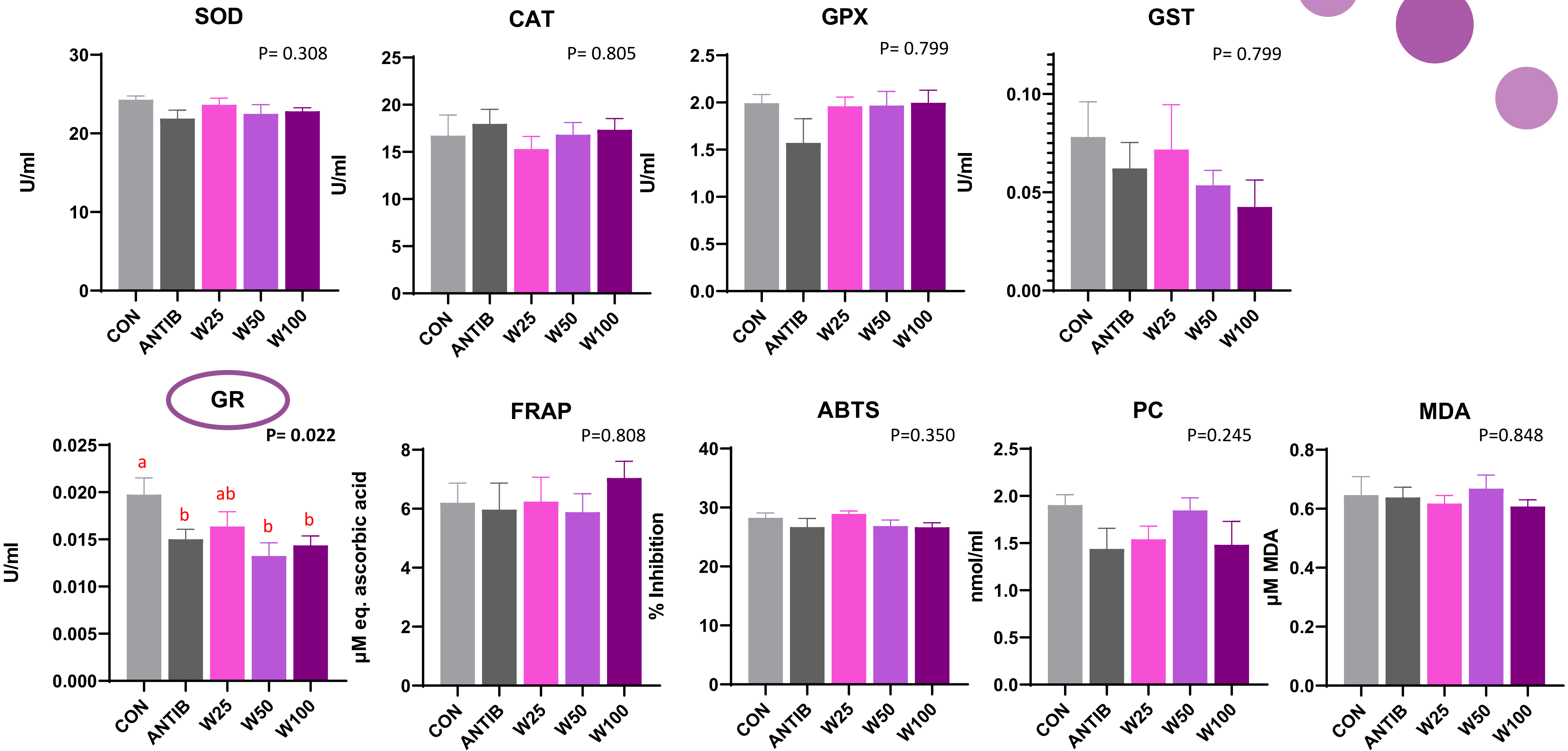
	CON	ANTIB	W25	W50	W100	SEM	PVALUE
Initial BW (g)	43.8	44.0	43.6	43.6	43.6	0.106	0.793
BW starter (g)	304.1	311.0	305.6	306.6	301.7	2.043	0.730
BW grower (g)	**1413^{ab}**	**1445^{a}**	**1381^{b}**	**1429^{ab}**	**1392^{b}**	**8.512**	**0.088**
BW finisher (g)	**2762^{a}**	**2727^{a}**	**2565^{b}**	**2696^{ab}**	**2646^{ab}**	**23.769**	**0.056**
AFI starter (g)	319.2	319.6	310.8	316.1	316.9	2.111	0.731
AFI grower (g)	1386	1356	1352	1346	1347	6.226	0.233
AFI finisher (g)	**1934^{a}**	**1812^{ab}**	**1756^{b}**	**1849^{ab}**	**1822^{ab}**	**21.162**	**0.087**
AFI (0 to 35 days) (g)	**3640^{a}**	**3488^{b}**	**3419^{b}**	**3511^{b}**	**3486^{b}**	**23.142**	**0.021**
FCR (0 to 35 days) (g)	1.34	1.30	1.36	1.32	1.34	0.007	0.109

Blood analysis

Blood biomarkers

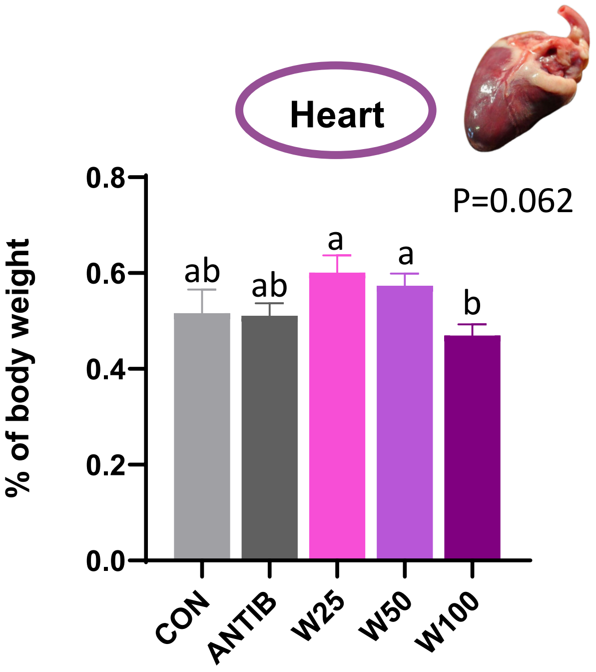


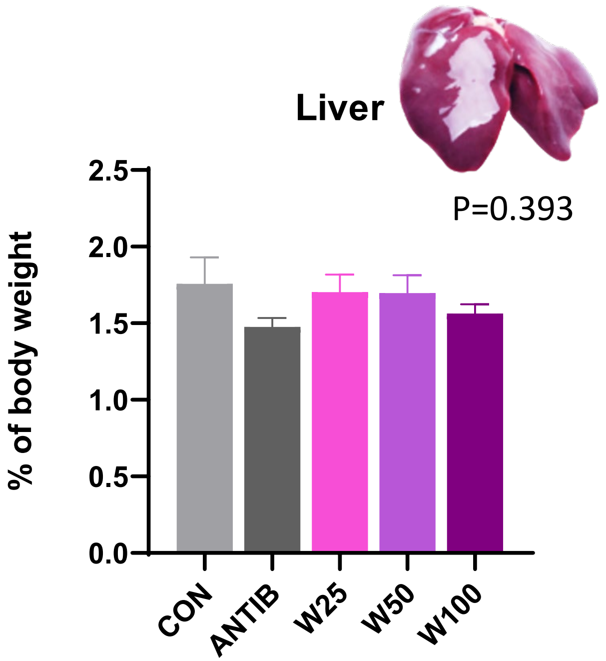
Blood antioxidant analysis

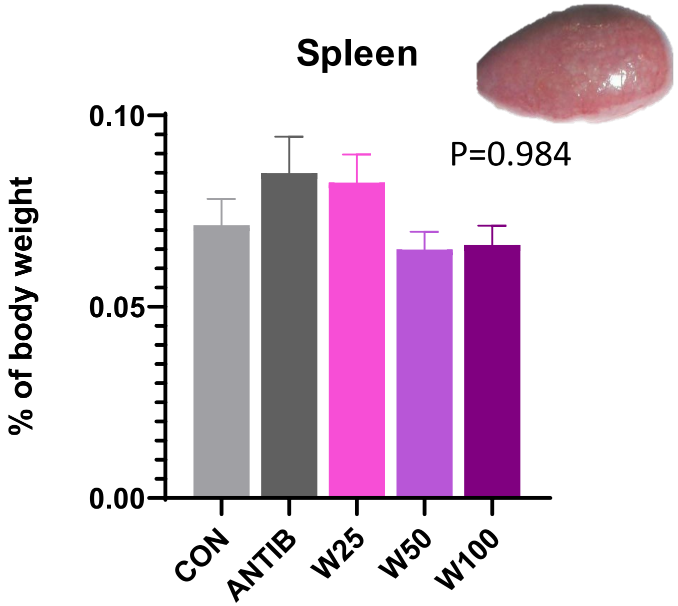


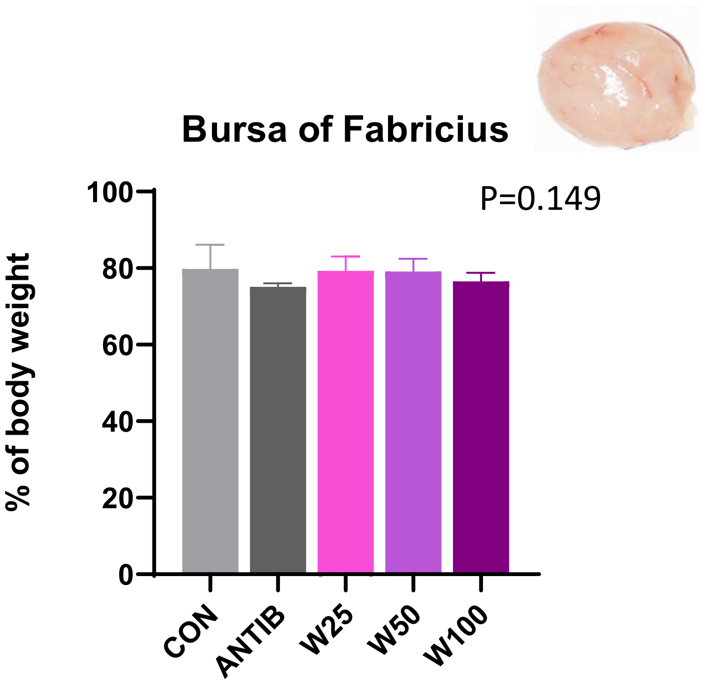
Carcass traits

Organ Relative Weights



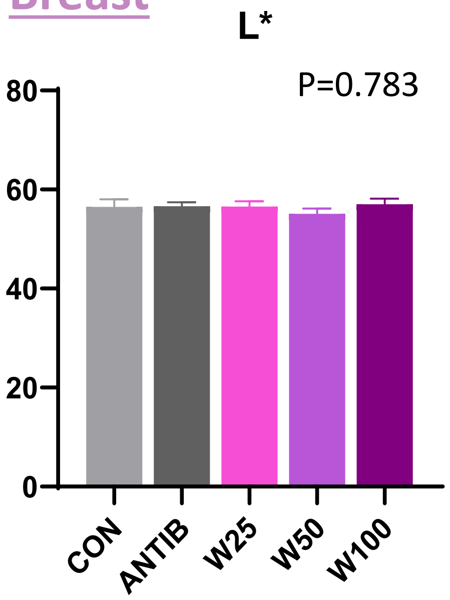


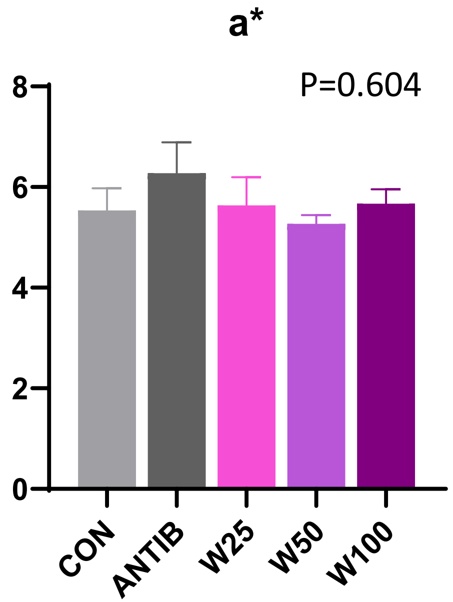


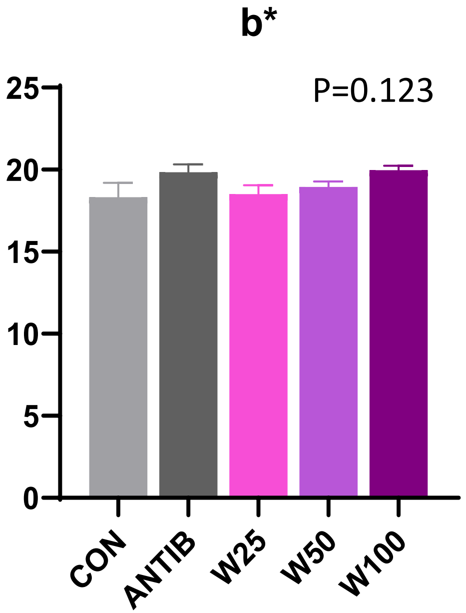


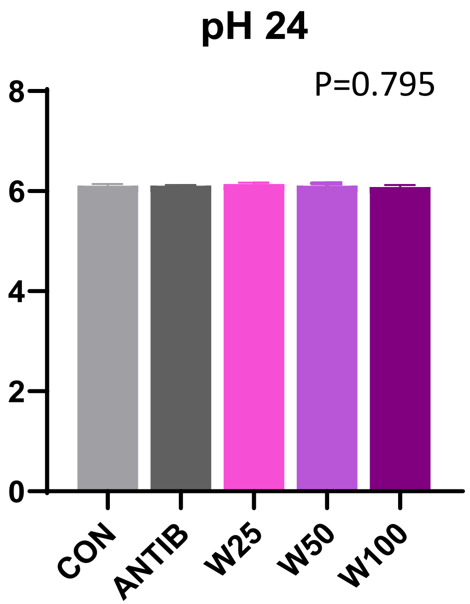
Quality parameters (1)

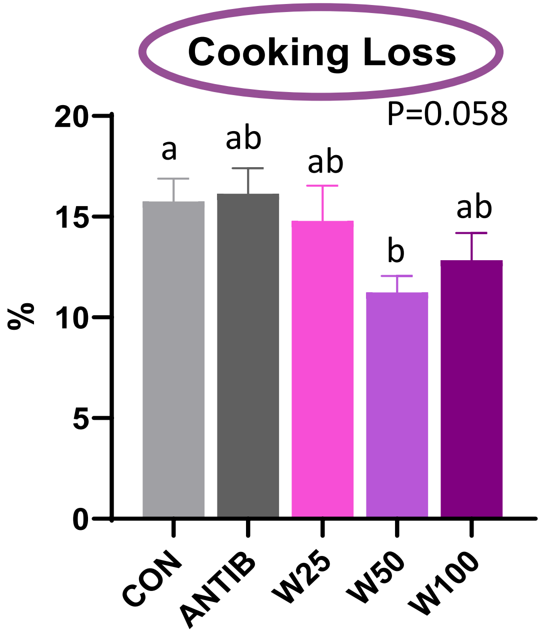
Breast

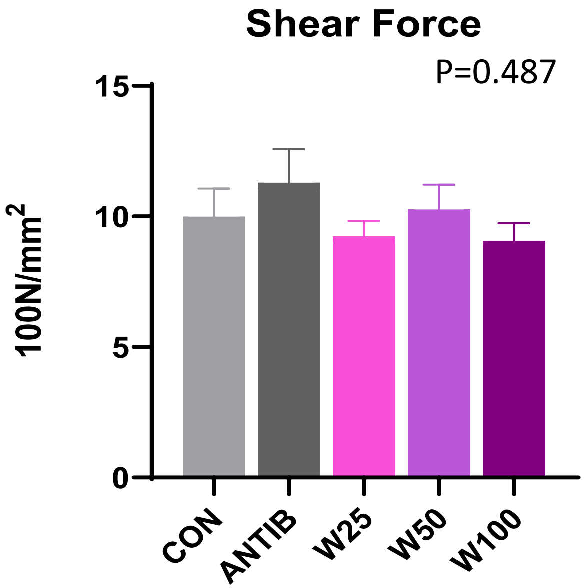






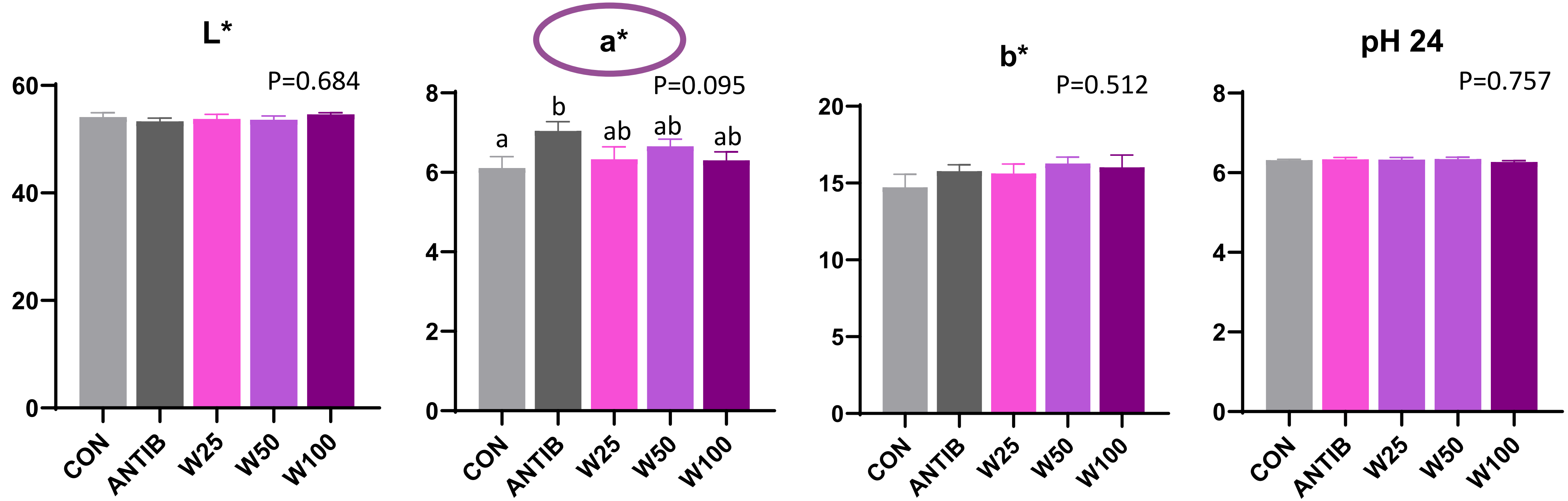






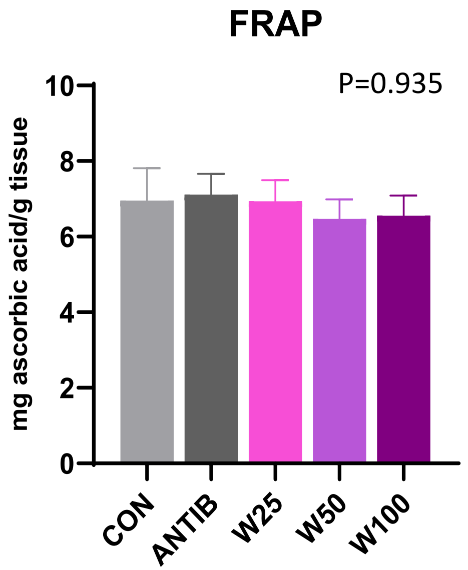
Quality parameters (2)

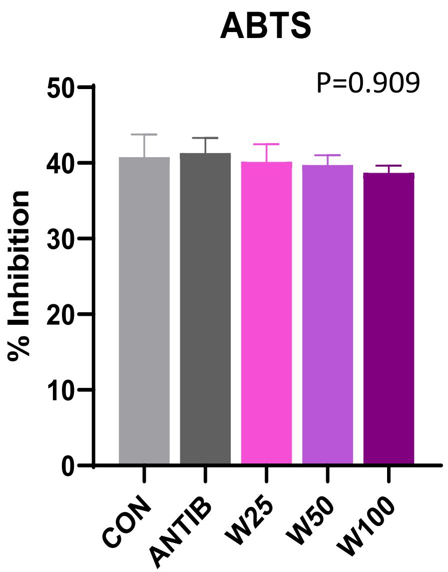
Thigh muscle

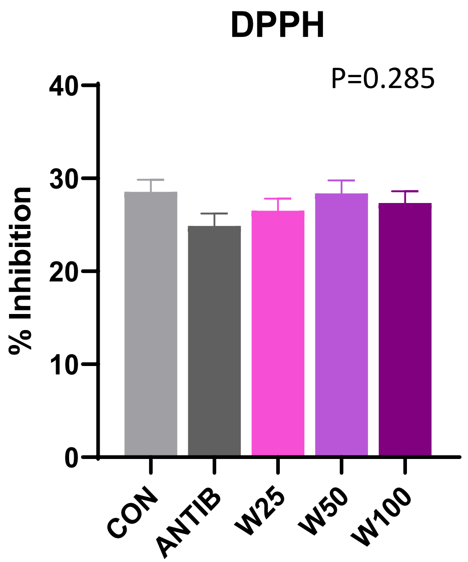


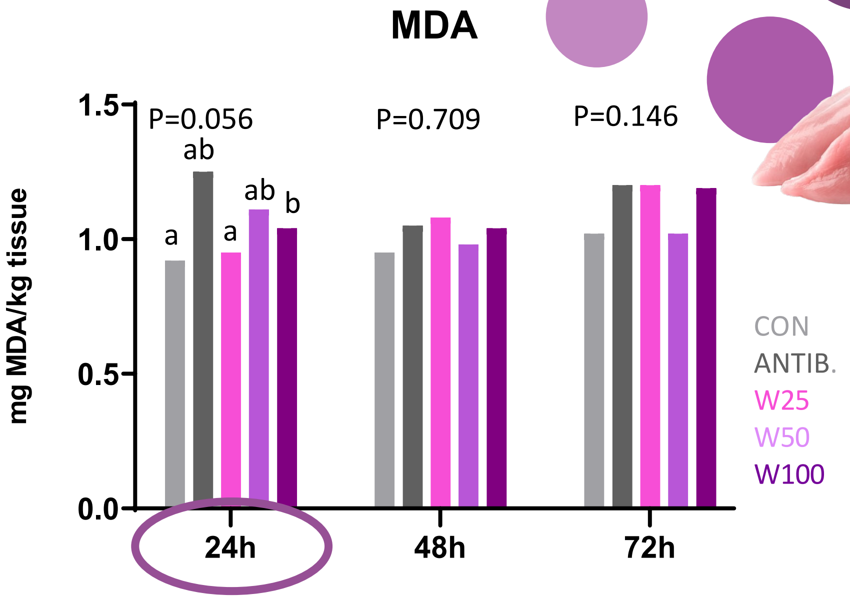
Antioxidant activity

Breast

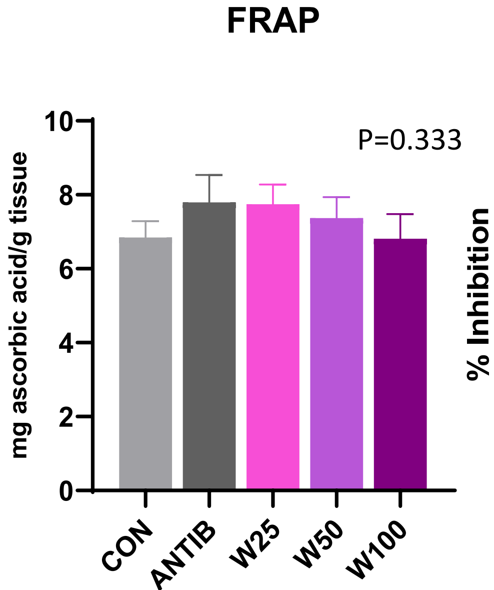


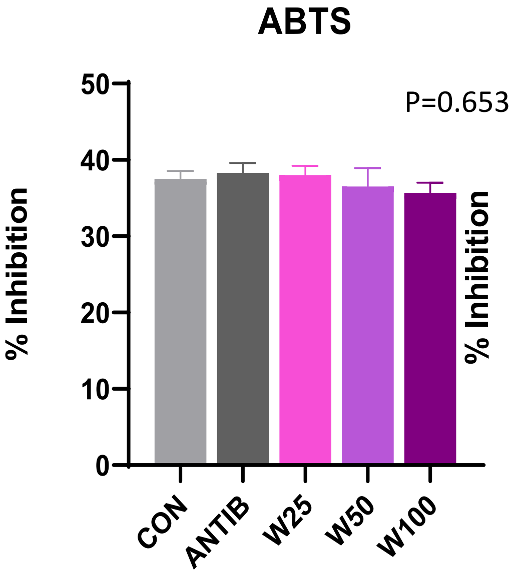


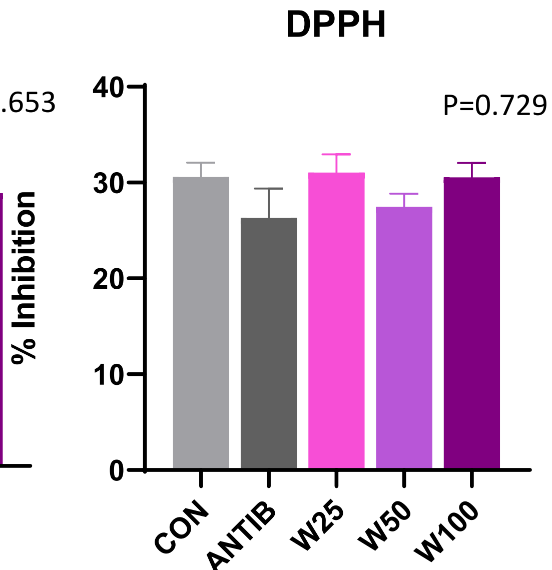


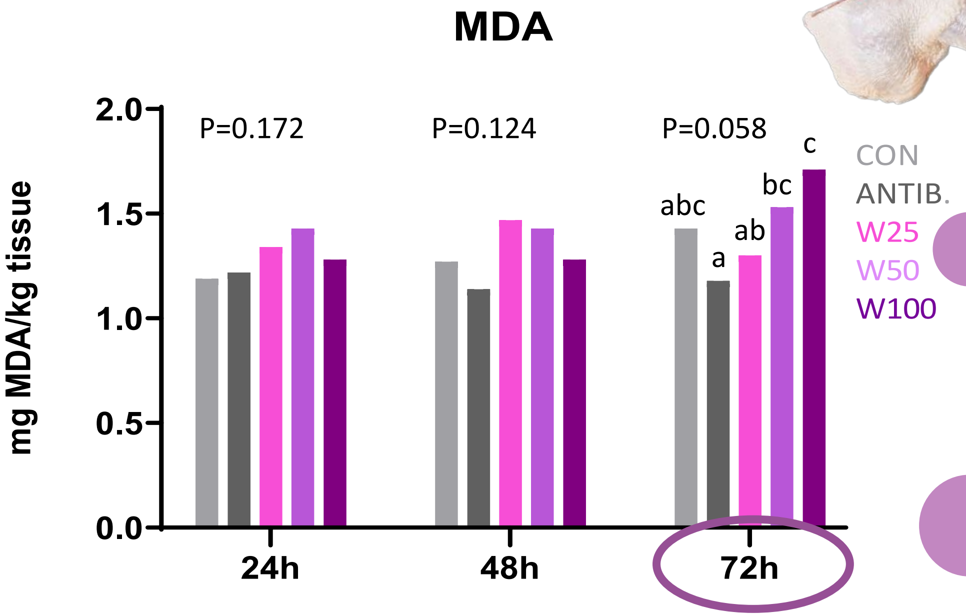


Thigh muscle



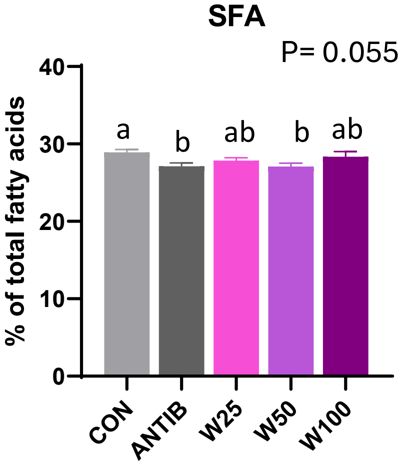


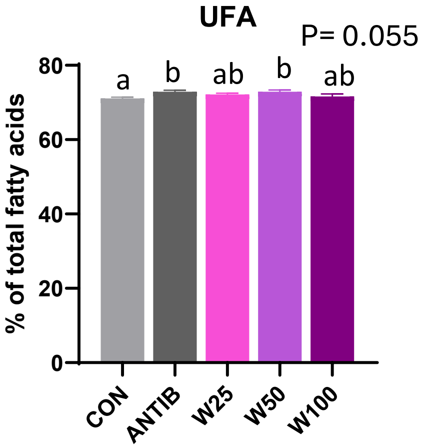


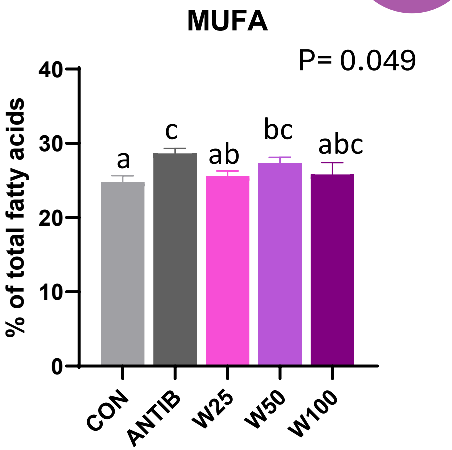


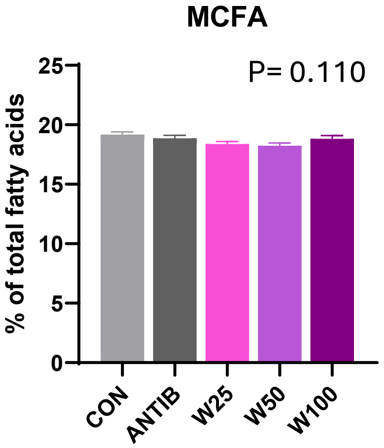
Fatty acids (1)

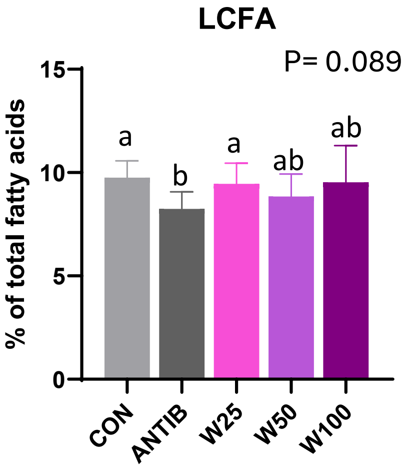
Breast

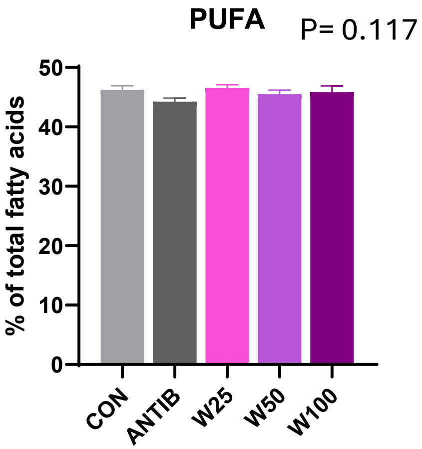


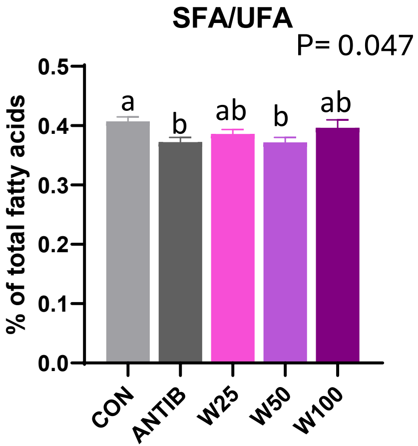


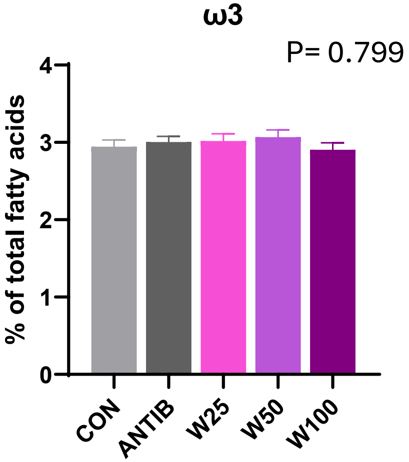


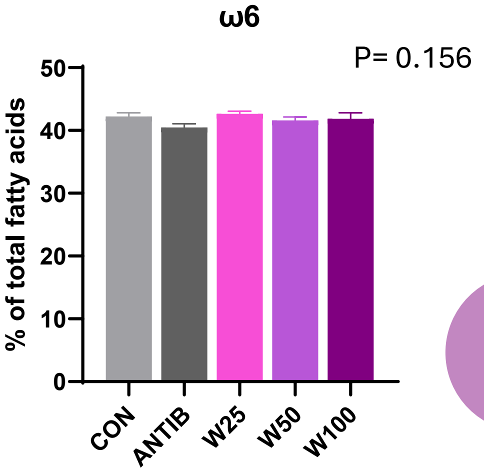






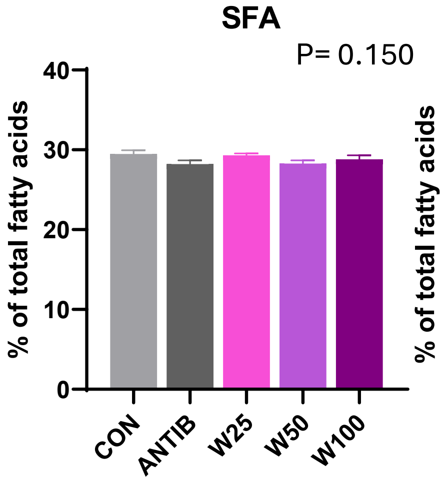


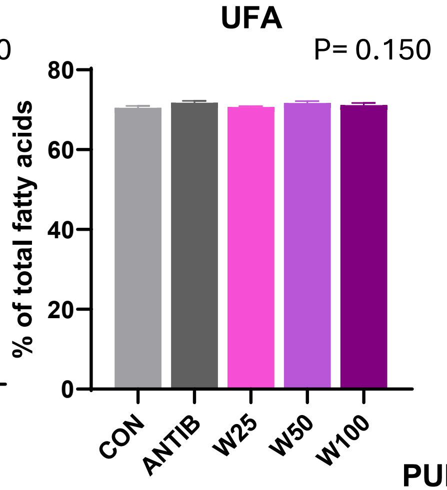


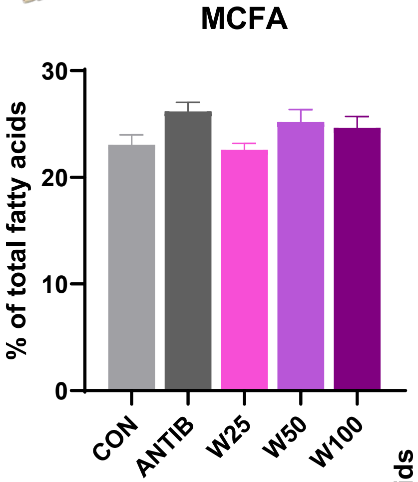


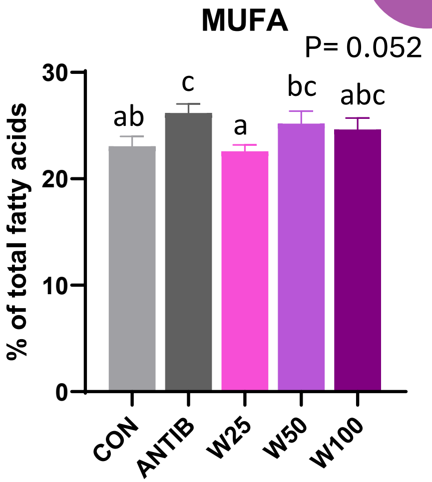
Fatty acids (2)

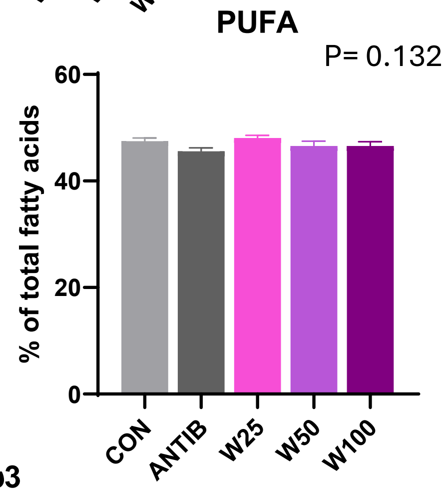
Thigh muscle

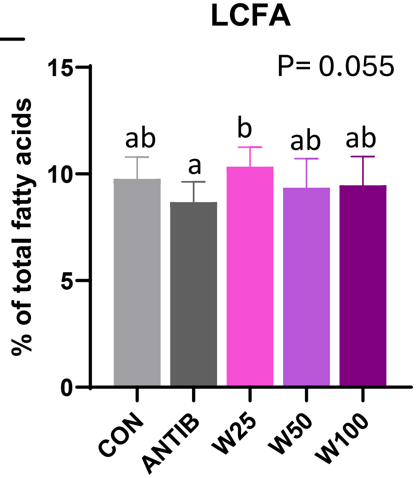


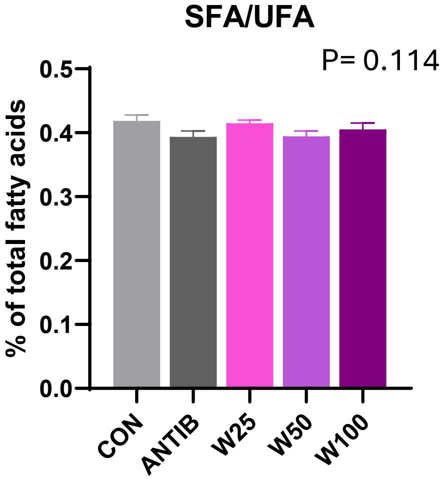


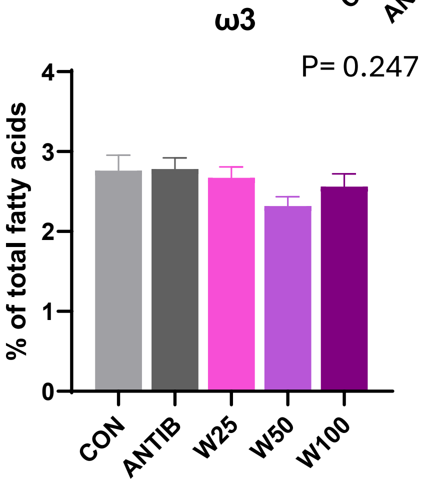


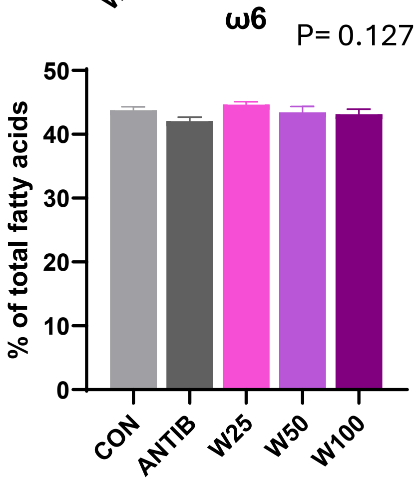






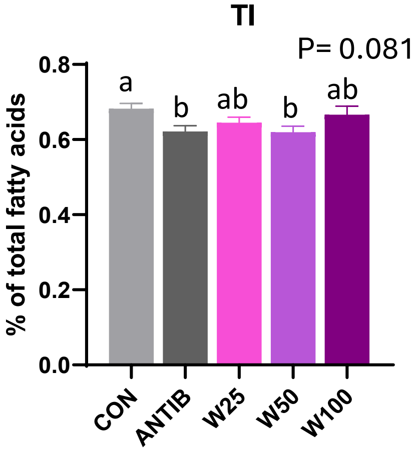


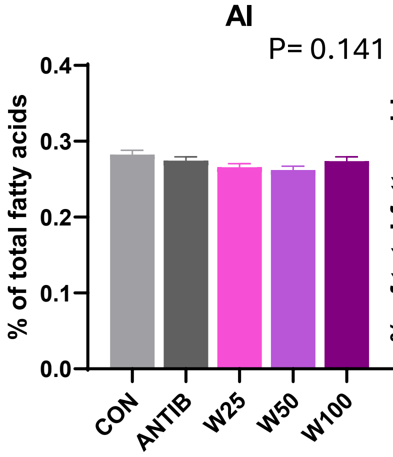


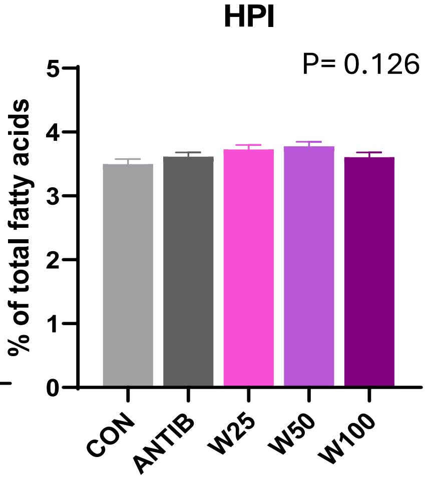


Health indexes

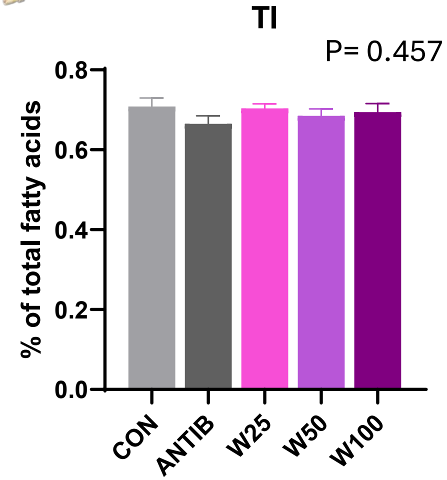
Breast

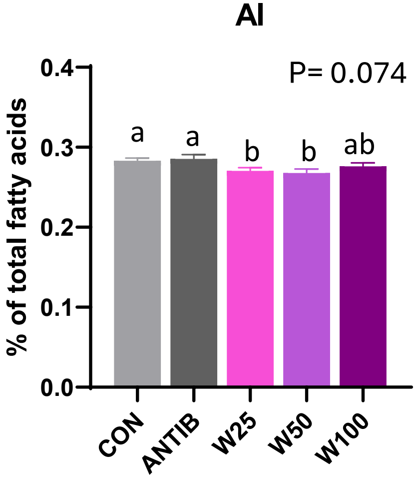


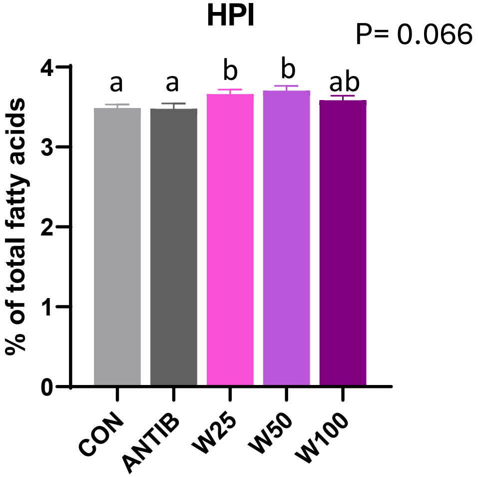




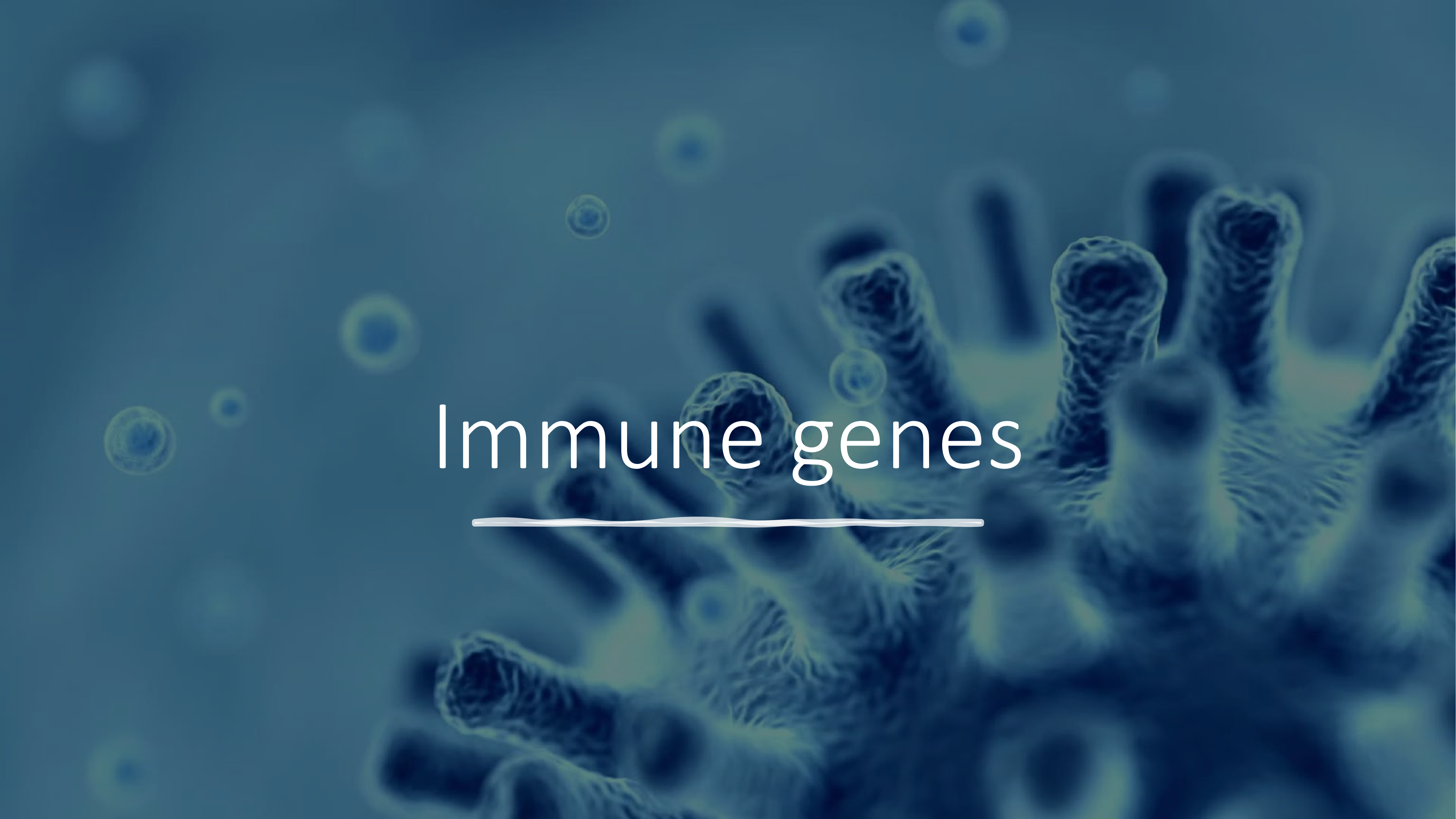
Thigh muscle





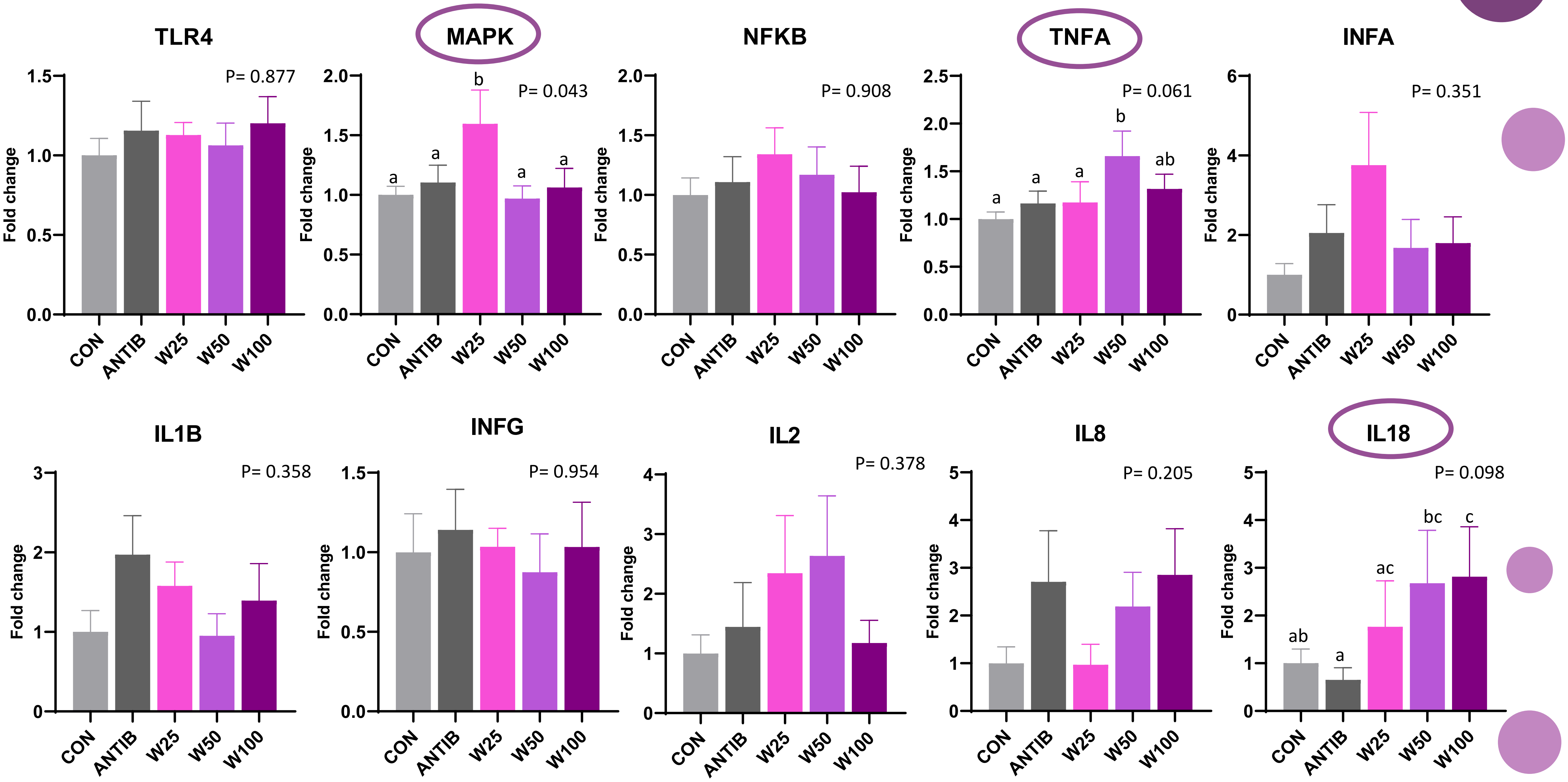


Immune-oxidative genes expression



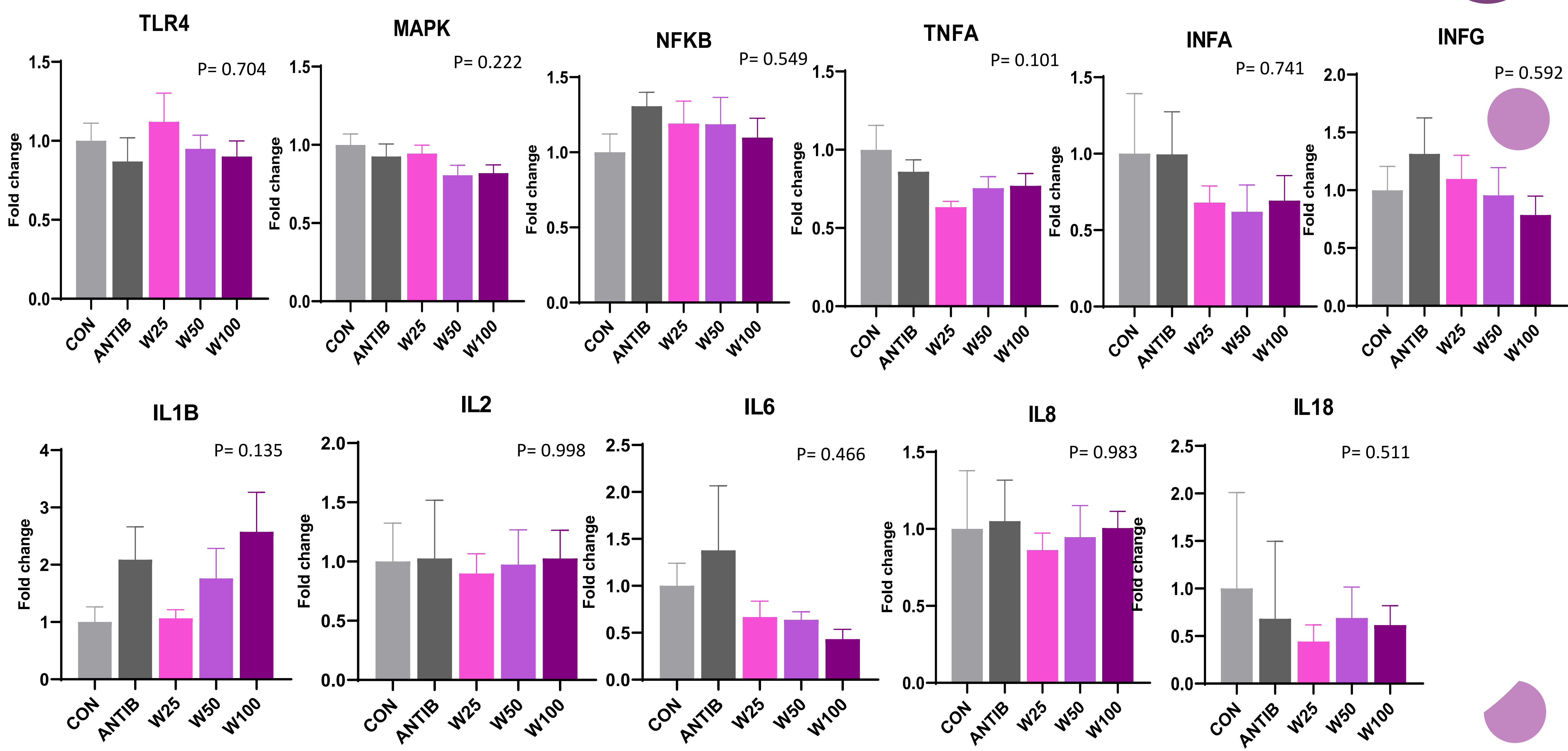
Immune genes

Liver

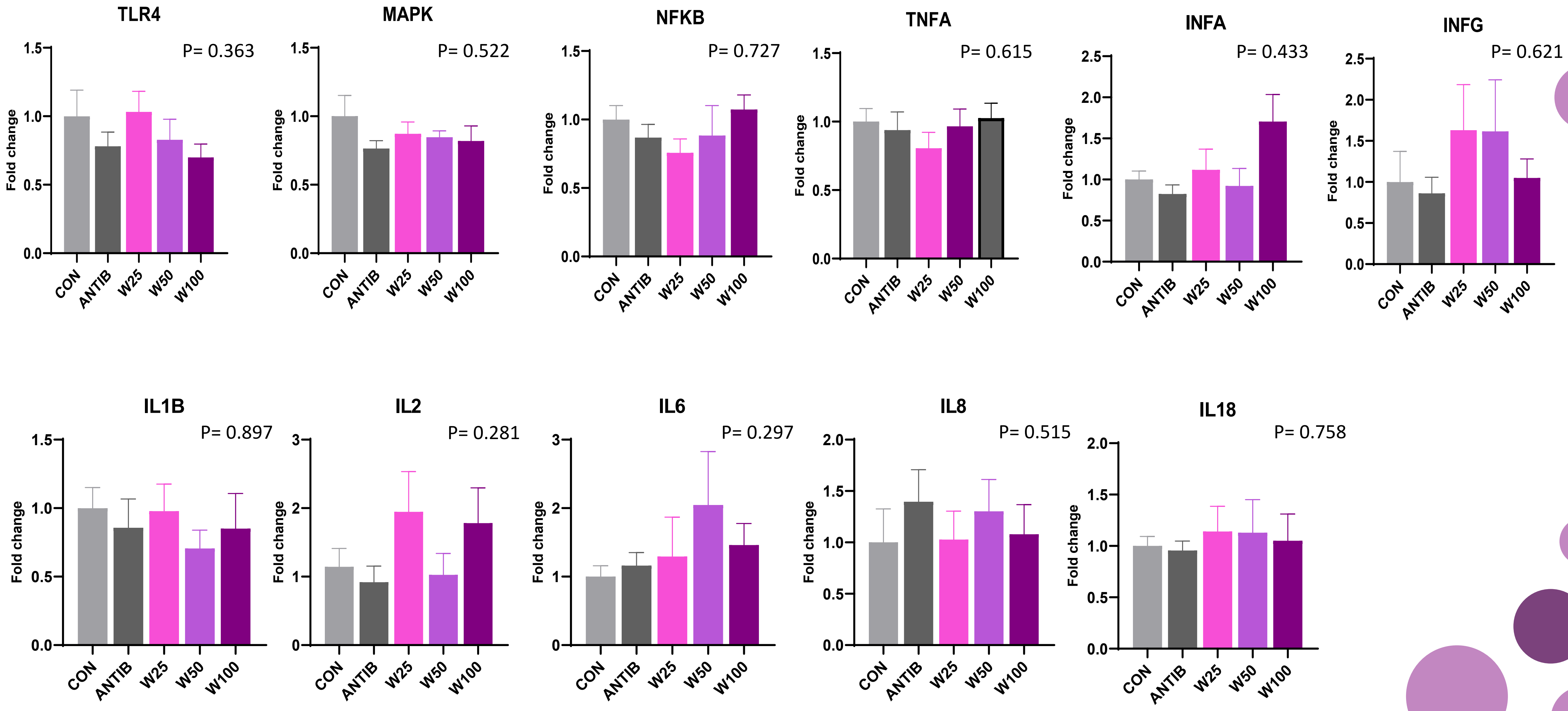


TLR4
P= 0.877
MAPK
P= 0.043
NFKB
P= 0.908
TNFA
P= 0.061
INFA
P= 0.351
IL1B
P= 0.358
INFG
P= 0.954
IL2
P= 0.378
IL8
P= 0.205
IL18
P= 0.098
Fold change
CON
ANTIB
W25
W50
W100
a
b
ab
ac
bc
c

Spleen

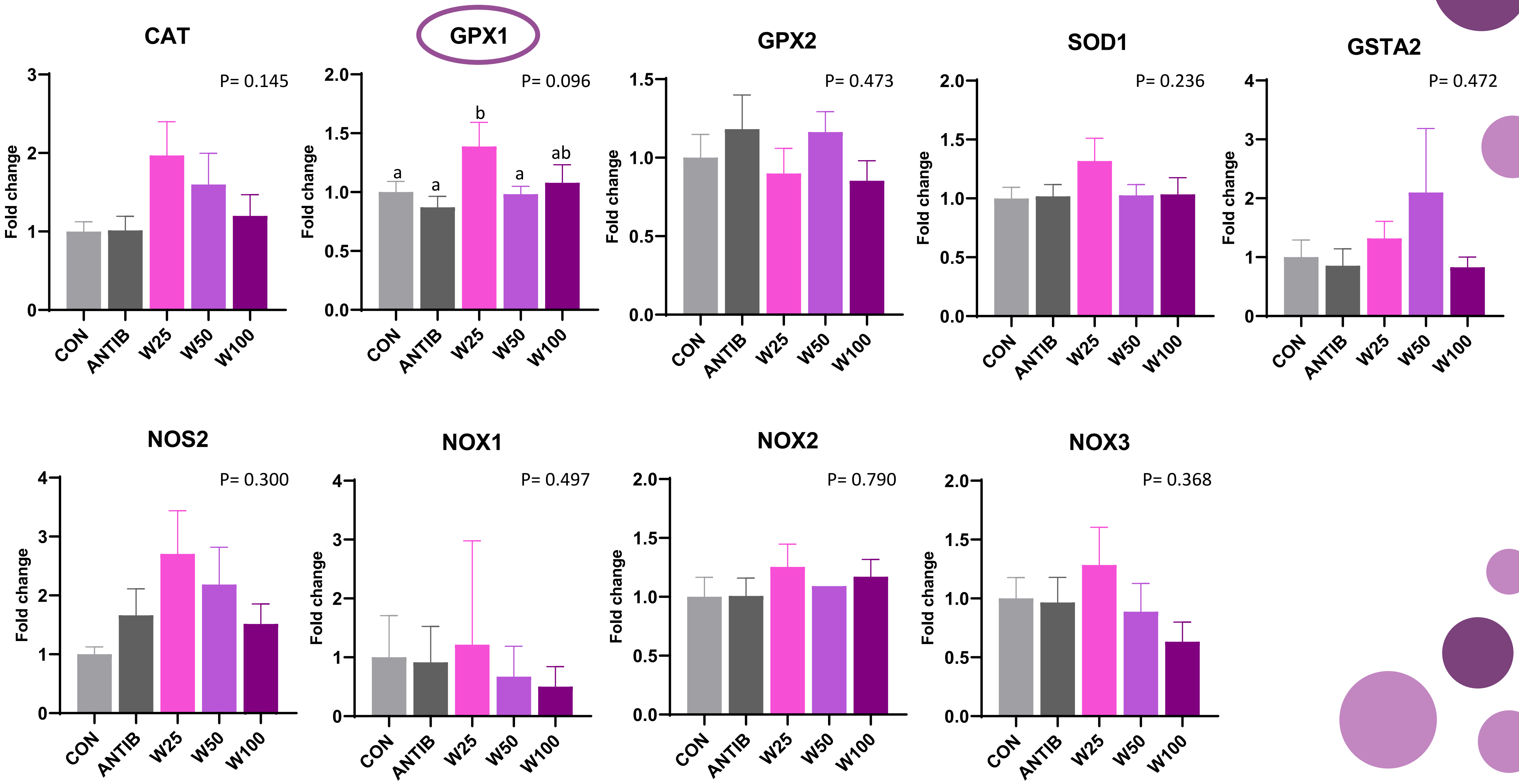

TLR4
P= 0.704
MAPK
P= 0.222
NFKB
P= 0.549
TNFA
P= 0.101
INFA
P= 0.741
INFG
P= 0.592
IL1B
P= 0.135
IL2
P= 0.998
IL6
P= 0.466
IL8
P= 0.983
IL18
P= 0.511
Fold change
CON
ANTIB
W25
W50
W100

Bursa of Fabricius



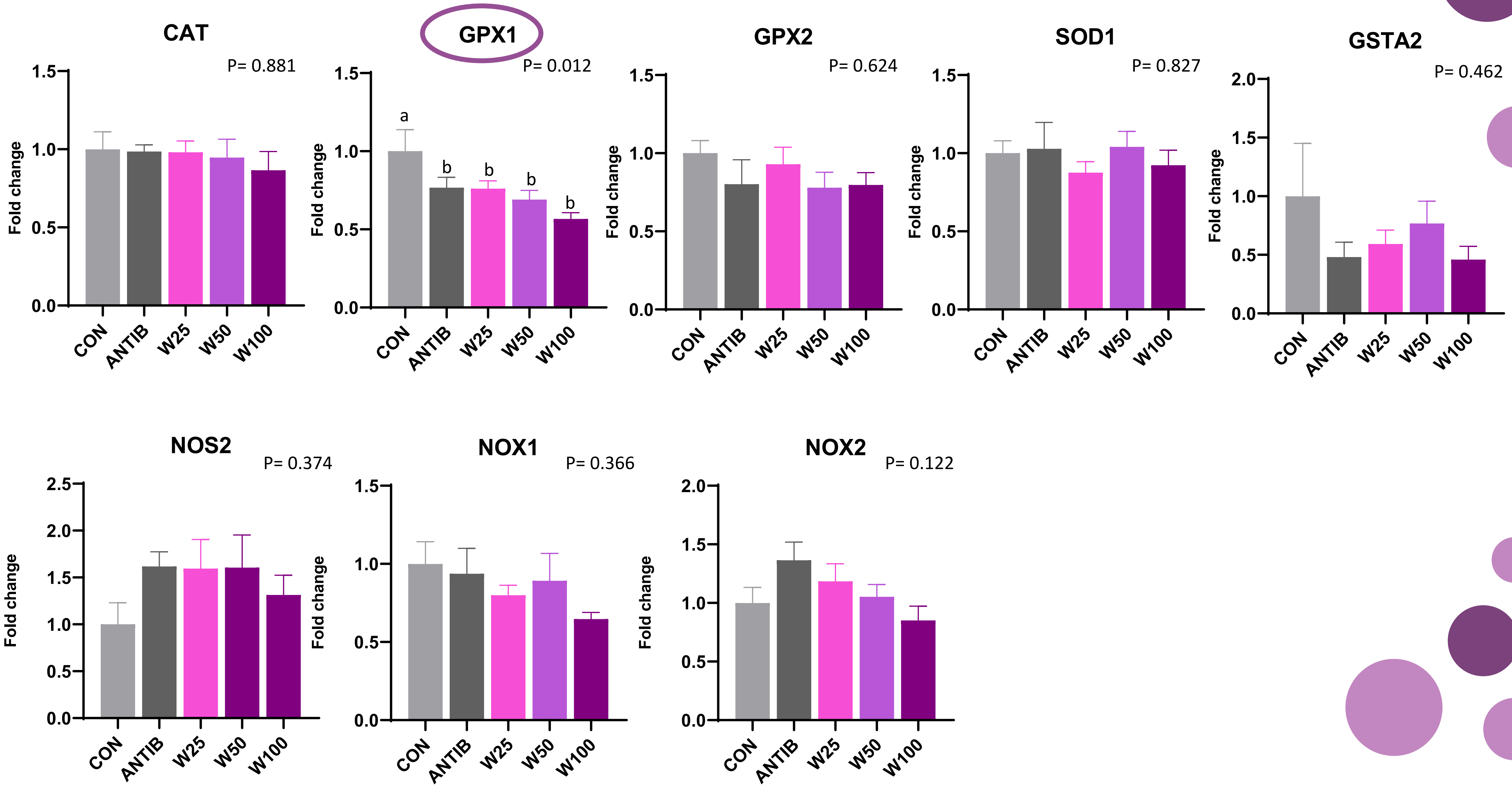
Oxidative genes

Liver



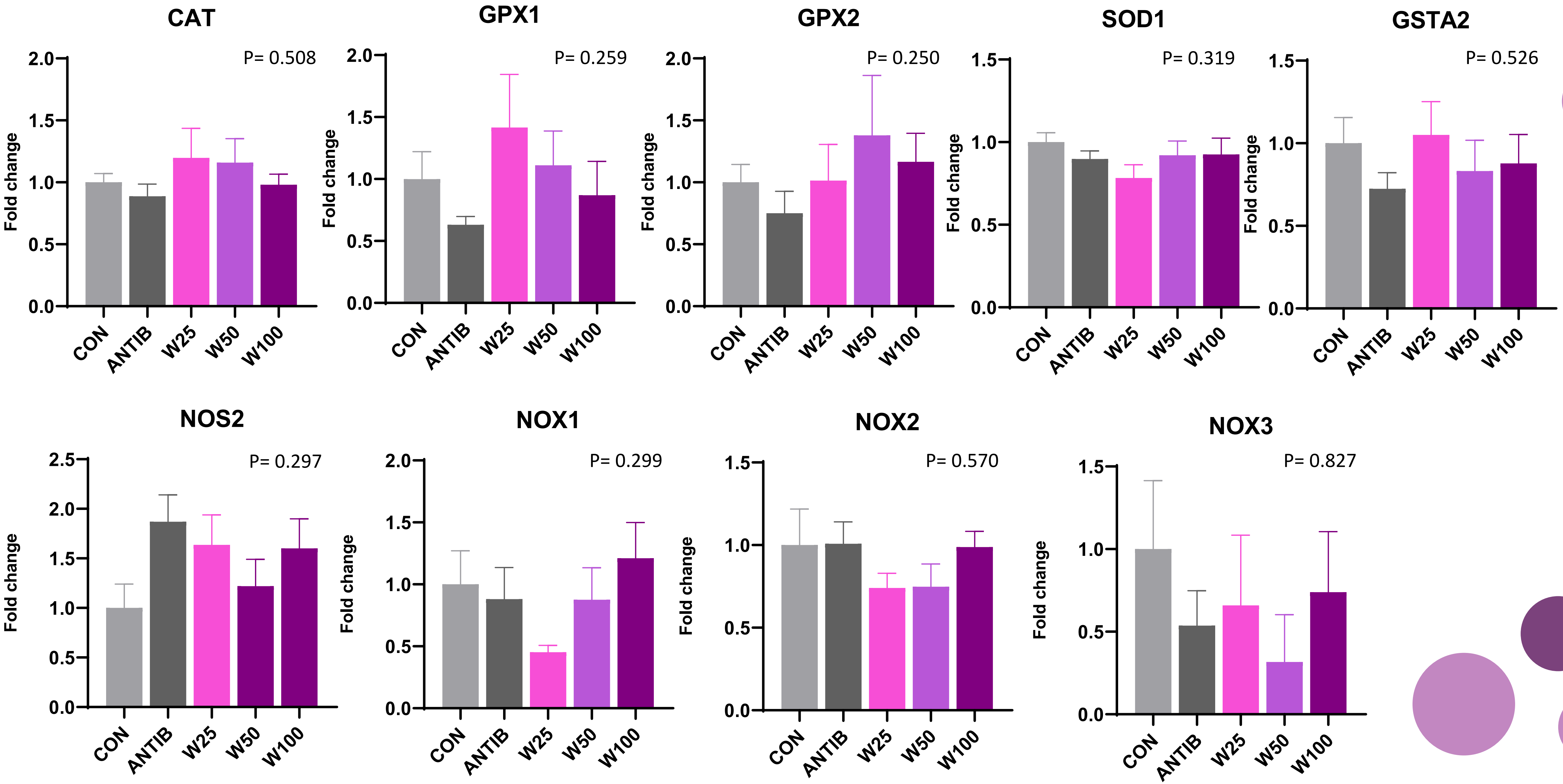
CAT
P= 0.145
GPX1
P= 0.096
a
a
b
a
ab
GPX2
P= 0.473
SOD1
P= 0.236
GSTA2
P= 0.472
NOS2
P= 0.300
NOX1
P= 0.497
NOX2
P= 0.790
NOX3
P= 0.368
Fold change
CON
ANTIB
W25
W50
W100

Spleen



CAT
P= 0.881
GPX1
P= 0.012
GPX2
P= 0.624
SOD1
P= 0.827
GSTA2
P= 0.462
NOS2
P= 0.374
NOX1
P= 0.366
NOX2
P= 0.122
Fold change
0.0
0.5
1.0
1.5
2.0
2.5
a
b
CON
ANTIB
W25
W50
W100

Bursa of Fabricius



Conclusions

01

-Complete substitution of synthetic vitamin E by winery by-products did not affect broilers productivity.
-Hematological parameters, blood health indices, and carcass traits were not substantially affected by partial and complete substitution of vitamin E.
-Inclusion of AGP did not further support broilers performances.

02

HOWEVER

-Transcriptional signatures of immune-oxidative indices revealed non-linear effect of vitamin E substitution indicating that 25mg of polyphenols were not enough to substitute by 25% the Vit. E requirements.

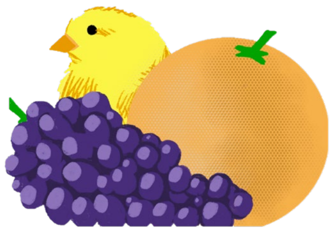
03

-Both 50 and 100 mg of polyphenols by grape pomace extract in combination with 150 mg b-glucans from wine lees assessed as rational strategies to substitute by 50 and 100% vitamin E, respectively, in broilers diet.

04

Recommendation

Considering the results as a whole, the 50% substitution of Vitamin E is more sustainable.



Next steps

30%
COMPLETED

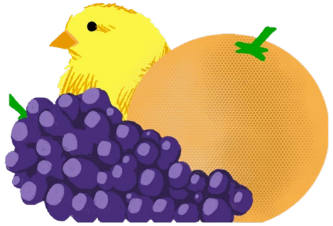
Stage 1
Screening with targeted and untargeted tools the polyphenols presented in winery by-products.

Stage 2
Assess the transfer efficiency of the polyphenolic compounds into the edible meat muscles of chickens.

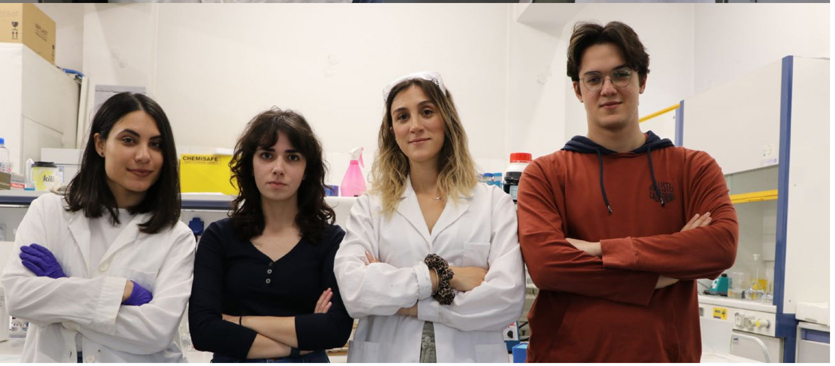
Stage 3
Monitor changes in the gut microbiome structure in ceca using Whole Genome Sequencing. The omics analytical tools will aim to assess the changes in ceca microflora and provide insights for the gut resistome pool (ARG). This was the main reason why the treatment of antibiotics as GPs was enrolled.

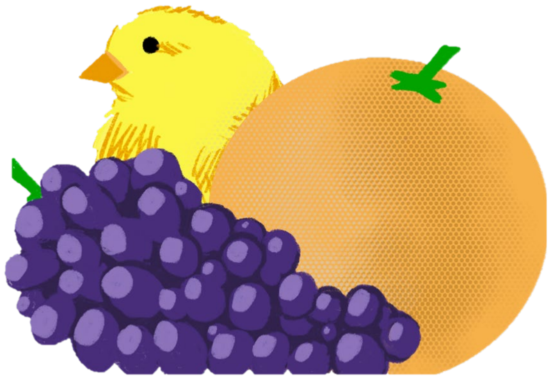
Stage 4
Monitor changes in the gut integrity including cytoprotective mechanisms lumen from duodenum, jejunum, ileum, and ceca using RNA-sequencing.

Stage 5
Combining winery by-products and citrus CPEO carotenoids extracts to substitute vitamin E.



Science Is A Team Sport!





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Greece 2.0 NATIONAL RECOVERY AND RESILIENCE PLAN

Funded by the European Union NextGenerationEU

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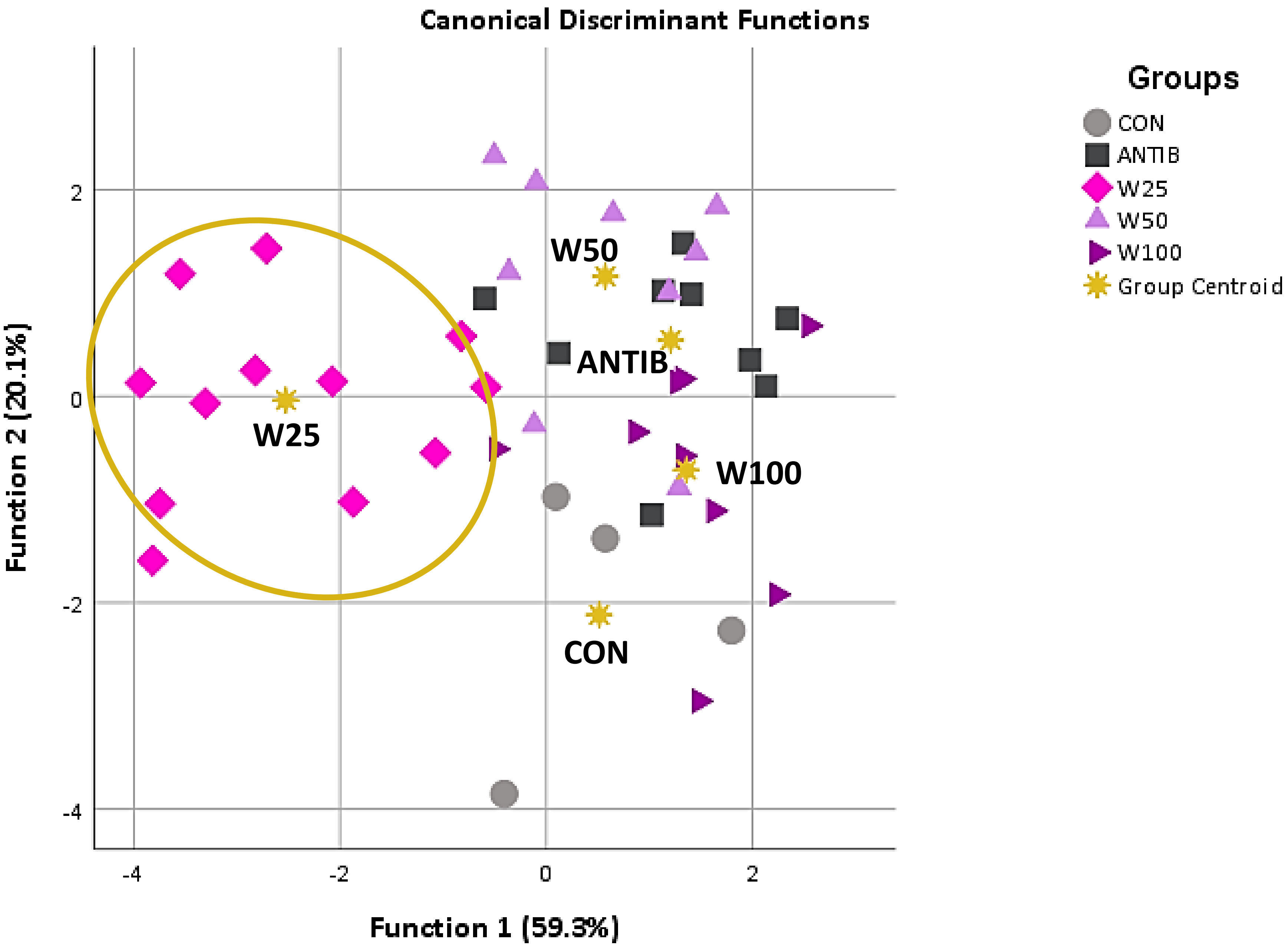

Discriminant analysis performed for pooled immune-oxidative genes expression data

Feeds fatty acids

	SFA	MCFA	LCFA	MUFA	PUFA	UFA	SFA/UFA	Ω3	Ω6	AI	TI	HPI
CON	16,12	12,48	3,64	24,47	59,41	83,88	0,19	5,04	54,00	0,15	0,29	6,55
ANTIB	16,16	12,46	3,71	24,89	58,94	83,84	0,19	4,99	53,54	0,15	0,29	6,55
W25	15,95	12,33	3,63	24,44	59,61	84,05	0,19	5,12	54,09	0,15	0,29	6,64
W50	14,51	12,59	1,92	24,89	60,61	85,49	0,17	5,14	55,05	0,15	0,26	6,62
W100	16,01	12,34	3,67	24,46	59,53	83,99	0,19	5,13	53,99	0,15	0,29	6,63