



南京农业大学消化道微生物研究室
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Flavonoids and other plant substances enhance the immunity of the animal



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Past concept of food:

- survival, hunger satisfaction, and absence of the classical nutrient deficiency diseases

New concept—Functional foods:

- better health and well-being
- reduce the risk of chronic illness and conditions such as cardiovascular diseases, cancers and obesity

Flavonoids as Functional Foods

Primary Actions:

- ◆ Antioxidation
- ◆ Immunomodulation
- ◆ Anti-inflammatory
- ◆ Antimicrobial

Secondary Actions:

- ◆ Anticancer effects
- ◆ Cardioprotective effects
- ◆ Gastrointestine protective effects
- ◆ Production performance

two large groups of flavonoids

Isoflavone (Soy flavonoid):

Occurrence: Soybeans and soy products

Example: Genistein

Flavanones (Citrus flavonoid):

Occurrence: Citrus fruits and tomatoes

Example: Hesperidin

Overview of flavonoids research

- ◆ Most studies have been performed **in vitro** using **high concentrations** compared with the amounts possibly present in animal tissues or the diet
- ◆ Some animal studies have also been performed, mostly on growth performance, the results varied between experiments and between animals

Objective

To elucidate the role and mechanisms of action of hesperidin (a member of citrus flavonoids) and genistein (a member of phytoestrogens) in immunity in vivo (broilers)

Exp. 1, Hesperidin, genistein, separately

Exp. 2, Hesperidin, genistein, individual and combined, +/- LPS challenge

Exp. 1, Treatments

Control 0mg flavonoid/kg feed

Hesperidin, 2.5, 5, 10, 20 mg/kg feed

Genistein, 2.5, 5, 10 mg/kg feed

Measurements

Growth performance, not affected

Immunity parameters

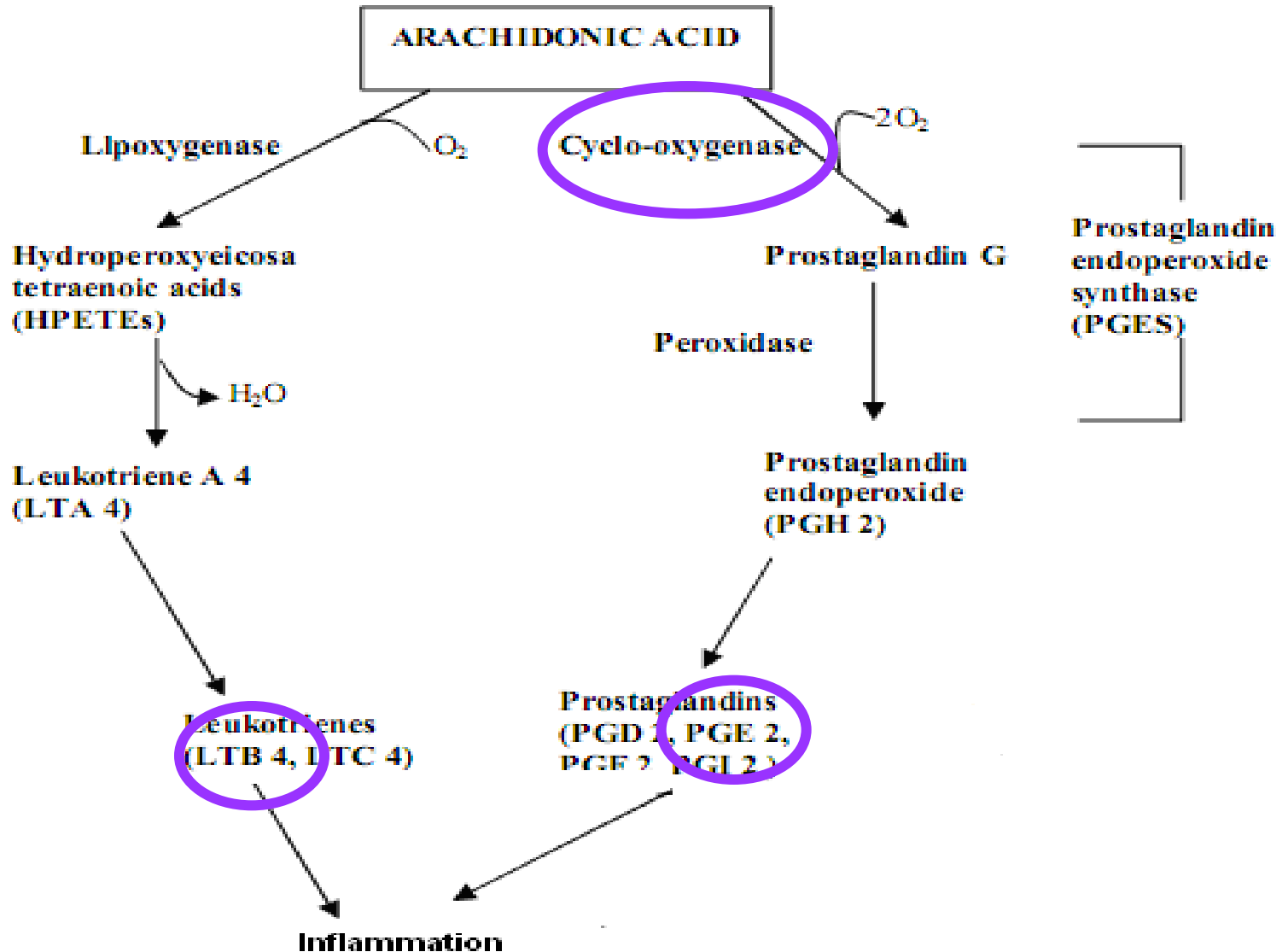
Anti-inflammatory Parameters

- ◆ **Prostaglandin E2 (PGE2)**
- ◆ **Leukotriene B4 (LTB4)**

Hepatic gene expression

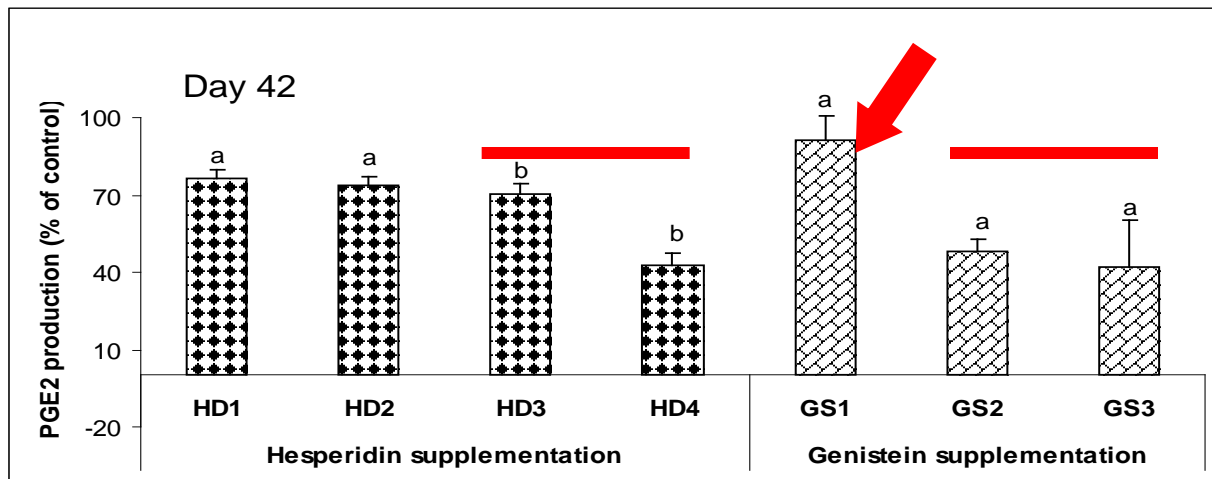
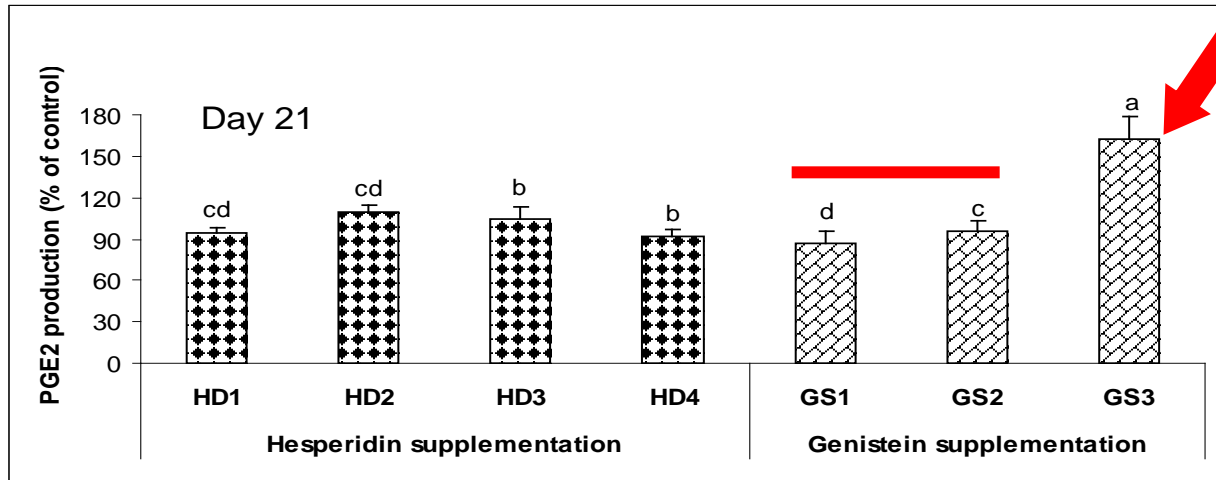
- ◆ **Cyclooxygenase-2 (COX-2)**
- ◆ **Inducible Nitric Oxide Synthase (iNOS)**

Network of inflammation



Day 21: Elevated PGE2 in GS3 group

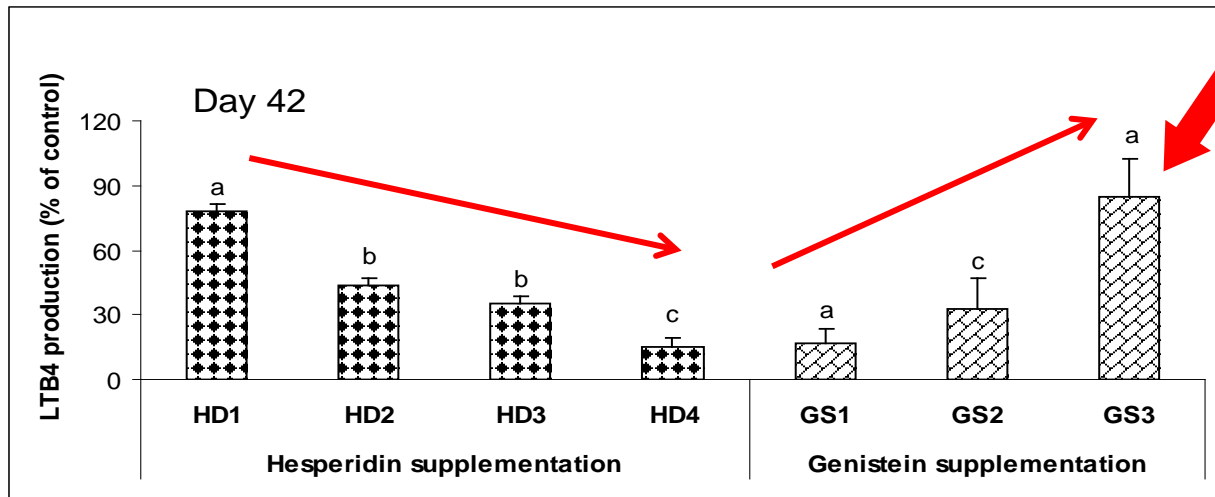
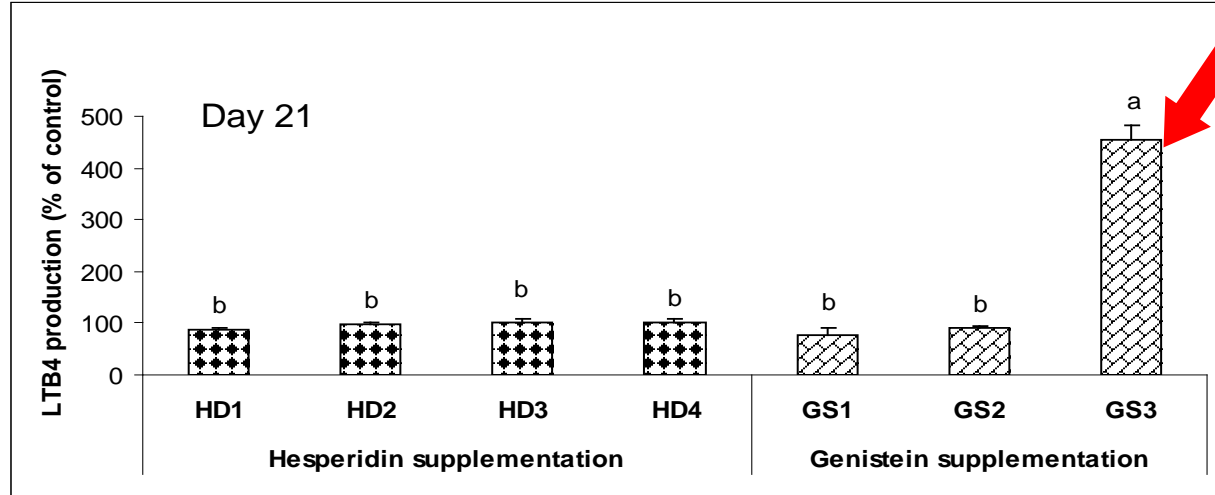
PGE2



Day 42: Hesperidin and Genistein decreased PGE2 except elevation in (GS1) group

Day 21: Elevated LTB4 in GS3 group

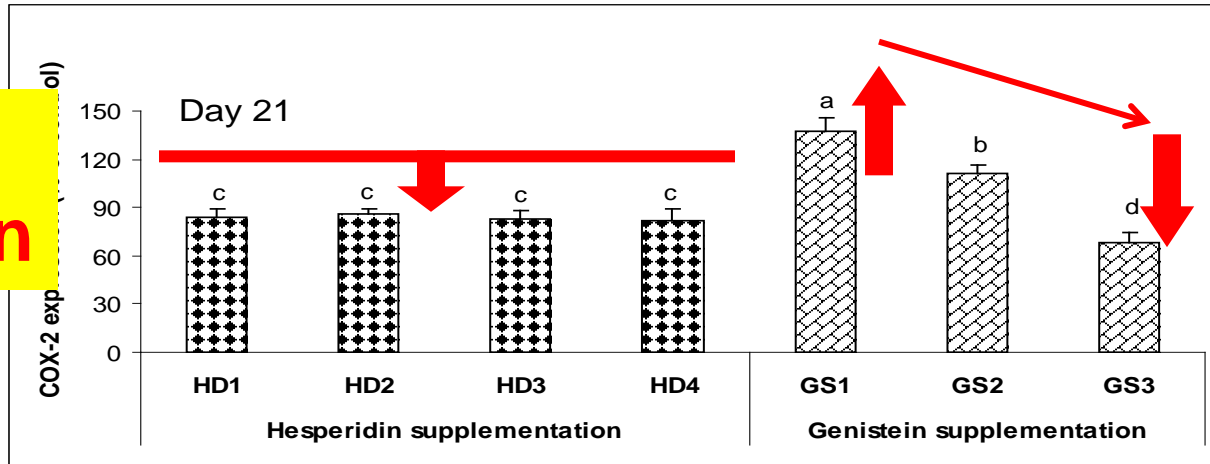
LTB4



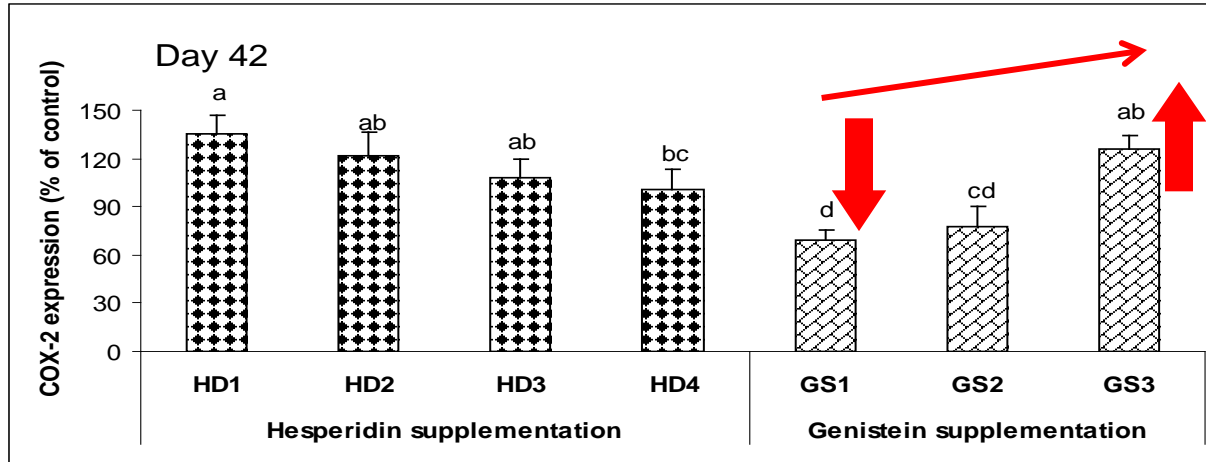
Day 42: Hesperidin and Genistein decreased LTB4 except no change in (GS3) group

Day 21: Hesperidin decreased COX-2 expression
Genistein ↑ expression in GS1 while down regulated in GS3

COX-2 expression



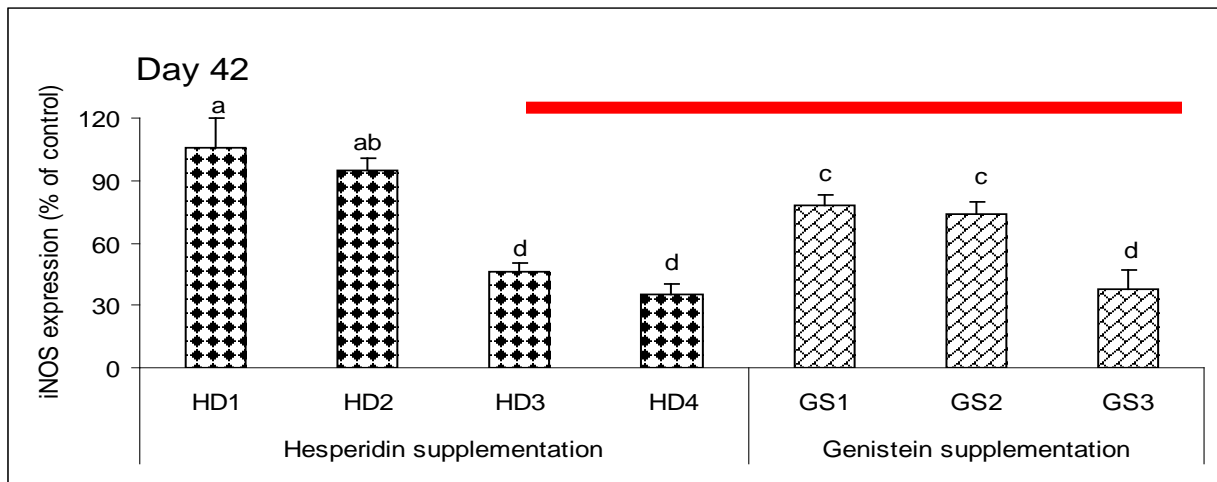
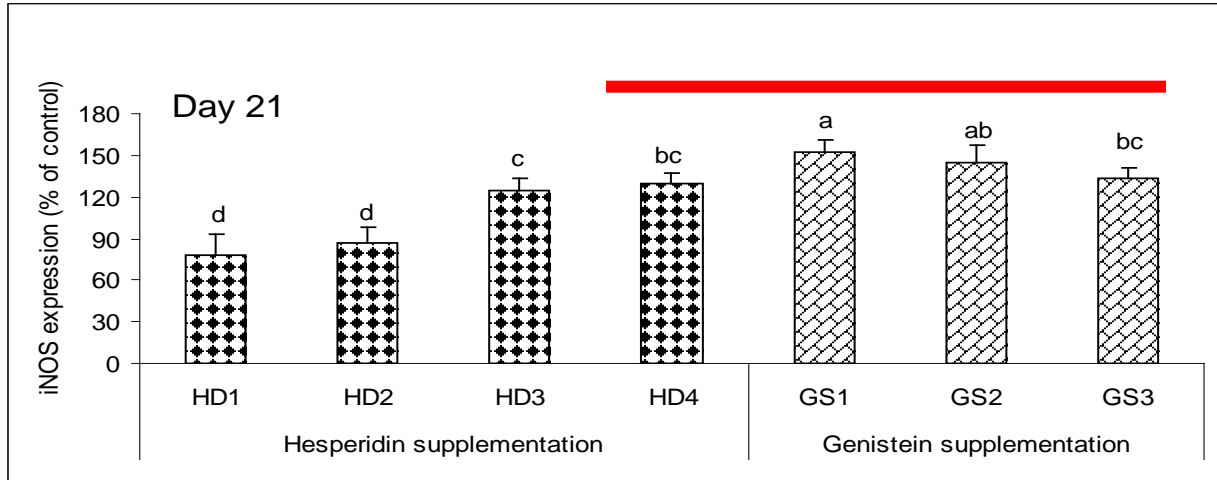
Dose-dependent



Day 42: No effect of Hesperidin
Genistein response was reversed from day 21

Day 21: low level Hesperitin decrease, high level increase; Elevated iNOS expression in all genistein

**iNOS
expression**



Day 42: dose - dependent decrease

Immune-modulating effects

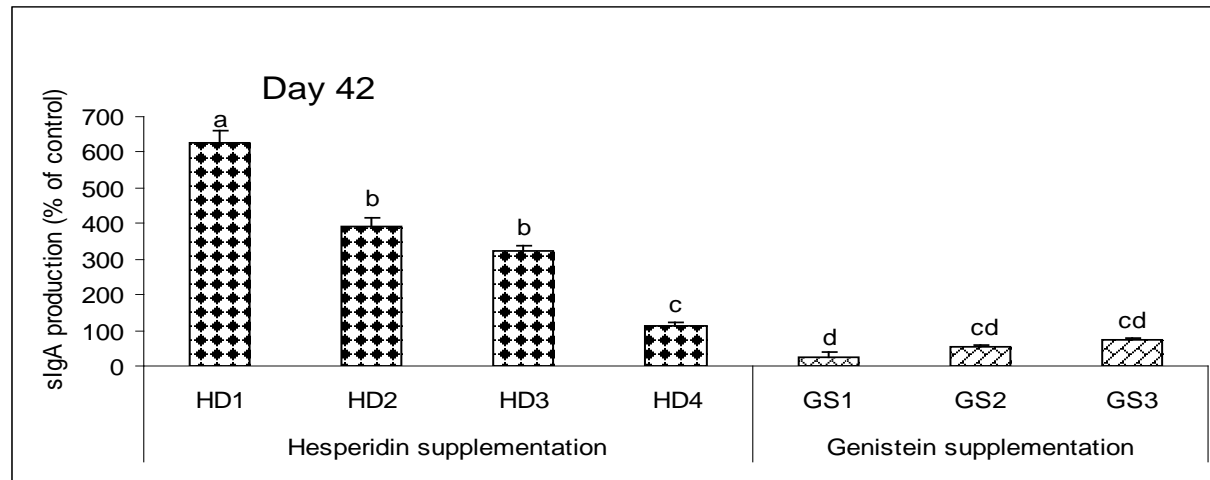
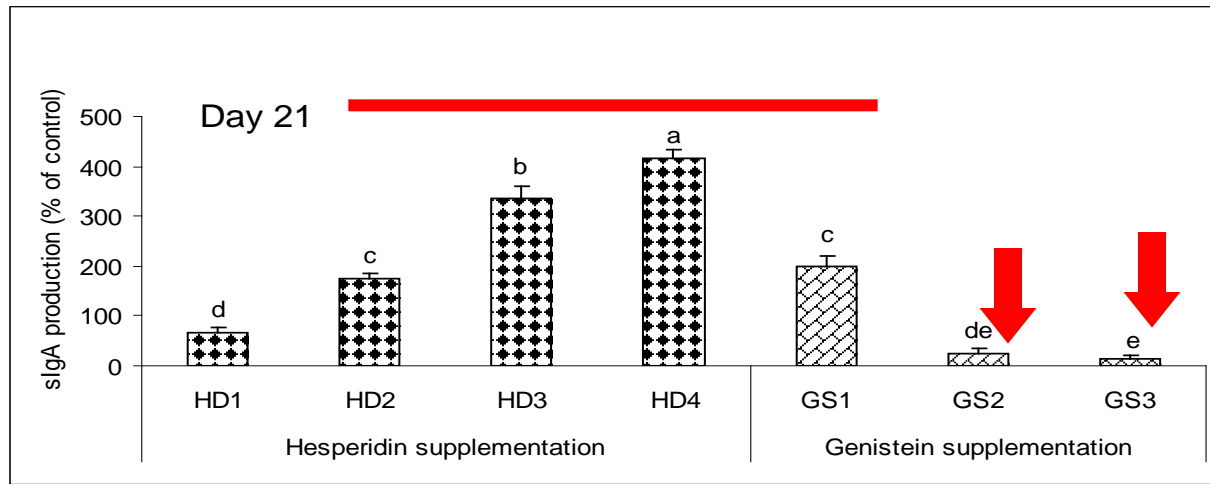
- ◆ **Secretory IgA (sIgA)**
- ◆ **Intraepithelial lymphocytes (IEL)**

Gene expression

- ◆ **Interferon-gamma (IFN- γ)**
- ◆ **Interleukin-10 (IL-10)**
- ◆ **Interleukin-4 (IL-4)**

Day 21: high level hesperidin \uparrow sIgA;
low level genistein \uparrow sIgA in GS1, high level \downarrow

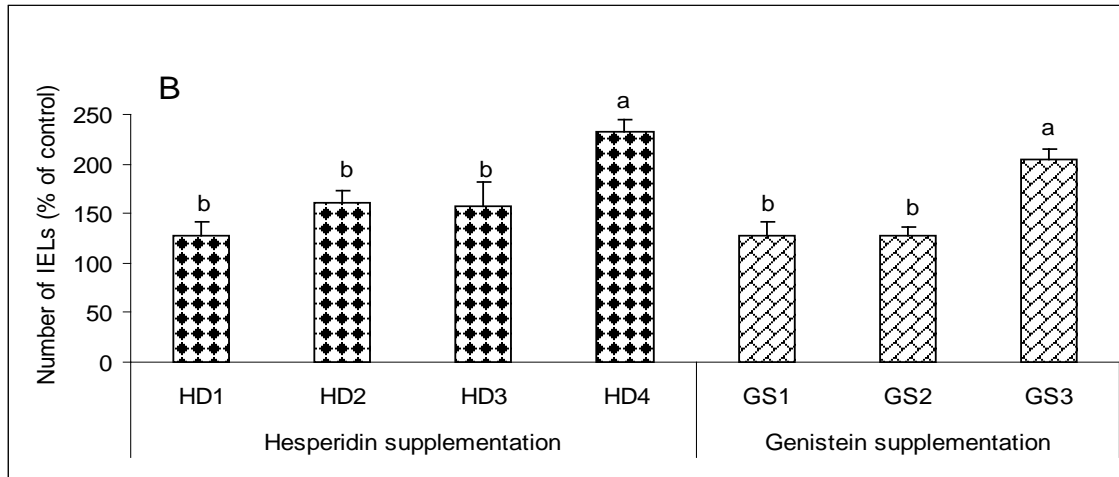
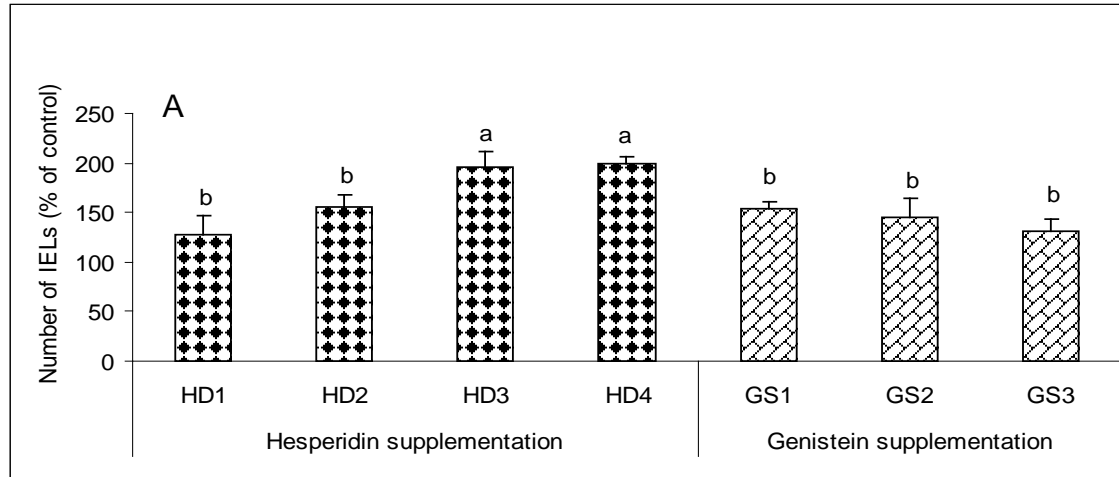
sIgA



Day 42: low level hesperidin \uparrow sIgA production;
all genistein \downarrow sIgA

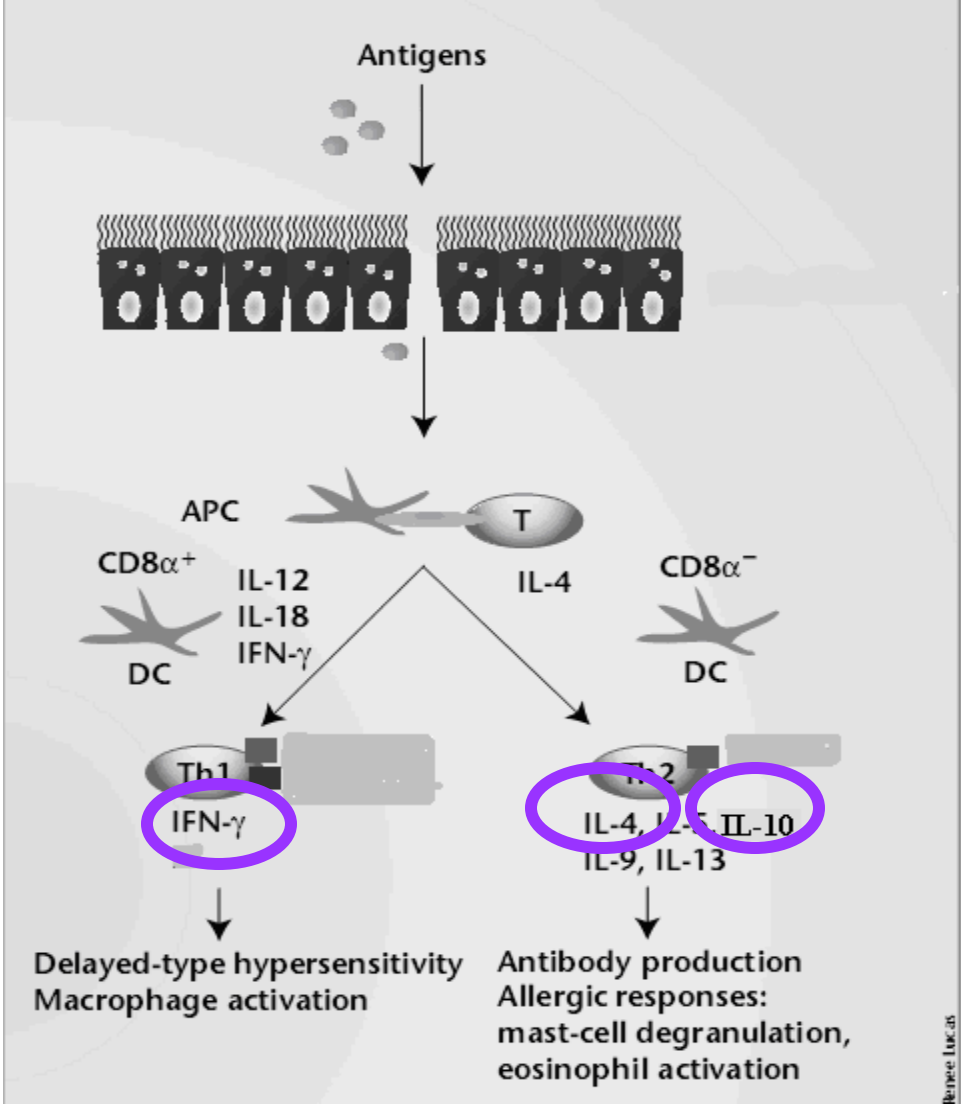
Hesperidin: increased IELs on both 21 and 42 days

IEL



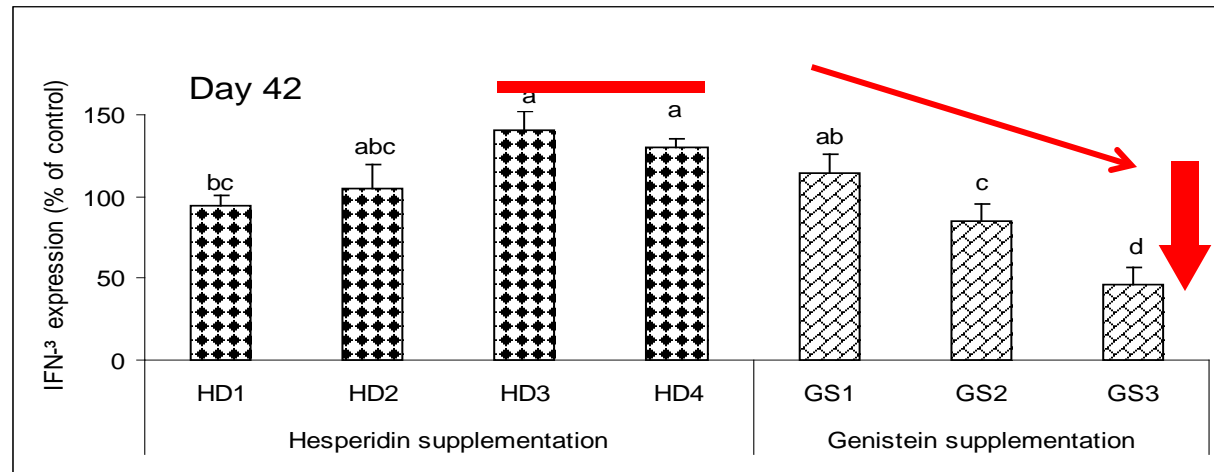
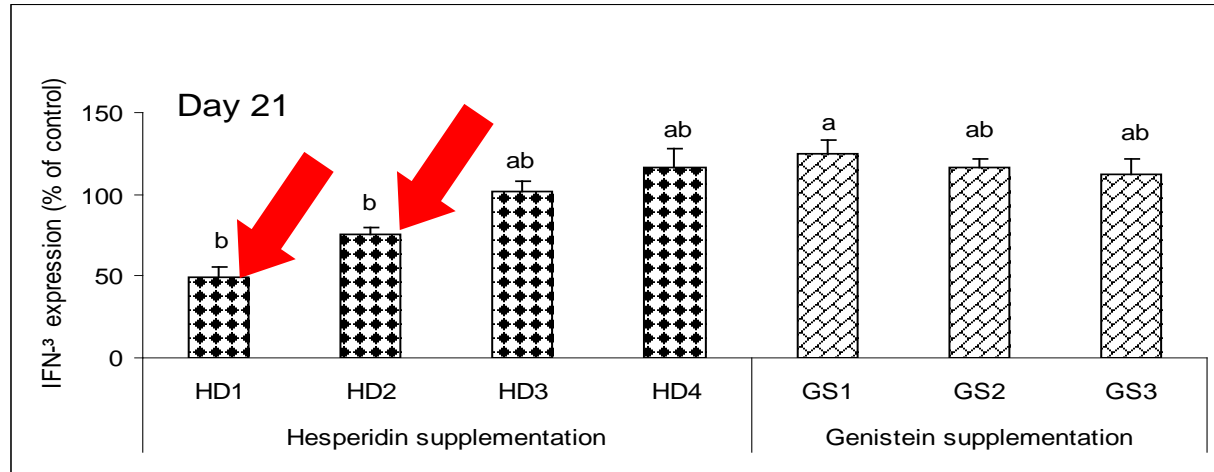
Genistein: high level GS3 increased IELs

Th1 and Th2 cytokines and Immunity



Day 21: low level hesperidin reduced IFN- γ expression

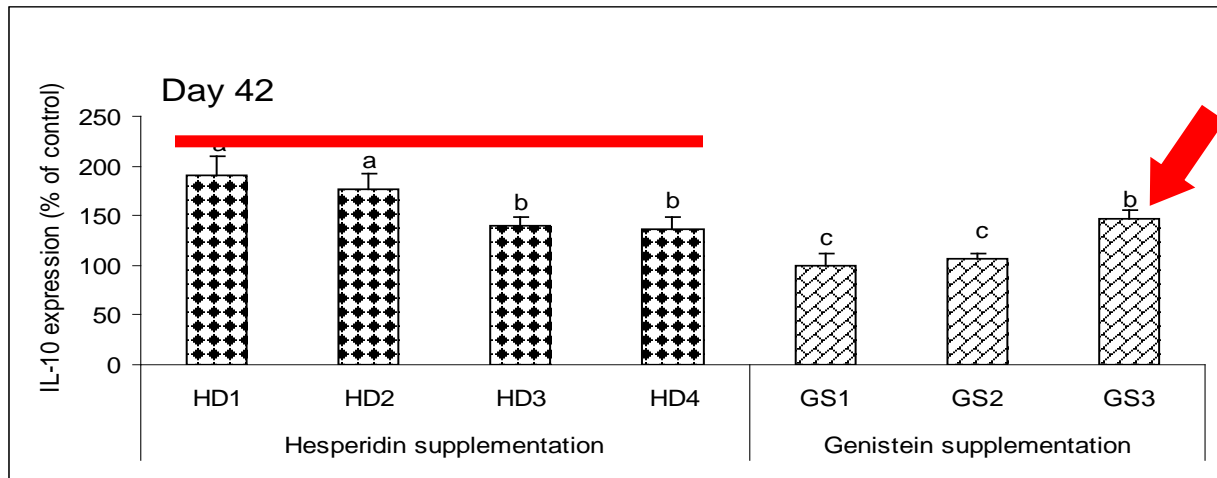
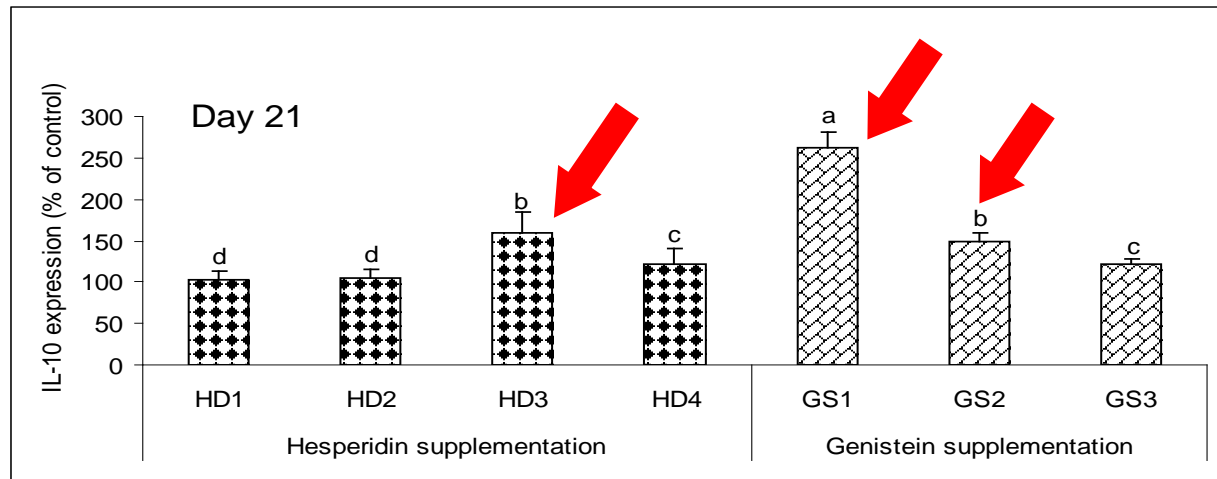
Hepatic
IFN- γ



Day 42: high level hesperidin \uparrow expression;
high level genistein \downarrow in expression

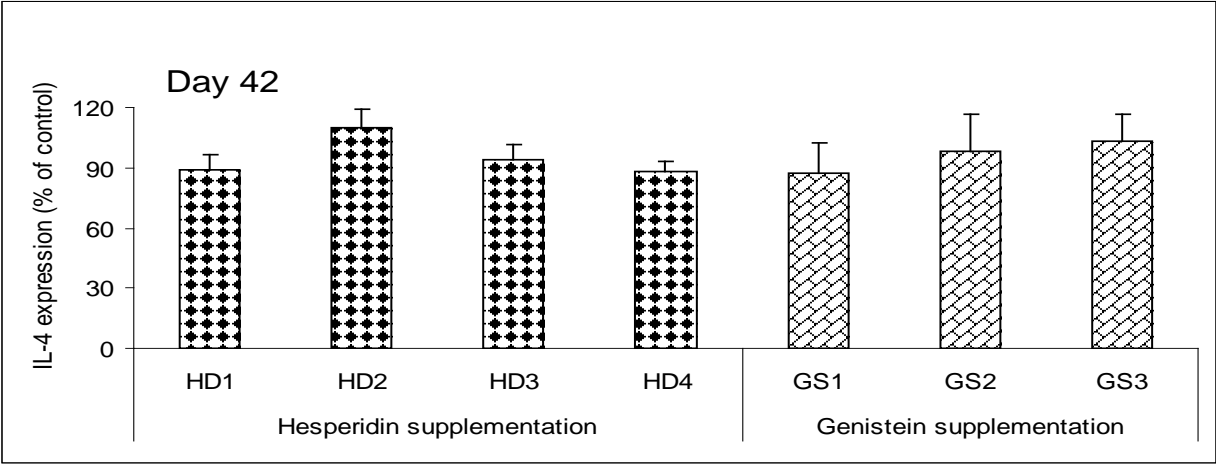
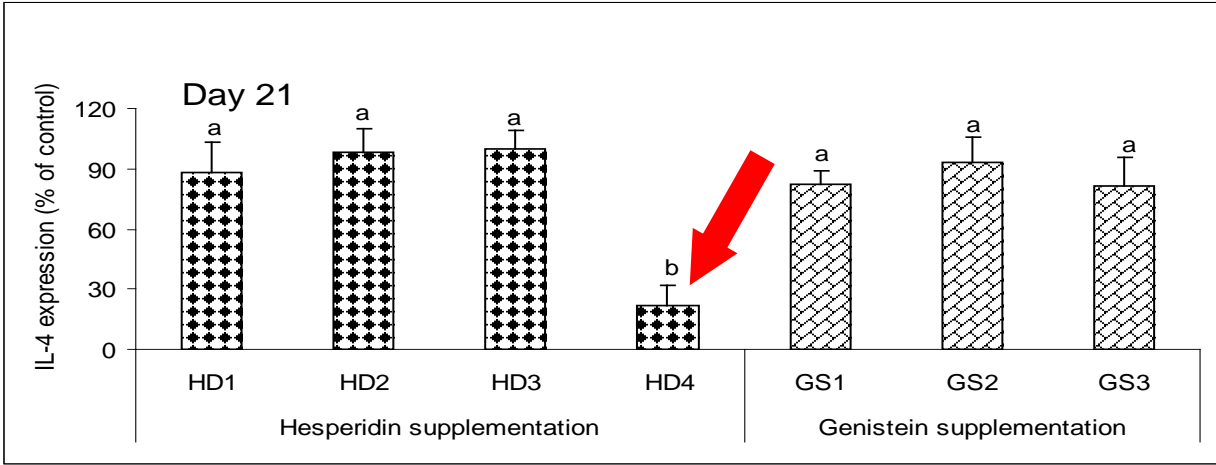
Day 21: Hesperidin ↑ Expression in HD3 group, low level genistein ↑ expression

IL-10



Day 42: all hesperidin ↑ expression; high level
genistein ↑ expression

IL-4



All treatments had no effect, except high level hesperidin suppressed IL-4 gene expression at 21 days

Summary

- ◆ Overall, growth was not affected
- ◆ Generally, the anti-inflammatory and immunity responses were shifted towards positive direction; however, an increasing trend for iNOS, sIgA and IFN- γ on 21 day and decreasing trend for LTB₄, iNOS, COX-2, sIgA and IL-10 on 42 days

- ◆ **Hesperidin suppressed eicosanoid synthesis and modulate immune related gene expression**
- ◆ **Genistein was not efficient immunity enhancer; however, an ↑ sIgA production and ↑ IL-10 mRNA gene expression with low dose of genistein (2.5 mg/kg) was observed on 21 days.**

- ◆ **A strong relationship between dietary genistein concentration and COX-2, and IL-10 was observed with decreasing trend on day 21 and increasing trend on day 42 of broiler life**

Overall:

Flavonoids effects were animal age specific, and dose - dependent

Exp. 2, Treatments

720 birds, 6 groups

CON	6 x20=120	Basal diet
G5	6 x20=120	Basal diet + 5mg/kg genistein
H20	6 x20=120	Basal diet + 20mg/kg hesperidin
*GH5	6 x20=120	Basal diet + 5mg/kg genistein /hesperidin
*GH10	6 x20=120	Basal diet + 10mg/kg genistein / hesperidin
*GH20	6 x20=120	Basal diet + 20mg/kg genistein / hesperidin

GH: Genistein/Hesperidin (1:4)

Half numbers of birds LPS challenge

on day 16, 18 & 20

Measurements

Growth performance

Antioxidant activity

Immunity parameters

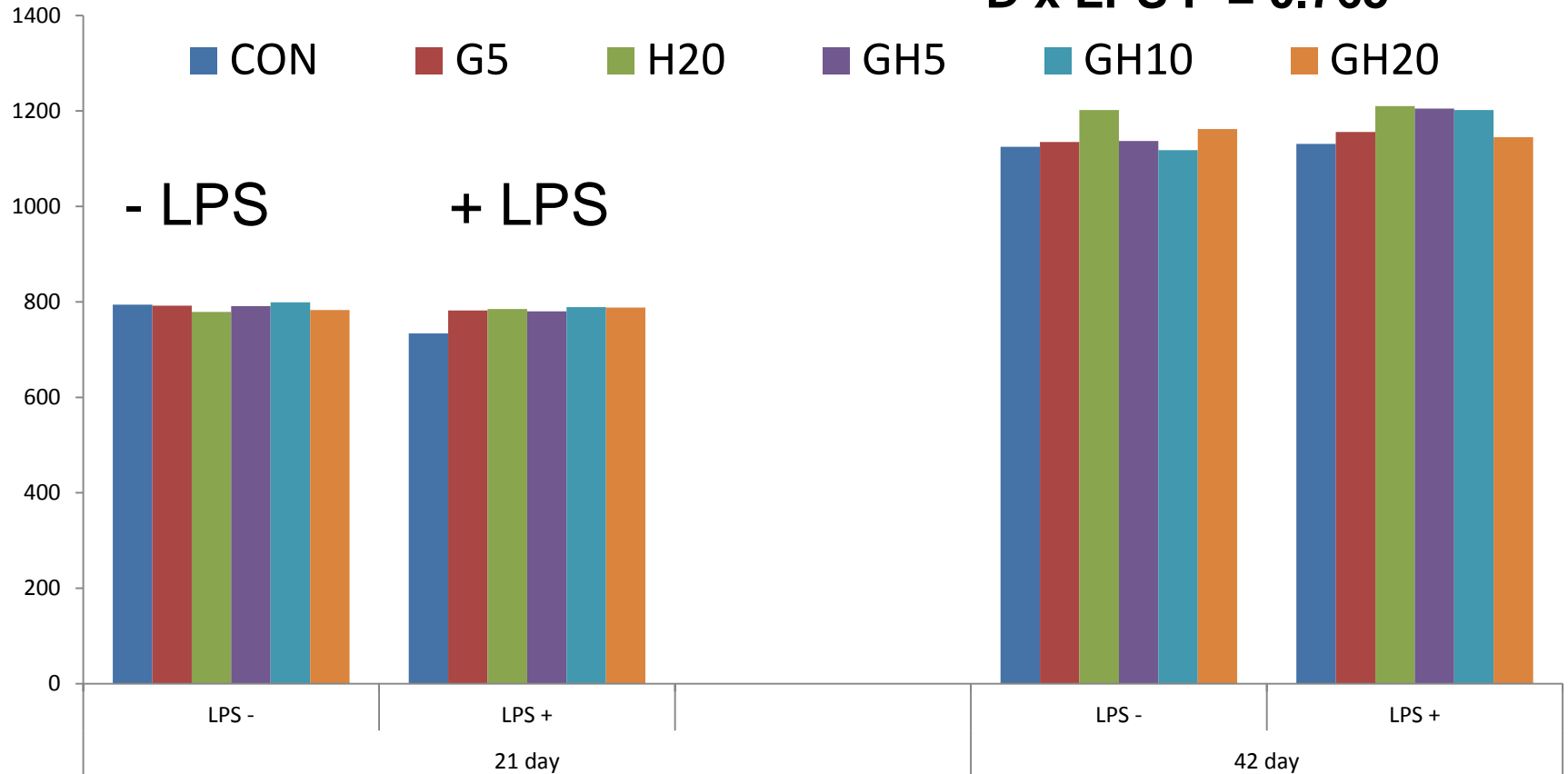
Lipid metabolism

Effect of genistein and hesperidin on growth performance +/- LPS

Body weight gain, g

Diet P = 0.064
LPS P = 0.056
D x LPS P = 0.033

Diet P = 0.074
LPS P = 0.957
D x LPS P = 0.765

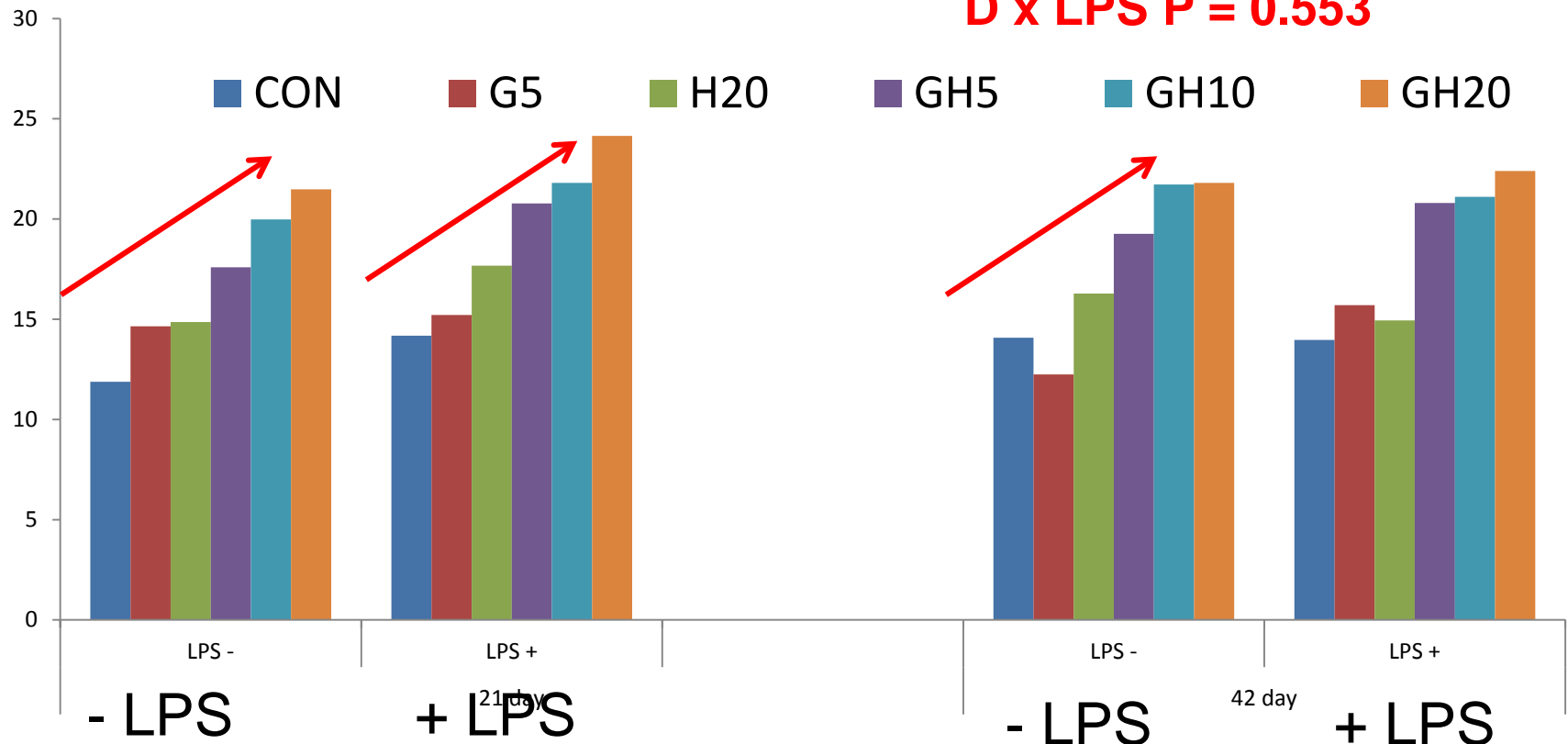


Effect of genistein and hesperidin on plasma antioxidant status +/- LPS

T-AOC, U/ml

Diet P < 0.001
LPS P < 0.001
D x LPS P = 0.383

Diet P = 0.001
LPS P = 0.221
D x LPS P = 0.553

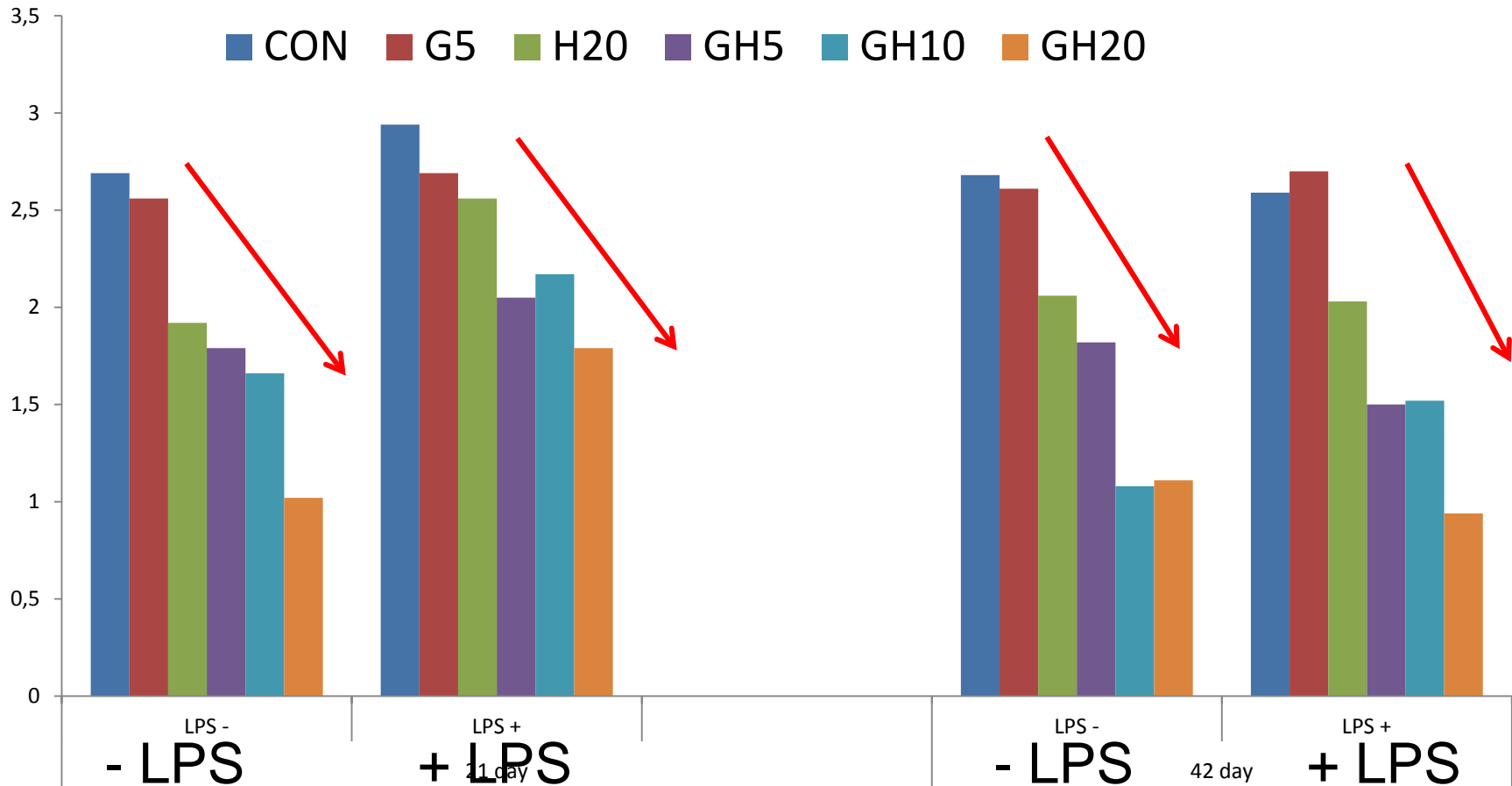


Effect of genistein and hesperidin on plasma antioxidant status +/- LPS

MDA, n mol/ml

21 day, Diet P < 0.01

42 day, Diet P < 0.01

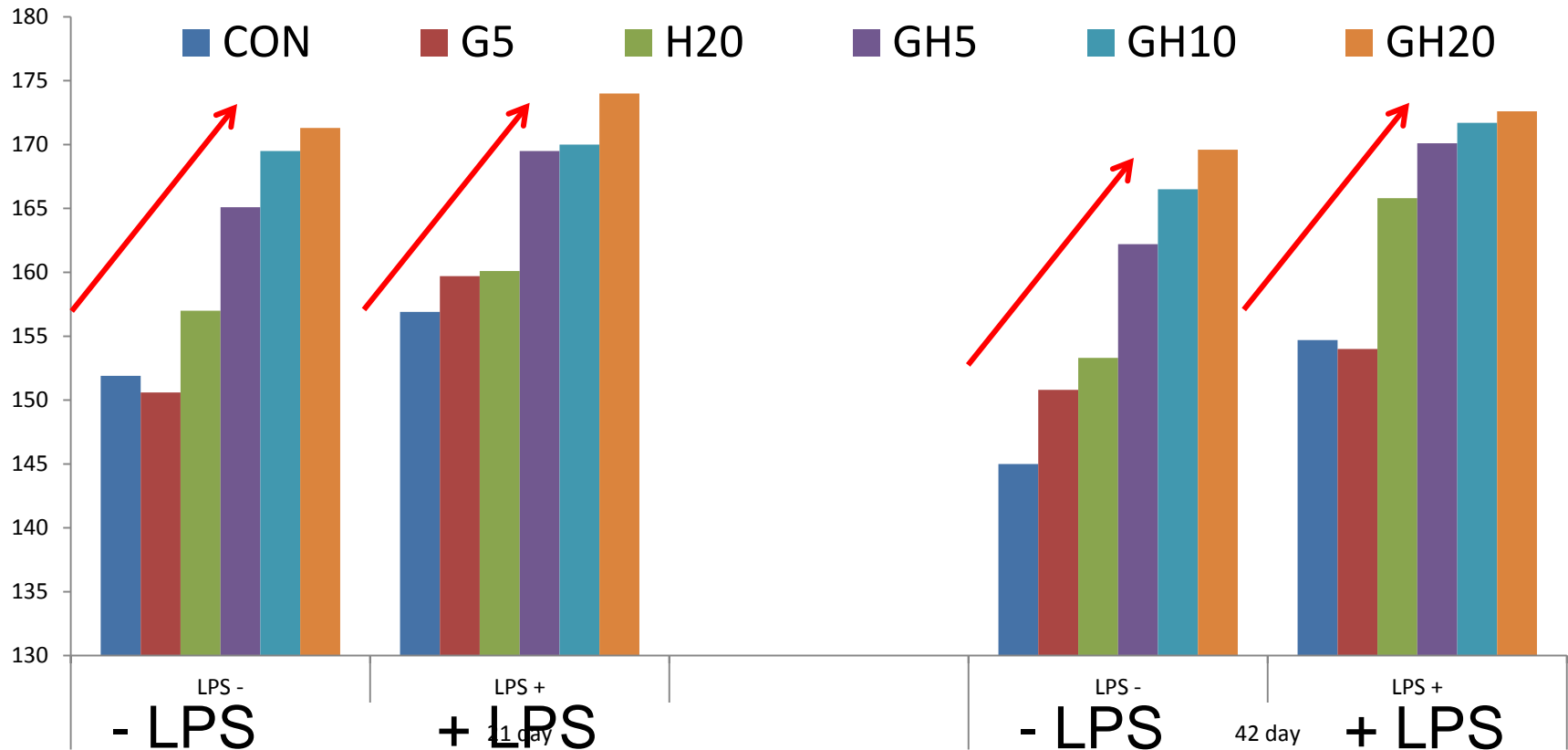


Effect of genistein and hesperidin on plasma antioxidant status +/- LPS

SOD, U/ml

21 day, Diet P < 0.01
LPS P < 0.05

42 day, Diet P < 0.01
LPS P < 0.01



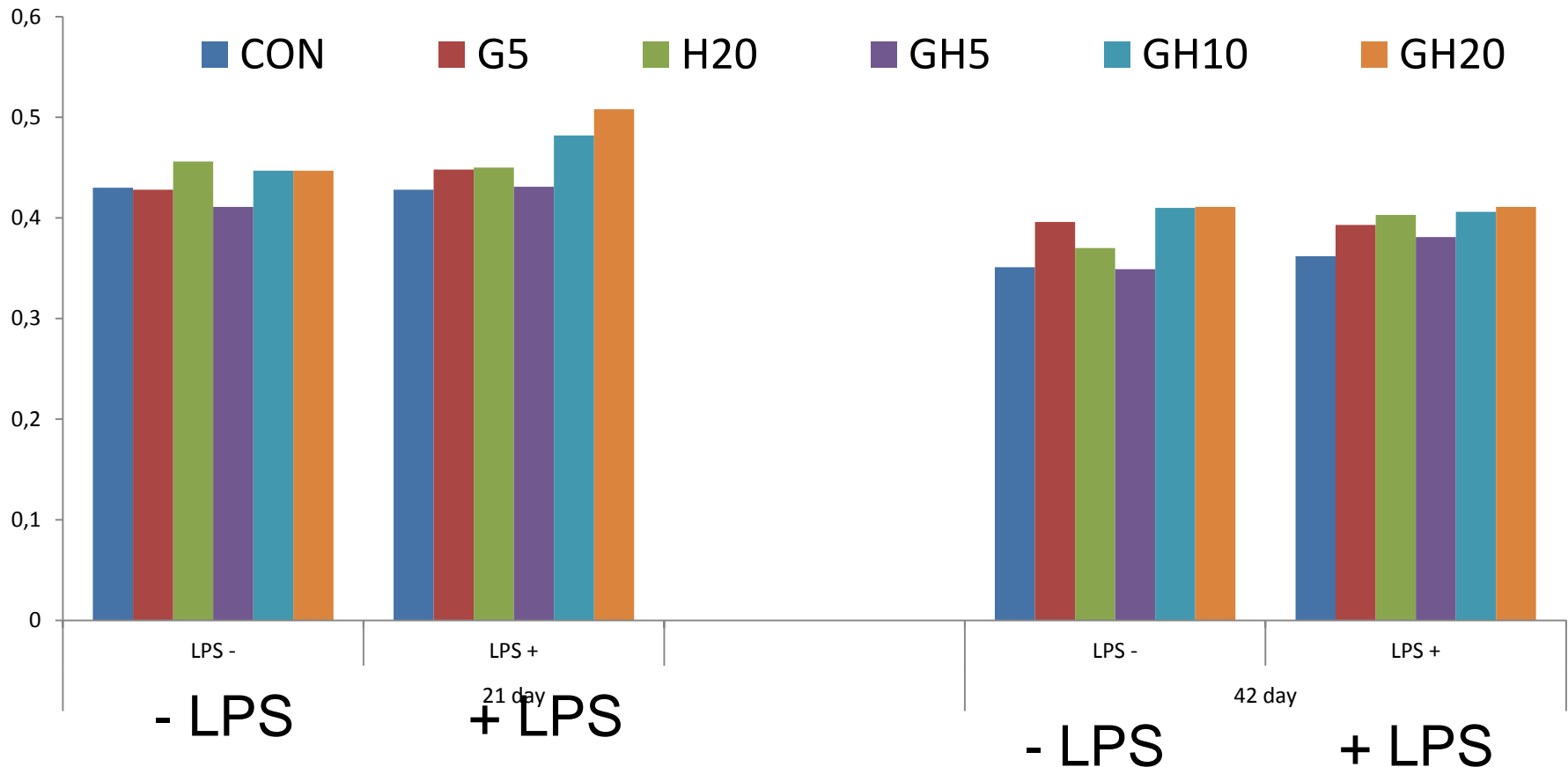
Effect of genistein and hesperidin on immune organ indices (relative organ weight) +/- LPS

Combined effect

Thymus, g/g

21 day, No effect

42 day, Diet P < 0.01

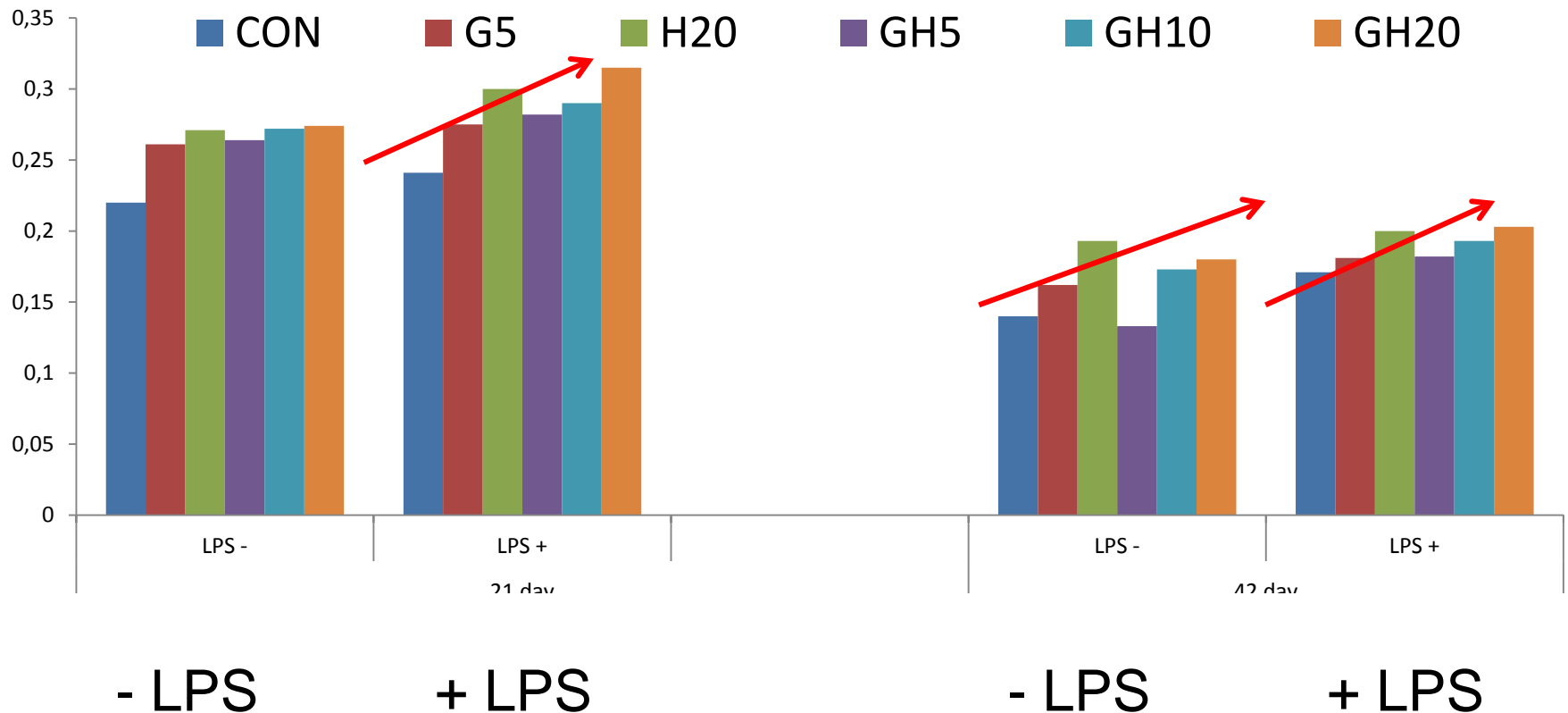


Effect of genistein and hesperidin on immune organ indices (relative organ weight) +/- LPS

Bursa, g/g

Diet P < 0.01
LPS P < 0.01
D x LPS P < 0.01

LPS P < 0.05

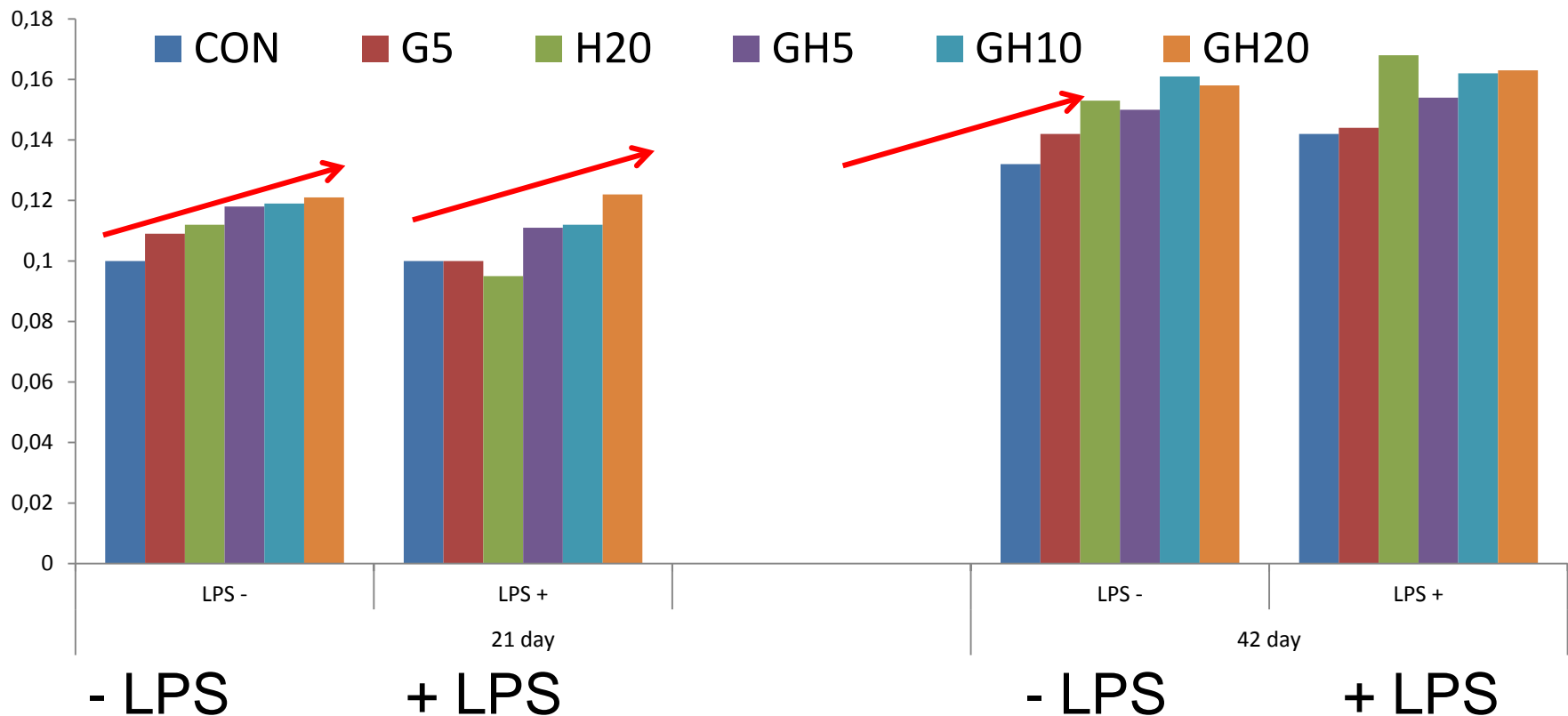


Effect of genistein and hesperidin on immune organ indices (relative organ weight) +/- LPS

Spleen, g/g

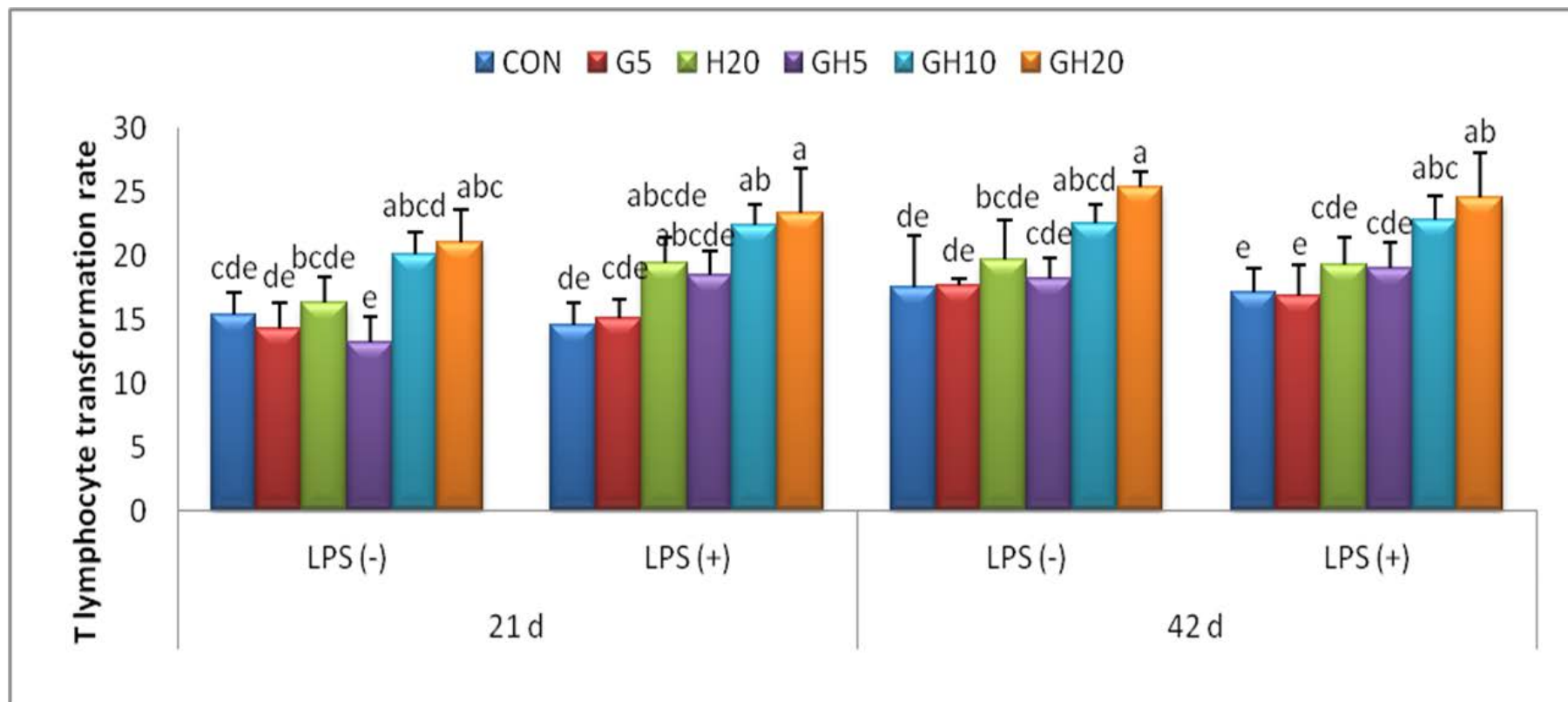
Diet P < 0.01
LPS P < 0.05

Diet P < 0.05



Effect of genistein and hesperidin on T lymphocyte transformation rate (TLTR)

Combined effect

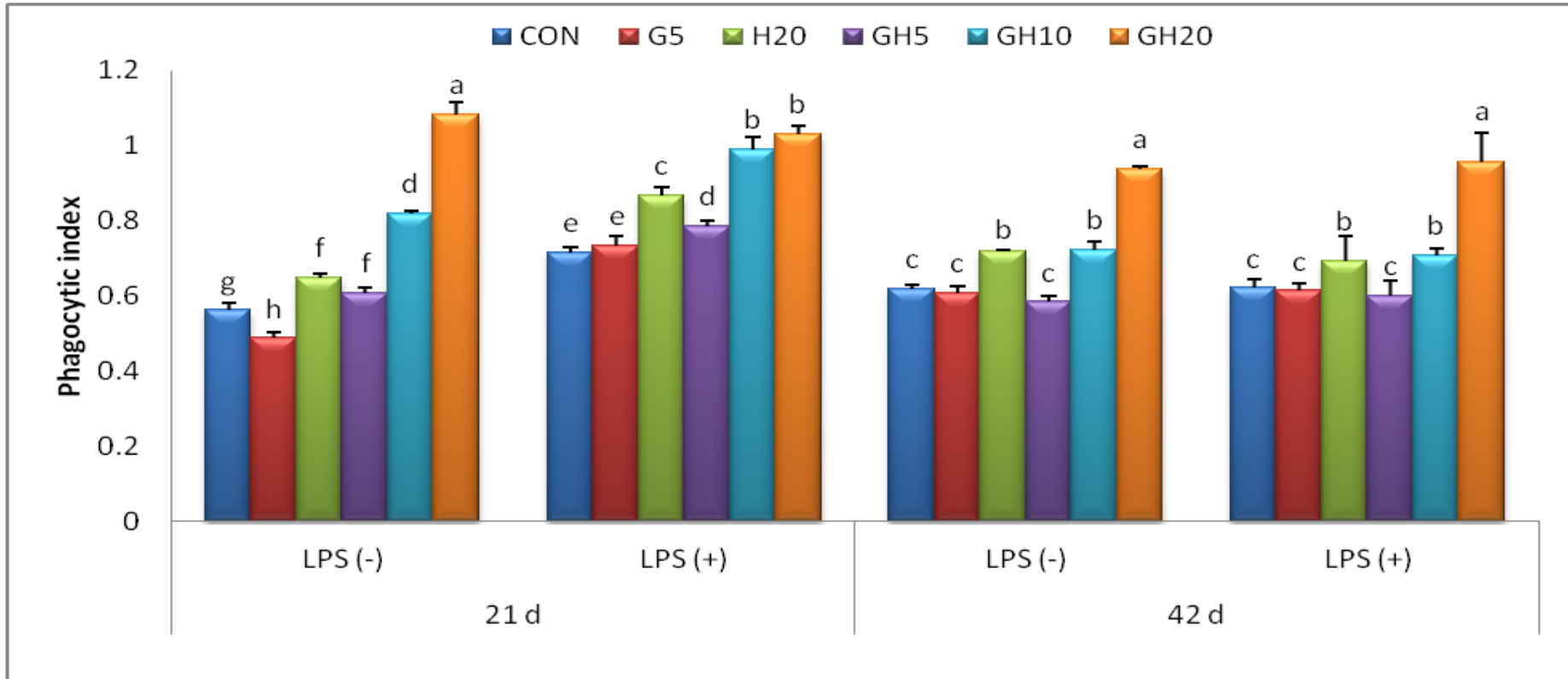


Diet P <0.001
LPS P <0.001

Diet P <0.001

Effect of genistein and hesperidin on phagocytic index +/- LPS)

Combined effect

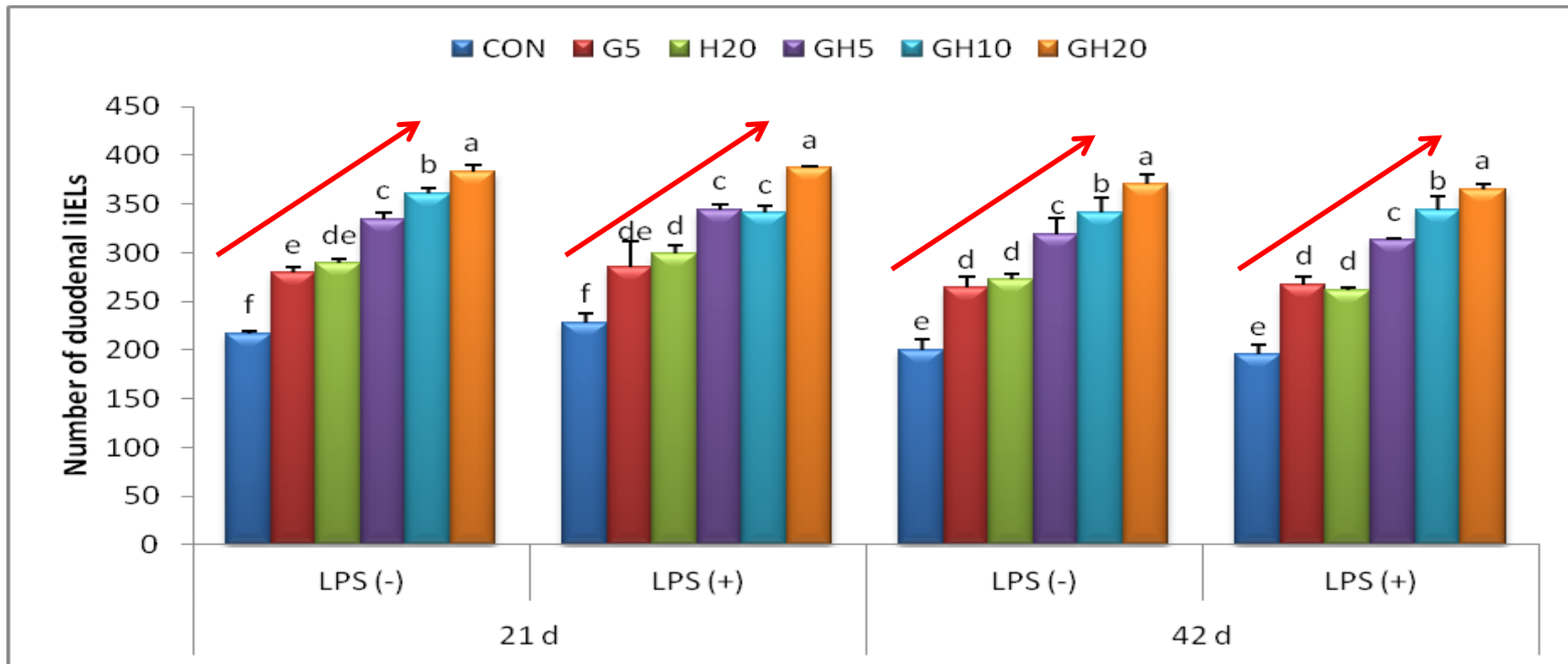


Diet P <0.001
LPS P <0.001
D x LPS P <0.001

Diet P <0.001
Dose-dependent

Effect of genistein and hesperidin on intestinal intraepithelial lymphocytes (iIELs)

duodenum

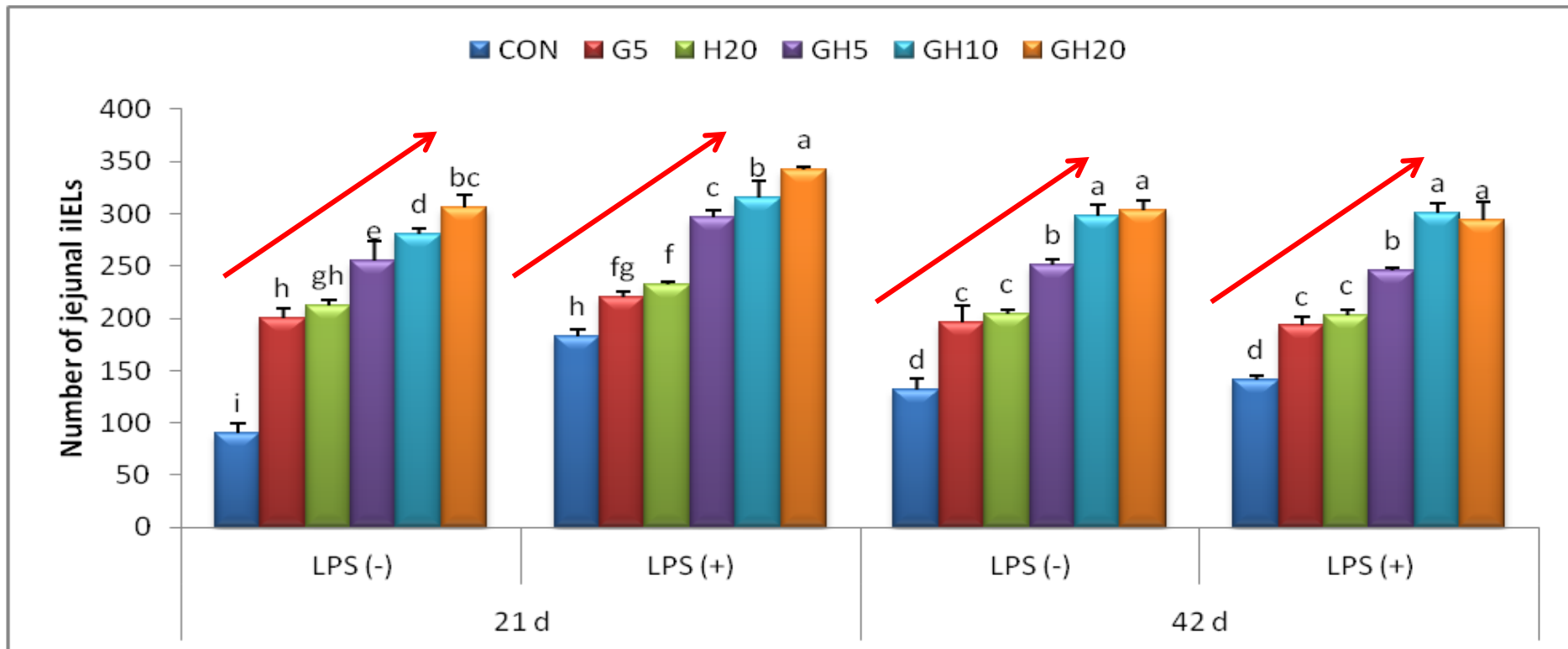


Diet P <0.001

Diet P <0.001

Effect of genistein and hesperidin on intestinal intraepithelial lymphocytes (iIELs)

jejunum

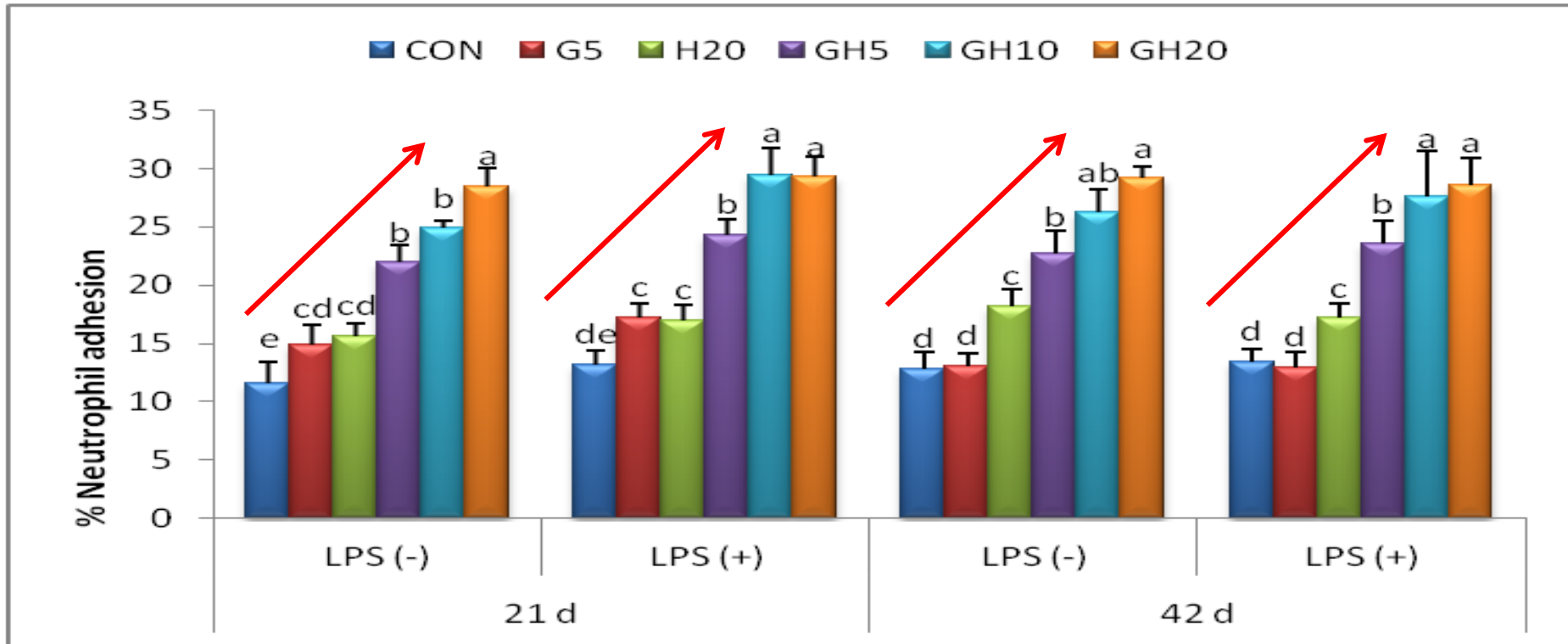


Diet P <0.001
LPS P <0.001

Diet P <0.001

Effect of genistein and hesperidin on neutrophil adhesion rate +/- LPS

Combined effect



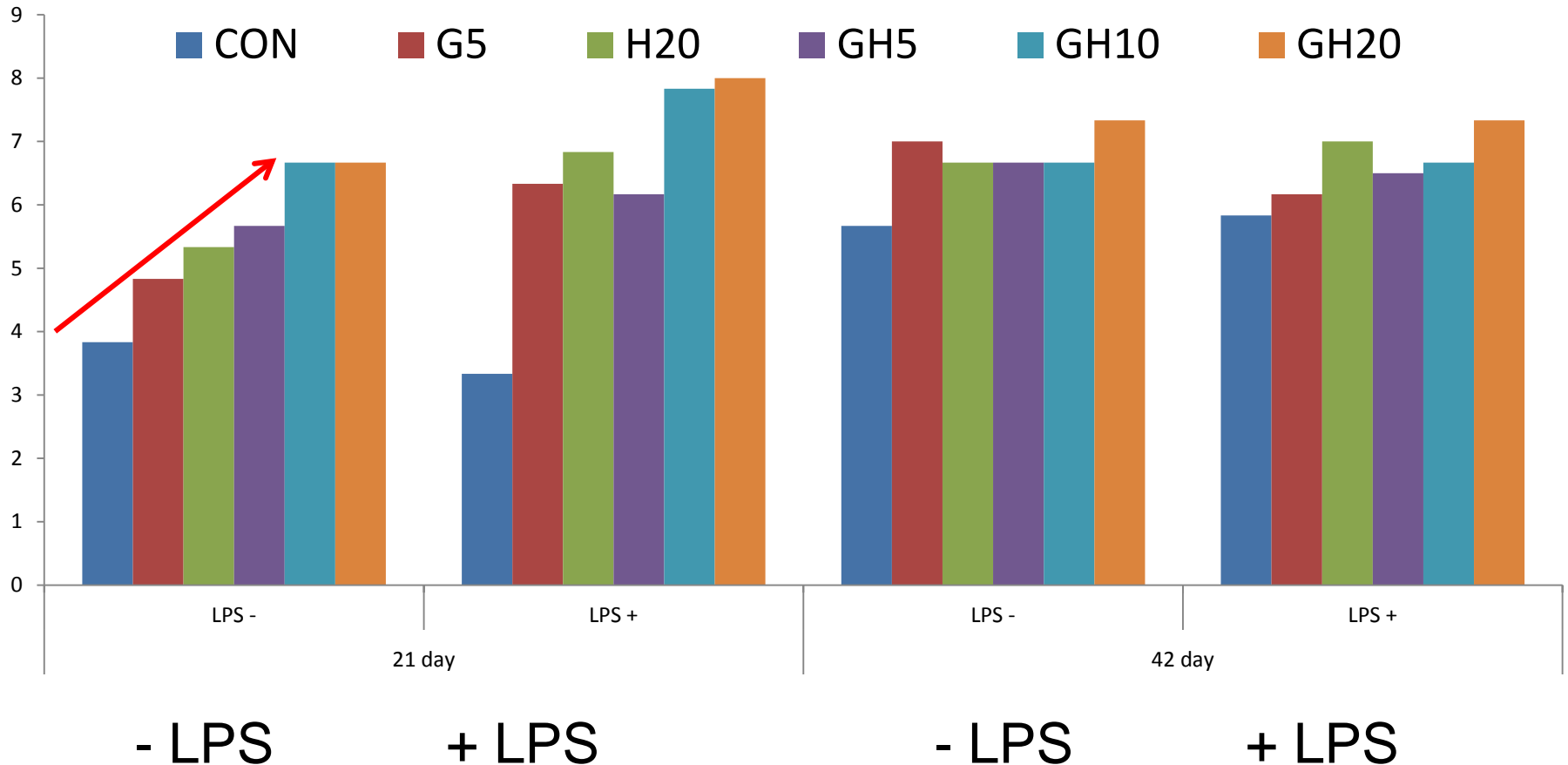
Diet P <0.001
LPS P <0.001

Diet P <0.001

Effect of genistein and hesperidin on serum anti-NDV (Newcastle disease virus) antibody titers

21 day, Diet P <0.001
LPS P <0.001

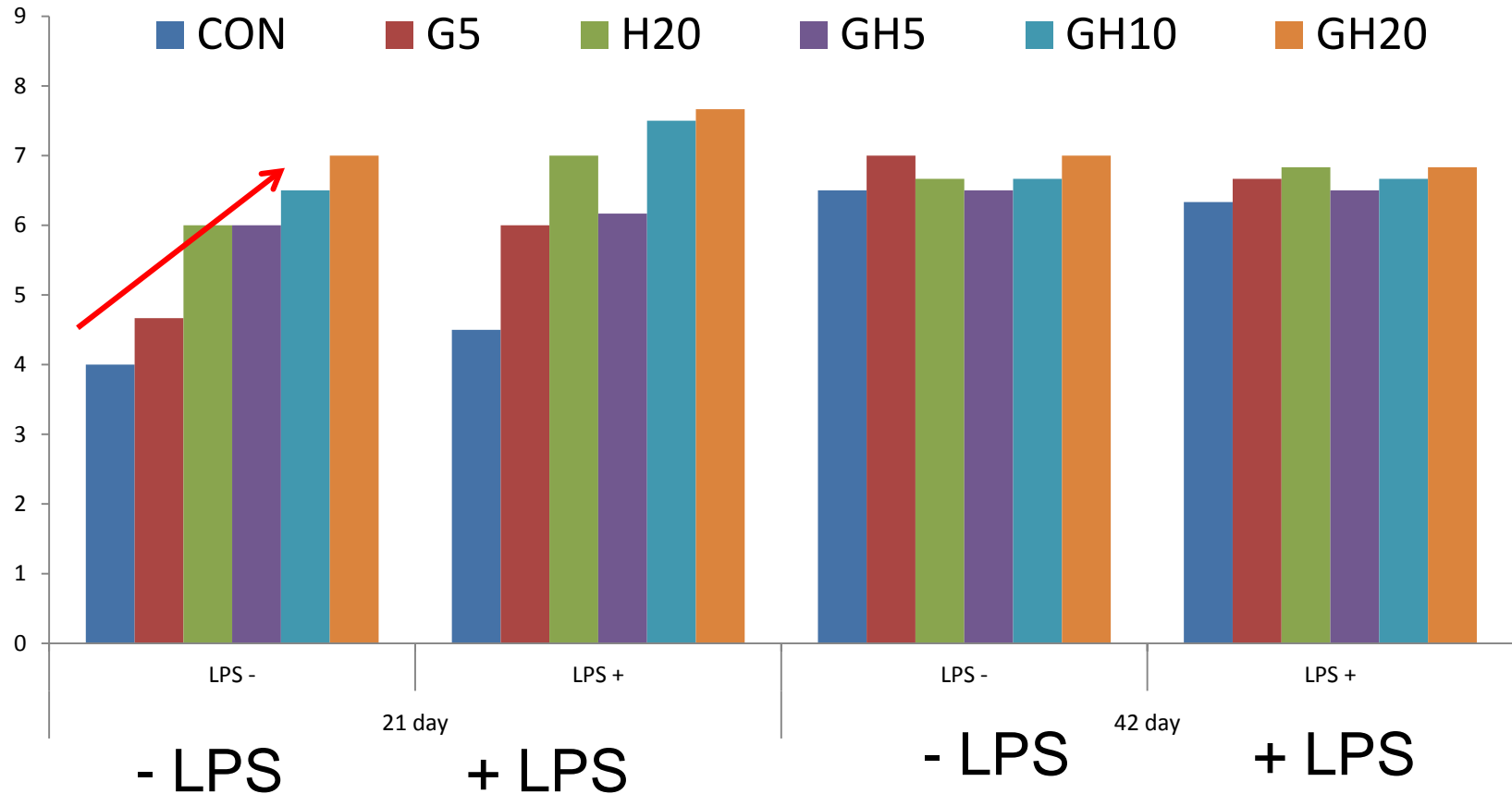
42 day, Diet P <0.001



Effect of genistein and hesperidin on serum anti-AIV (avian influenza virus) antibody titers

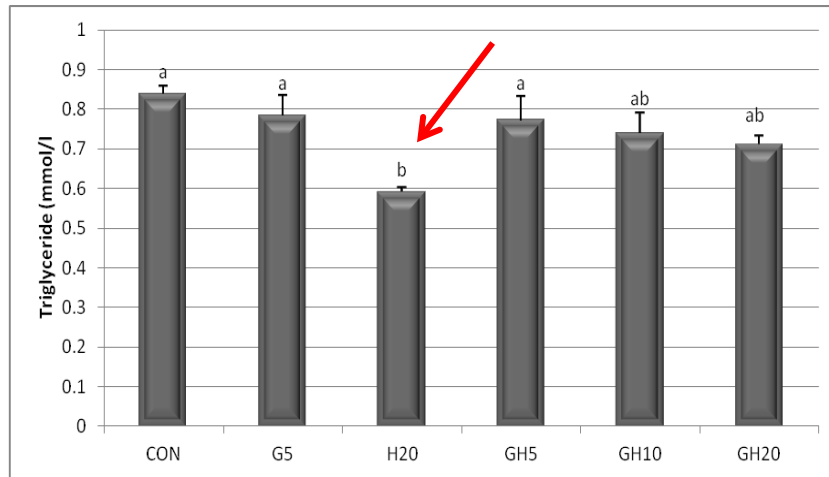
21 day, Diet P <0.001

42 day, no effect

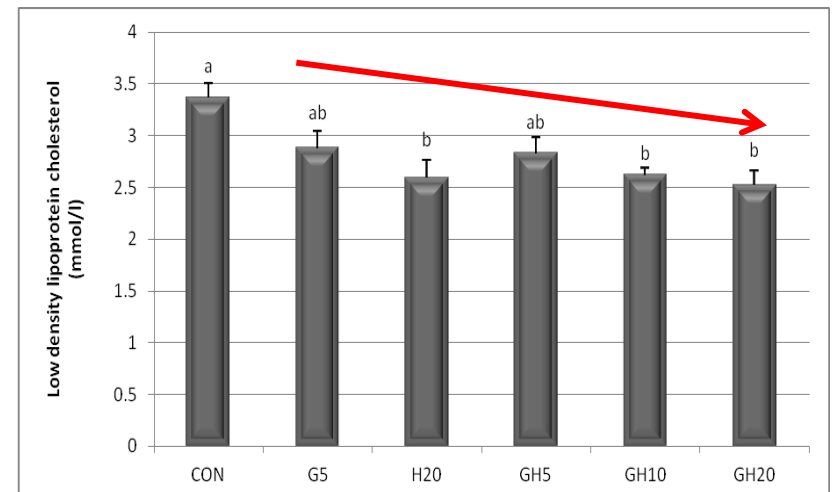
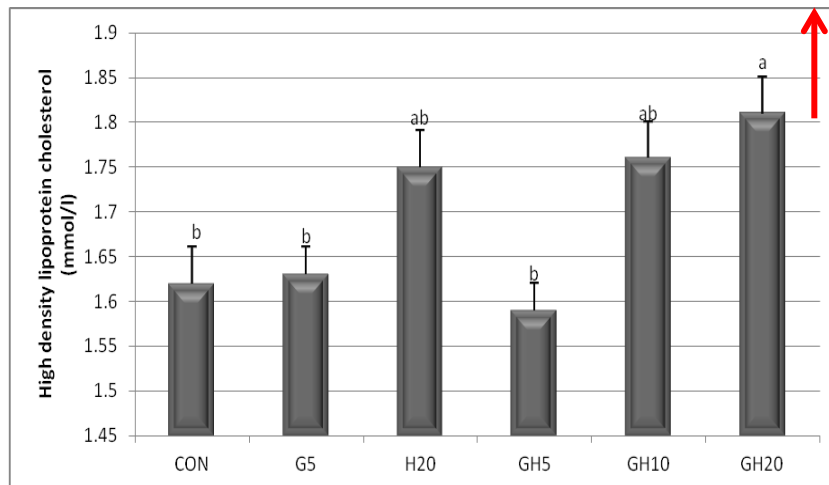
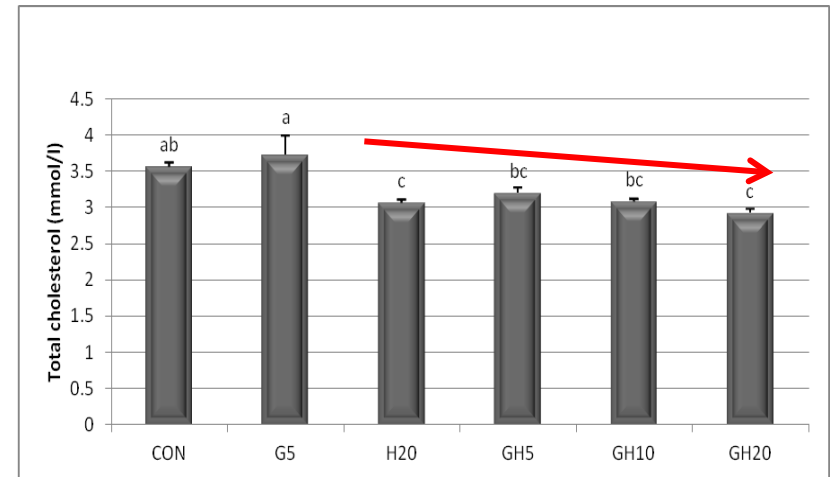


Effect of flavonoids on serum lipid profile

total cholesterol (CHO)



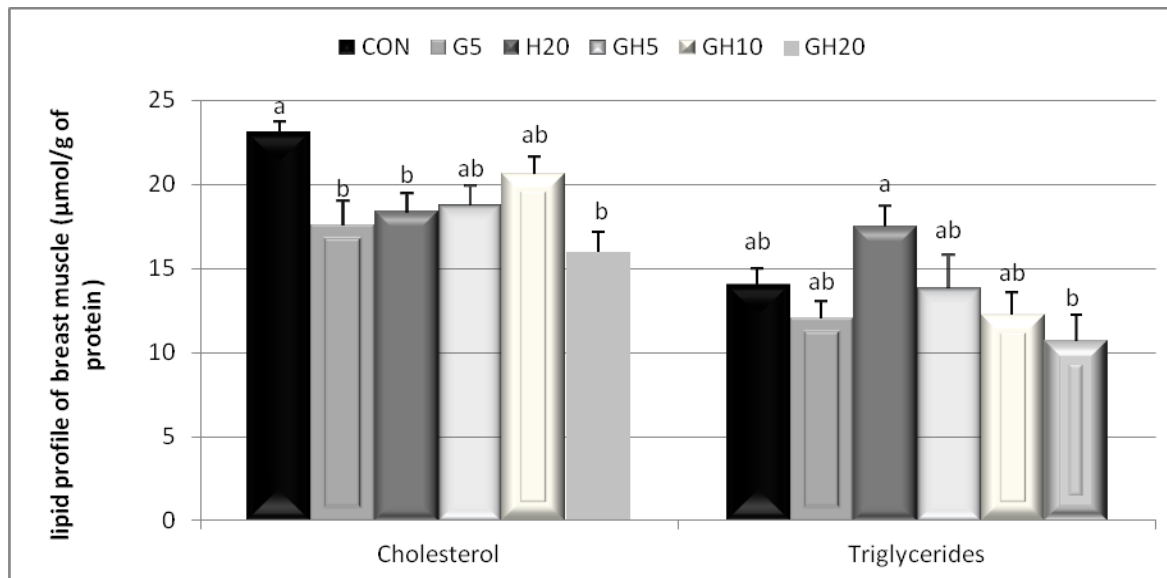
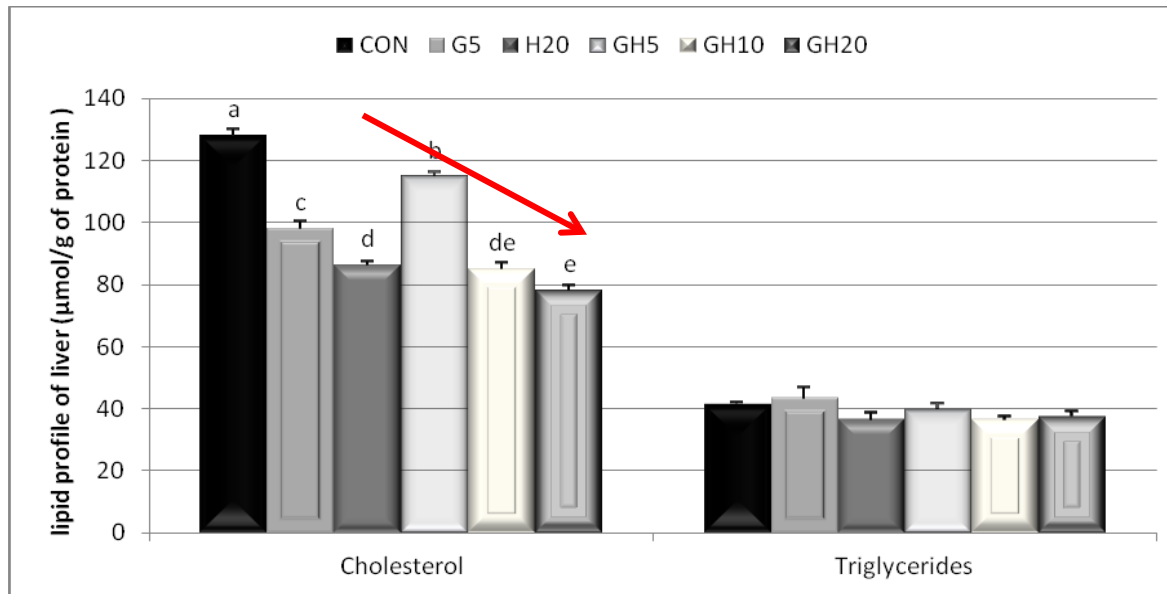
triglyceride (TG)



high density lipoprotein cholesterol (HDLC)

low density lipoprotein cholesterol (LDLC).

Effect of flavonoids on tissues total cholesterol (CHO) and triglyceride (TG)



Effect of flavonoids on fatty acid composition of breast muscle in broilers (% total fatty acids)

	CON	G5	H20	GH5	GH10	GH20	P value
ΣSFA ↓	37.06 ± 0.34 a	34.89 ± 0.2 7 ^{bc}	36.02 ± 0.23 ab	34.89 ± 0.15 bc	35.14 ± 0.39 bc	34.15 ± 0.22 c	<0.001
ΣMUFA ↓	45.58 ± 1.43 a	43.58 ± 0.7 4 ^{ab}	43.66 ± 0.81 ab	41.73 ± 0.85 ab	40.65 ± 0.32 b	40.97 ± 0.75 b	0.013
ΣPUFA ↑	22.40 ± 0.23 b	23.99 ± 0.3 2 ^{ab}	23.76 ± 0.76 ab	27.12 ± 0.53 ab	27.88 ± 2.95 ab	30.31 ± 1.72 a	0.018
Σn-6 ↑	19.13 ± 0.21 b	20.94 ± 0.3 1 ^b	21.20 ± 0.68 ab	24.74 ± 0.68 ab	25.35 ± 2.61 ab	27.37 ± 1.60 a	0.006
Σn-3 ↓	3.26 ± 0.02 ^a	3.04 ± 0.02 ab	2.56 ± 0.07 ^{bc}	2.38 ± 0.19 ^c	2.53 ± 0.14 ^{bc}	2.94 ± 0.17 ^a bc	0.002
n-6/n-3 ↑	5.85 ± 0.04 ^c	6.87 ± 0.11 bc	8.27 ± 0.05 ^{ab} c	10.59 ± 1.19 a	10.36 ± 1.16 a	9.31 ± 0.43 ^a b	0.002
PUFA/SFA ↑	0.604 ± 0.01 b	0.687 ± 0.0 1 ^{ab}	0.660 ± 0.03 ab	0.780 ± 0.04 ab	0.805 ± 0.10 ab	0.887 ± 0.05 a	0.017

Summary

Genistein and hesperidin could notably improve the antioxidative status

Both flavonoids seemed to improve the cellular, humoral and mucosal immunity of the animal

Both compounds seemed to affect lipid metabolism with more health-promoting metabolites

Summary

The effects of genistein and hesperidin were dose-dependent and animal age specific

Generally, combined effect of genistein and hesperidin was more profound than individuals, especially with LPS challenge, indicating mutual interaction of both compounds



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Thanks for attention !

