Methionine alone or combined with choline and betaine affects ewes' milk and antioxidant capacity

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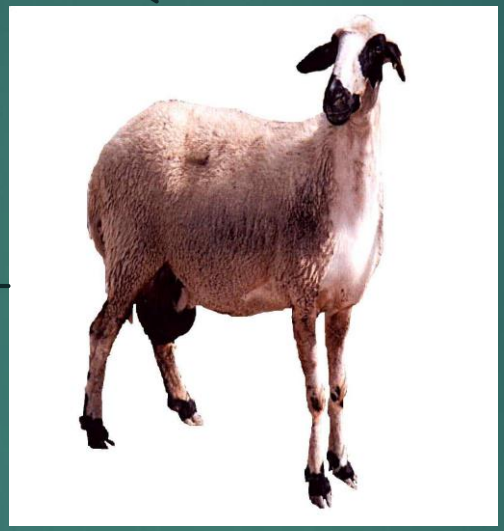
AA and especially methionine are the most limiting nutrients for milk production in high yielding ruminants



Choline availability determines the LCFA uptake from the mammary gland

Methionine affects the milk fatty acids profile

Choline affects positively the liver lipid metabolism

Participates:

- in the transportation of LCFA from blood to the udder
- on liver FA metabolism
- in the *de novo* synthesis of SCFA and MCFA

- However, in animal metabolism two are the principal methyl donors:

  -S-adenosyl-L-methionine (methionine metabolite)

  - betaine (choline metabolite)

Thus, methionine, choline and betaine are metabolically closely interrelated and their simultaneous role on milk fatty acids profile should be defined

- So far, the separate role of methionine , choline and betaine on milk yield and chemical composition has been extensively studied

- Moreover, recent papers with dairy cows confirm also, the significant role of methionine in glutathione synthesis (GST)

Objective

The objective of this study was to determine the effect of dietary supplementation with rumen-protected methionine alone (M) or in combination with rumen-protected choline and betaine (MCB) on:

- milk yield, chemical composition and fatty acids (FA) profile,
- blood plasma glutathione transferase (GST) activity and
- total antioxidant capacity in both plasma and milk

of periparturient dairy ewes.

- Forty five Chios breed dairy ewes, which were in the last fifth of their pregnancy were divided into three equal groups (n = 15) balanced according to their maximum milk yield from the previous lactation:

  -Control group (Control): no supplementation

  -Methionine group (M): 2.75 g methionine /animal/day

  -Methione/Choline/Betaine group (MCB): 2.20, 1.00 and 0.30 g methionine, choline and betaine /animal/day

- Each animal fed daily, in average, a basal diet consisted of 1.6 kg alfalfa hay, 0.2kg wheat straw and 2 kg concentrates

- The experiment started 15 days before lambing and lasted until 60 days in milk

- Individual milk samples were collected from the ewes at 10, 20 and 60 days after parturition for the analysis of milk chemical composition and fatty acids profile

- At the same days, in the individual milk samples, the total antioxidant capacity was determined by using the Ferric Reducing Ability of Plasma **(**FRAP) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays.
- Moreover, at the same intervals (10, 20 and 60 days after parturition) individual blood plasma were collected for glutathione transferase (GST) activity, FRAP and ABTS determination.
- Individual blood samples were also taken at 10 and 20 day after parturition for urea and β-hydroxybutyrate analysis

Statistical analysis

Data were analyzed using a general linear model (GLM) for repeated-measures analysis of variance (ANOVA) considering sampling time (S) as repeated measure, with fixed effects of dietary treatments (D = CONTROL, M, MCB), sampling time (S), the interactions among them (D x S) and ewes as random effect. Post hoc analyses were performed when appropriate using Duncan's multiple range test. Statistical analysis was performed using the statistical packages SPSS (version 20.0).


RESULTS

Table 1. Mean milk yield and chemical composition of ewes fed a basal diet supplemented with rumen-protected methionine alone (M) or in combination with rumen-protected choline and betaine (MCB)

	Treatments				Effect	
	Control	**M**	**MCB**	**SEM**	**Diet**	**Sampling time**
Milk yield (Kg)	2.37	2.26	2.48	0.416	NS	***
Fat (%)	4.90^{a}	5.74^{b}	5.45^{ab}	0.346	*	***
Crude protein (%)	5.34	5.54	5.40	0.177	NS	***
Lactose (%)	5.71	5.74	5.82	0.096	NS	***
Total solids (%)	16.74^{a}	17.82^{b}	17.48^{ab}	0.446	*	***
Total solids non fat (%)	11.81	12.08	12.04	0.156	NS	NS

Means with different superscript (a, b) in each row (between dietary treatments) for each parameter differ significantly ($P \leq 0.05$)

Table 2. The mean individual fatty acids (FA) (% of total FA)of ewes fed a basal diet supplemented with rumen-protected methionine alone (M) or in combination with rumen-protected choline and betaine (MCB)

	Treatment				Effect		
	Control	**M**	**MCB**	**SEM**	**Diet**	**Time**	**DxT**
$C_{4:0}$	4.14	4.15	4.14	0.141	NS	NS	NS
$C_{6:0}$	3.60	3.48	3.57	0.190	NS	NS	NS
$C_{8:0}$	3.72	3.80	3.63	0.212	NS	*	NS
$C_{10:0}$	10.96	11.25	10.75	0.660	NS	NS	NS
$C_{12:0}$	6.06	6.13	5.76	0.408	NS	NS	NS
$C_{14:0}$	11.37	11.63	11.32	0.389	NS	NS	NS
$C_{14:1}$	0.21^{a}	0.32^{b}	0.34^{b}	0.021	***	***	***
$C_{15:0}$	1.02	0.94	0.97	0.044	NS	NS	NS
$C_{15:1}$	0.33	0.31	0.33	0.024	NS	NS	NS

Table 2 (cont.)

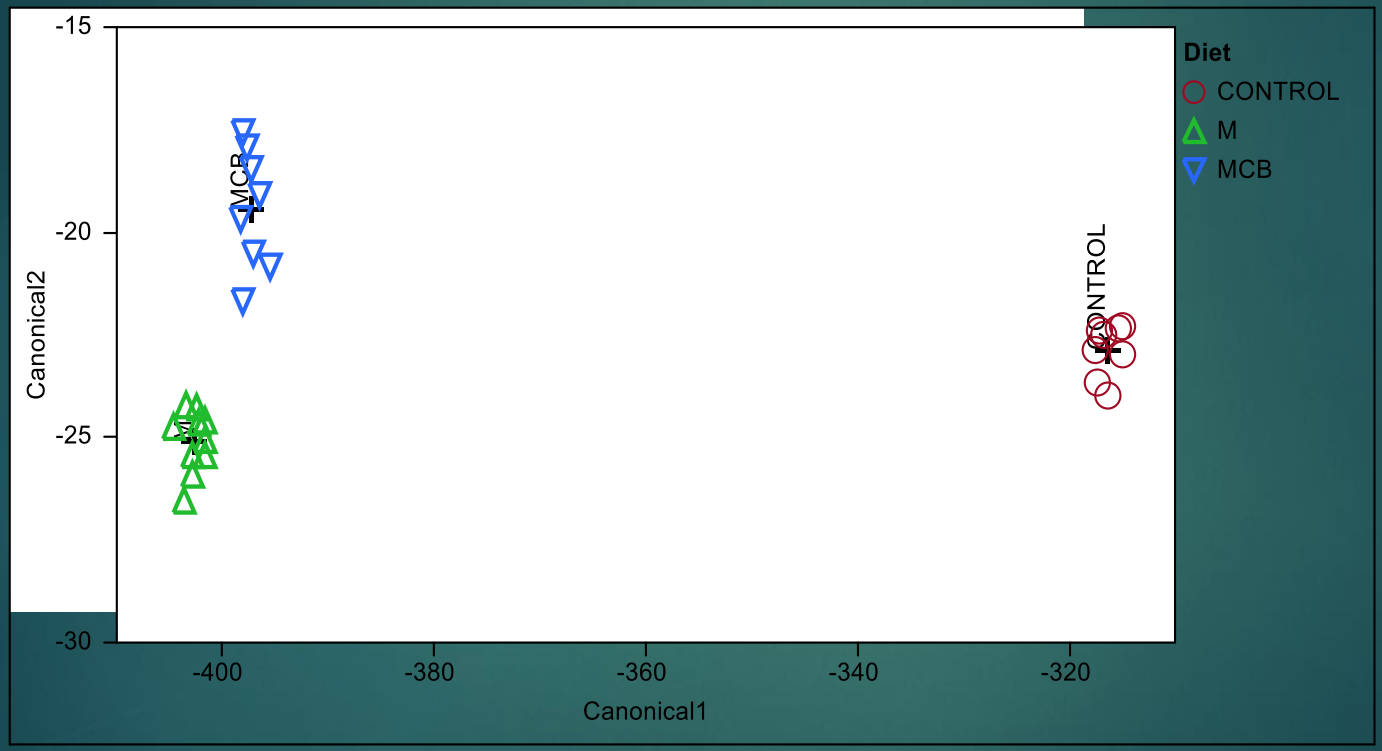
	Treatment				Effect		
	Control	**M**	**MCB**	**SEM**	**Diet**	**Time**	**DxT**
$C_{16:0}$	24.93	25.33	25.95	1.072	NS	NS	NS
$C_{16:1}$	0.44^{a}	0.58^{b}	0.91^{c}	0.052	***	**	***
$C_{18:0}$	7.52	7.58	6.89	0.648	NS	NS	*
trans-11 $C_{18:1}$	1.39	1.49	1.65	0.194	NS	NS	NS
trans 18:1	0.36^{ab}	0.33^{a}	0.42^{b}	0.029	*	NS	NS
cis-9 18:1	16.35	15.95	16.01	0.839	NS	NS	NS
$C_{18:2n6c}$	4.06	3.48	3.74	0.262	NS	NS	NS
*cis-9, trans-11*$C_{18:2}$	0.82	0.81	0.97	0.083	NS	NS	NS
C18:3n3	0.79^{a}	0.63^{b}	0.75^{a}	0.063	*	NS	NS

Table 2 (cont.)

	Treatment				Effect		
	Control	**M**	**MCB**	**SEM**	**Diet**	**Time**	**DxT**
SCFA	22.72	22.97	22.38	0.980	NS	NS	NS
MCFA	44.43	44.08	44.04	1.141	NS	NS	NS
LCFA	7.62	7.68	6.99	0.654	NS	NS	*
MUFA	19.99	19.84	20.55	0.789	NS	NS	NS
PUFA	6.24^{a}	5.42^{b}	6.03^{ab}	0.313	*	NS	*

Means with different superscript (a, b) in each row (between dietary treatments) for each parameter differ significantly ($P \leq 0.05$)

Figure 1. Discriminant plot separating the ewes (samples) according to the diet that they were fed (Control, M, MCB), based on milk FA profile.



Sign (+) is is the centroid of each group

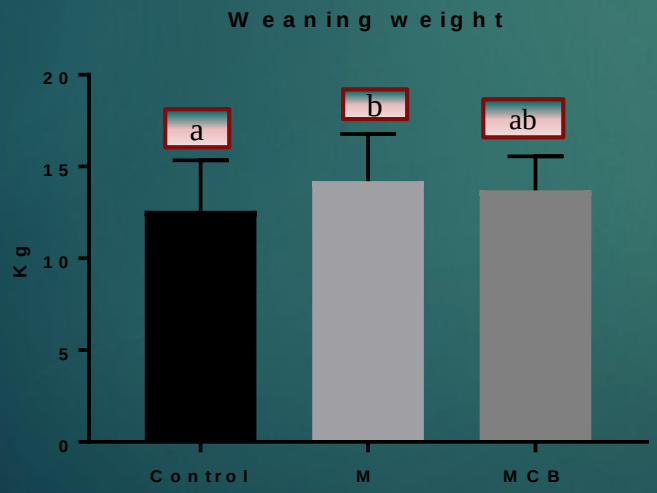
Table 3. Mean concentrations of blood plasma metabolites (BHBA and urea), mean total antioxidant capacity and glutathione transferase activity of ewes fed a basal diet supplemented with rumen-protected methionine alone (M) or in combination with rumen-protected choline and betaine (MCB)

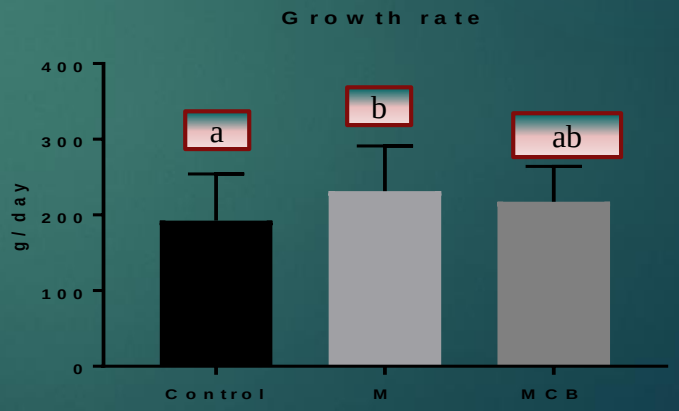
	Treatments				Effects	
	Control	**M**	**MCB**	**SEM**	**Diet**	**Time**
BHBA (mmol/l)	0.49^{a}	0.42^{ab}	0.32^{b}	0.06	*	NS
Urea (g/l)	0.43	0.48	0.44	0.036	NS	NS
Blood						
FRAP (µM ascorbic acid)	0.79^{a}	0.86^{a}	1.06^{b}	0.079	*	NS
ABTS (µM ascorbic acid)	90.16	85.60	91.66	3.045	NS	NS
GST (units/ml)	0.160^{a}	0.219^{b}	0.172^{ab}	0.020	*	NS
Milk						
FRAP (µM ascorbic acid)	1.05^{a}	1.42^{b}	1.33^{ab}	0.124	*	**
ABTS (µM ascorbic acid)	142.19	142.60	135.38	5.810	NS	*

Means with different superscript (a, b) in each row (between dietary treatments) for each parameter differ significantly (P≤0.05)

Figure 2. The mean body weight (Kg) and growth rate (g/day) of lambs derived from ewes fed a basal diet supplemented with rumen protected methionine alone (M) or in combination with rumen protected choline and betaine (MCB) during the 42 day suckling period

	Control	M	MCB
Body weight (Kg)	$12.59^{a}\pm0.631$	$14.21^{b}\pm0.64$	$13.71^{ab}\pm0.423$
Growth rate (g/ day)	$192.43^{a}\pm14.19$	$231.44^{b}\pm14.91$	$217.48^{ab}\pm10.68$





Conclusions

- The dietary supplementation with rumen-protected methionine revealed favorable alterations in the milk fat content of sheep and on the growth rate of suckling lambs, both of which affects the farm profitability.

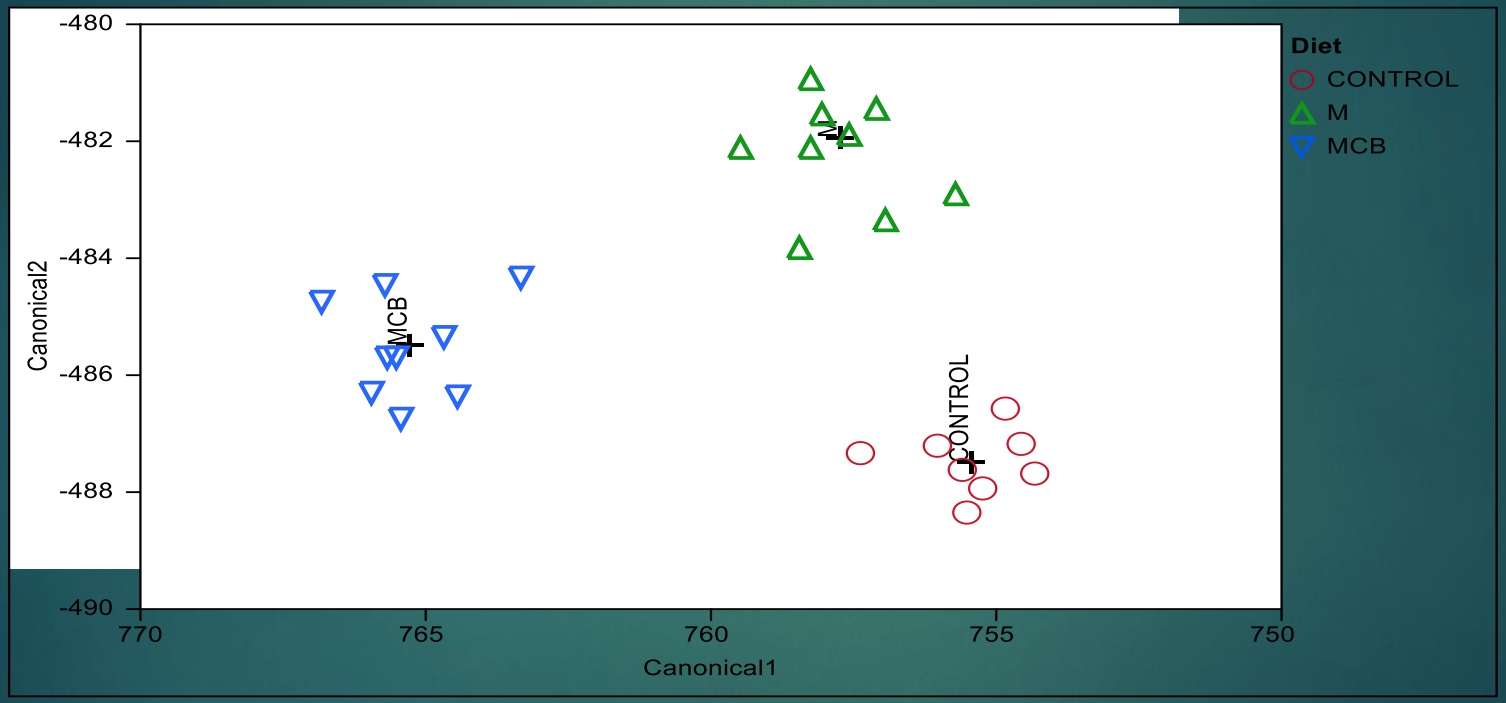
- The respective responses of the diets (M and MCB) on GST and FRAP activities suggest an improvement in the antioxidant defense system of the animals and consequently in its health.

Conclusions

- The significantly higher free radical scavenging activity measured either with FRAP or ABTS in the milk of ewes (10 days after parturition onwards) may reduce the risk of spoilage in this dairy product due to unpleasant oxidation.


תודה
Dankie
Gracias
Спасибо
شكراً
Merci
Takk
Köszönjük
Terima kasih
Grazie
Dziękujemy
Dėkojame
Ďakujeme
Vielen Dank
Paldies
Kiitos
Täname teid
谢谢
Thank You
Tak
感謝您
Obrigado
Teşekkür Ederiz
감사합니다
Σας ευχαριστούμε
ขอบคุณ
Bedankt
Děkujeme vám
ありがとうございます
Tack

Figure 2. Discriminant plot separating the ewes (samples) according to the diet that they were fed (Control, M, MCB), based on milk yield and chemical composition.



Sign (+) is is the centroid of each group

Figure 3. Discriminant plot separating the ewes (samples) according to the diet that they were fed (Control, M, MCB), based on free radical scavenging activities in milk and blood plasma.

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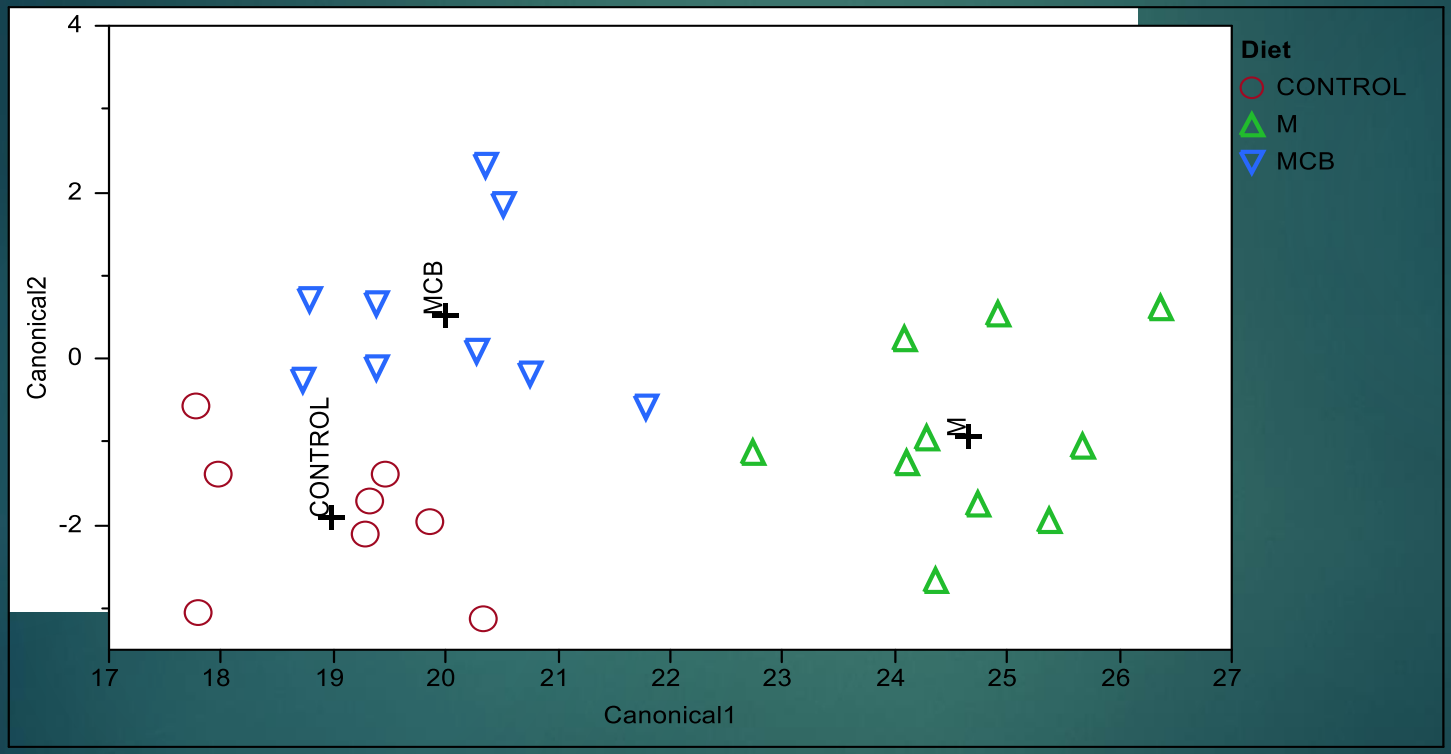


Sign (+) is is the centroid of each group

Materials and methods

- Concentrates consist of:

Ingredient (g/kg)	Control	M	MCB
Maize grain	340	340	340
Barley grain	200	200	200
Wheat bran	200	200	200
Soya bean meal	120	120	120
Sunflower meal	100	100	100
Calcium carbonate	20	20	20
Dicalcium phosphate	15	15	15
Salt	4	4	4
Minerals and vitamins mix	1	1	1
TIMET	-	2,5	
MECOVIT	-	-	5

Commercial Products

- TIMET: 550 g rumen -protected DL methionine/kg, at 2.5 g/kg concentrates

- MECOVIT: 220 g DL methionine, 100 chloride choline and 30 betaine, all in rumen –protected form at 5/kg concentrates

Materials and methods

- Chemical composition of experimental diets:

	Alfalfa hay	Wheat straw	Concentrates		
			Control	M	MCB
Dry matter (%)	88,01	92,85	88,78	89,96	88,62
Crude protein (%)	18,32	2,56	16,15	16,45	16,24
Crude fiber (%)	24,53	41,32	4,34	4,90	4,83
NDF (%)	462	715	207	211	205
ADF (%)	356	512	83	85	81
Ether extract (%)	1,96	1,54	3,08	2,78	3,22
Ash (%)	6,28	6,68	6,71	6,37	6,40

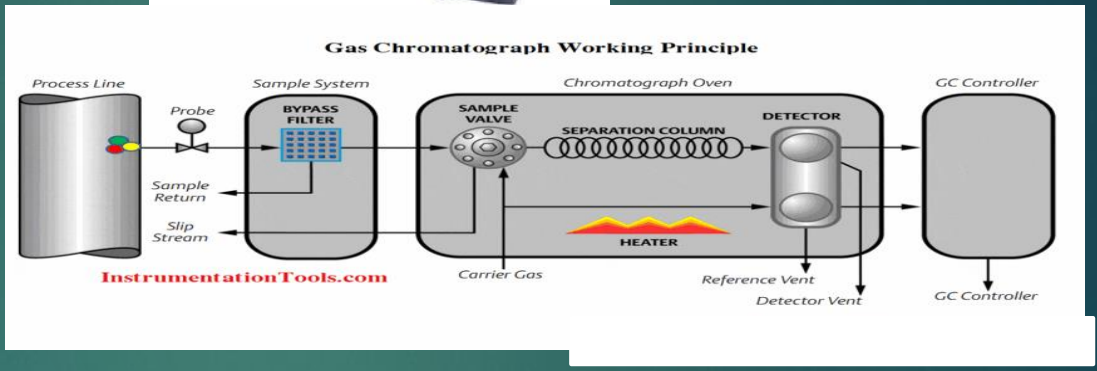
Materials and methods

Methodology

- Milk chemical composition:

Infrared analysis

after mixing the yield from the evening and the morning milking on a 5 per cent volume.

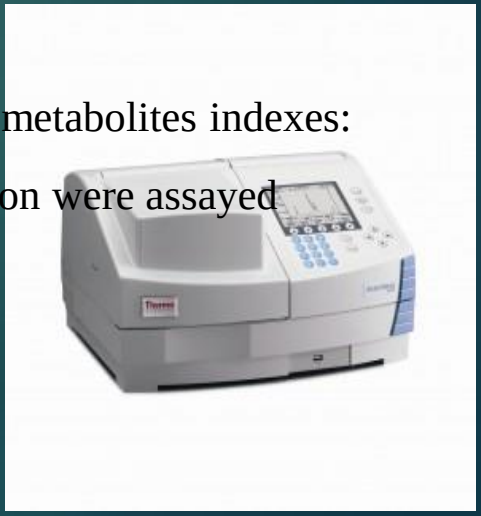


- Milk fatty acid profile:

GC/FID analyzed as fatty acids methyl esters

- Total antioxidant capacity (TAC), glutathione transferase (GSt) activity and metabolites indexes:

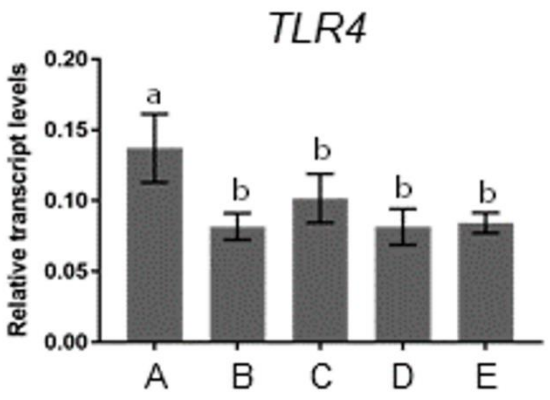
ABTS and FRAP method for TAC, GSt activity, Urea and B-HBA concentration were assayed spectrophotometrically

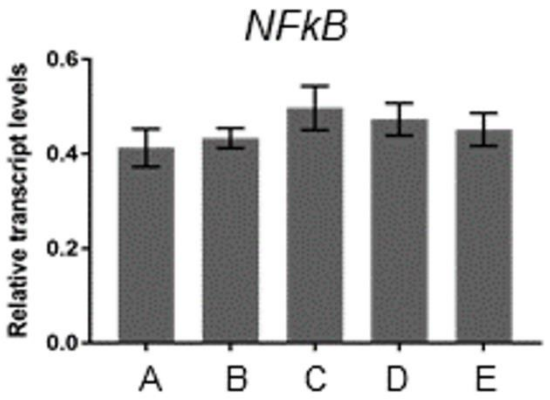


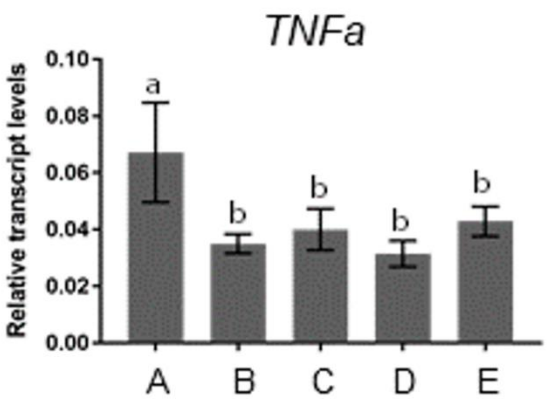
Statistics

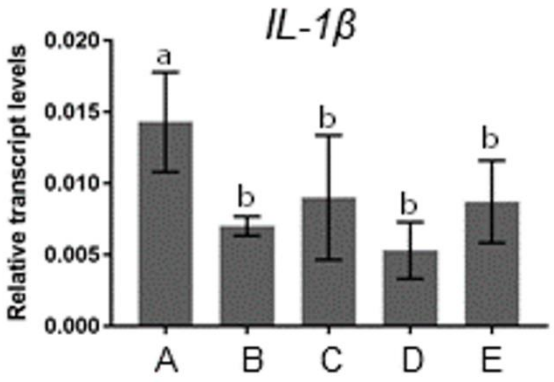
- Post hoc analyses were performed when appropriate using Duncan's multiple range test. Statistical analysis was performed using the statistical packages SPSS (version 20.0).
- Moreover, discriminant analysis was also applied to pooled data to establish those variables capable of distinguishing and classifying samples among the three dietary treatments. Wilk's lambda (λ) criterion was used for selecting discriminant variables.

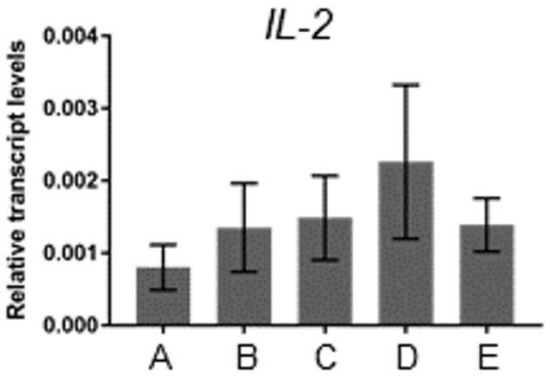
Macrophages

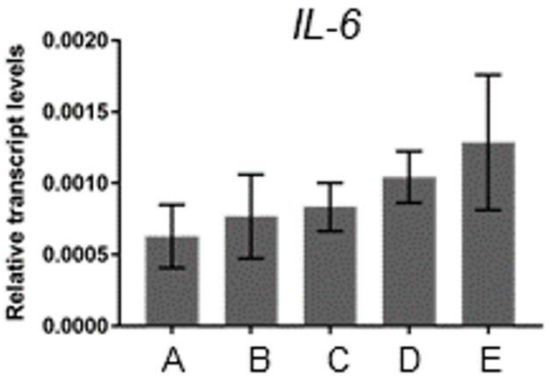


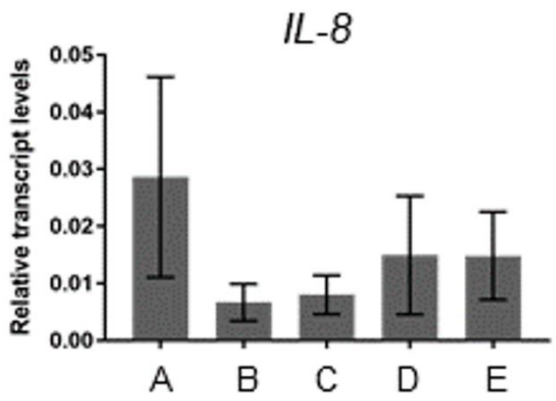


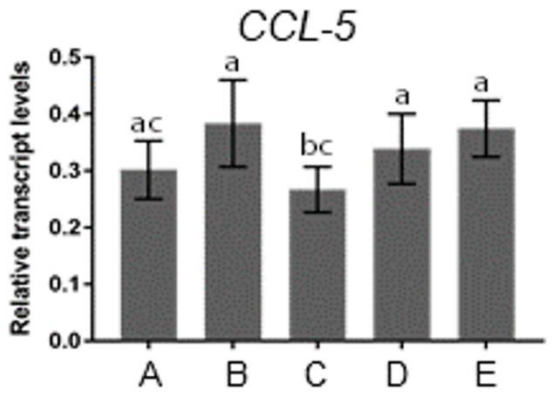


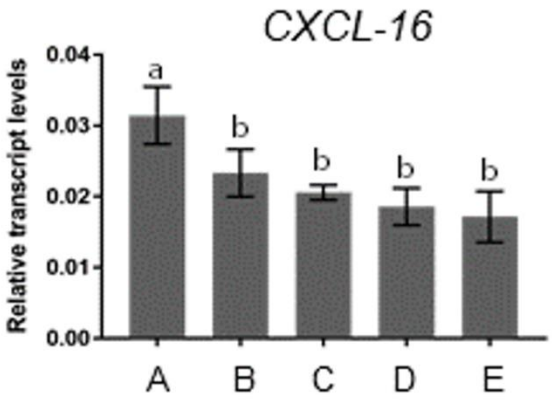


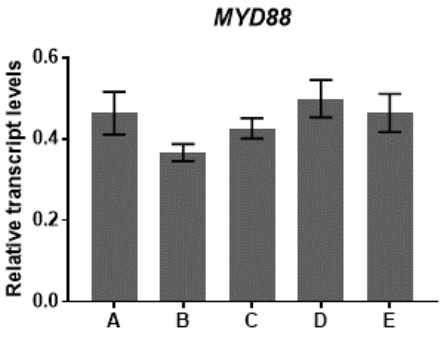








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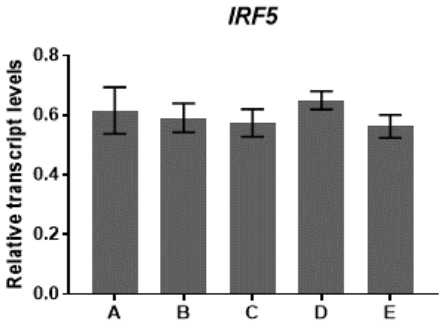
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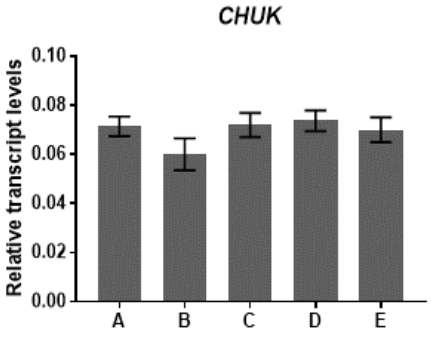
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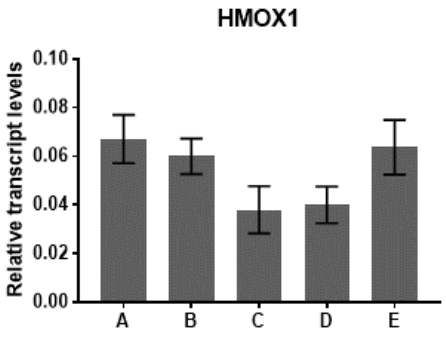
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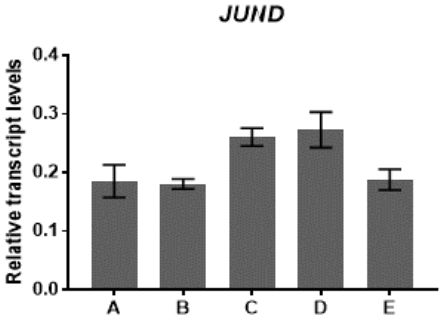
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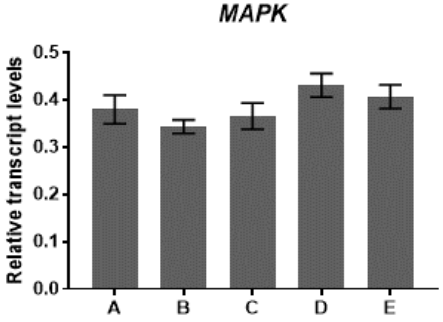
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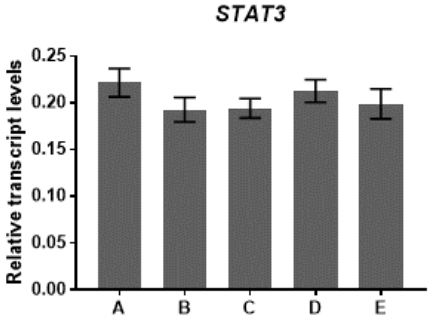
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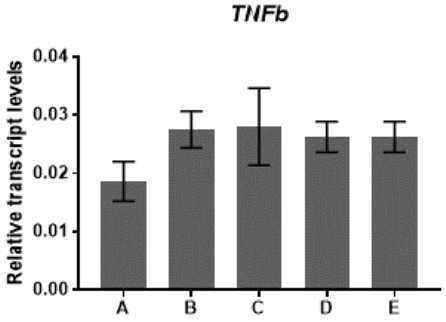
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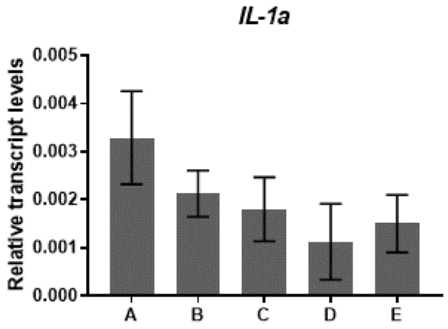
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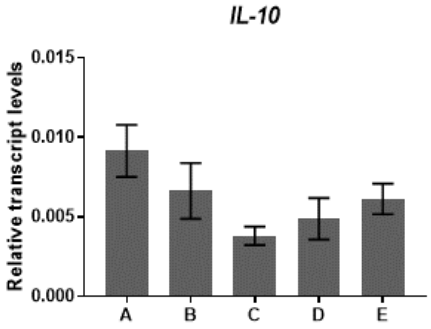
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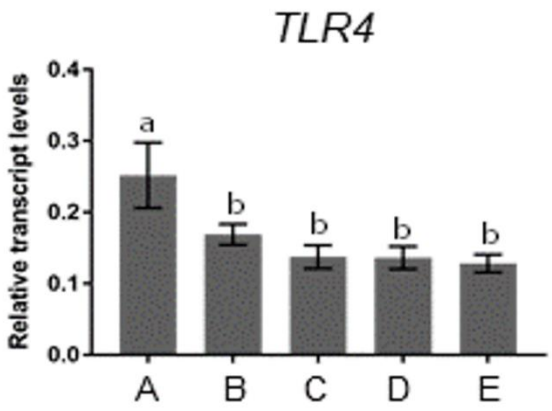
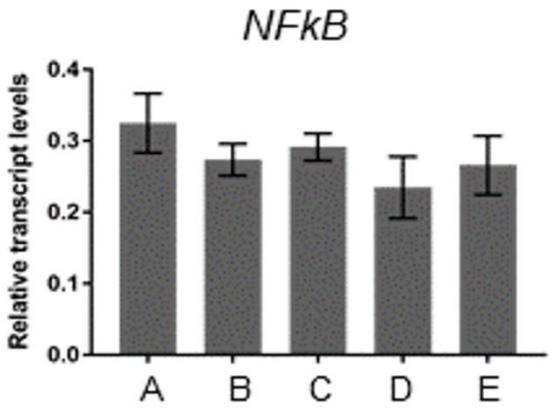
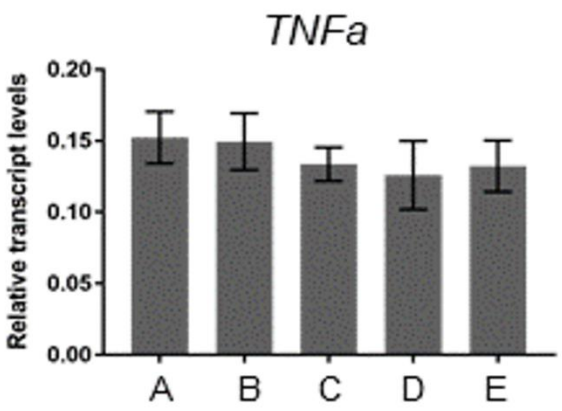
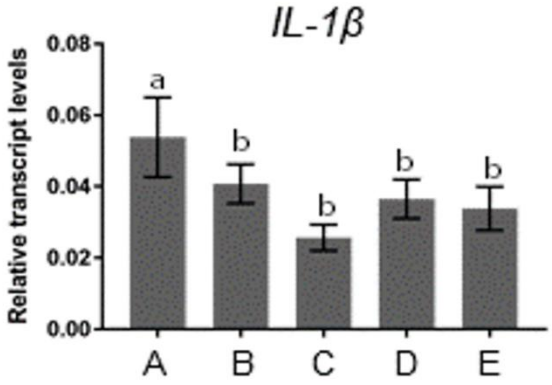
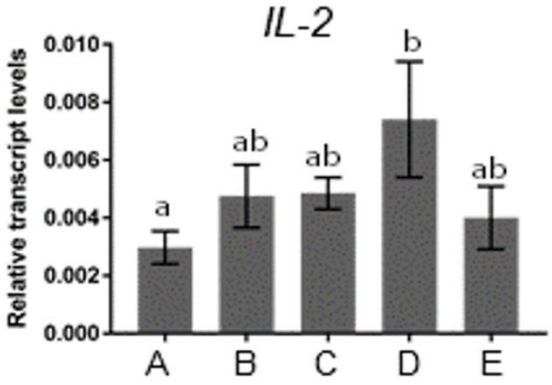
Neutrophils

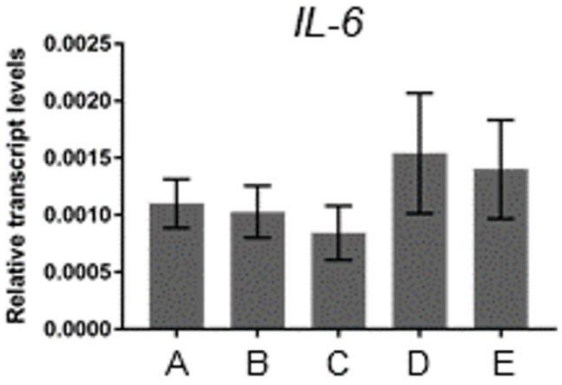


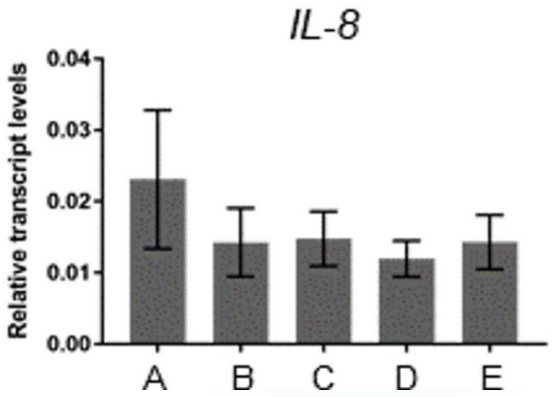


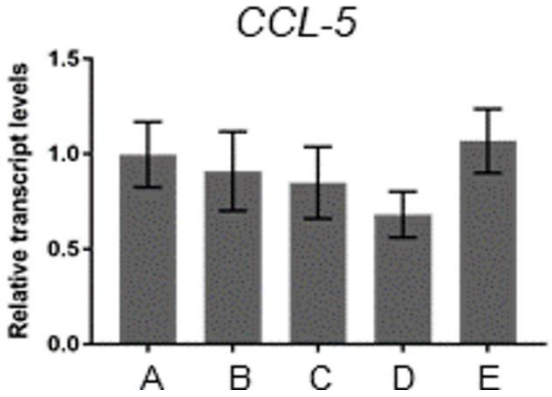


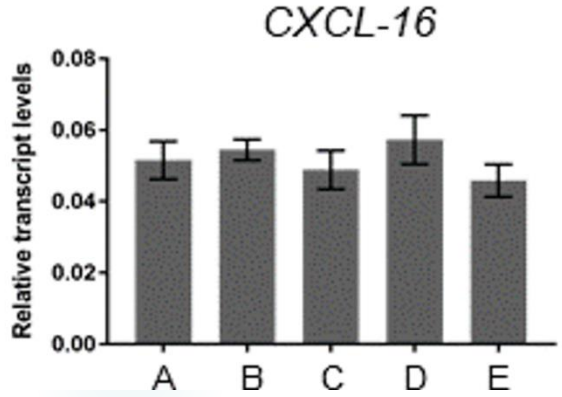


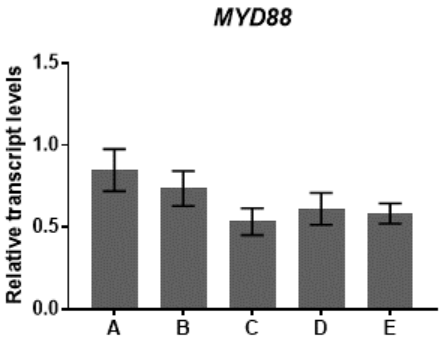











MYD88
Relative transcript levels
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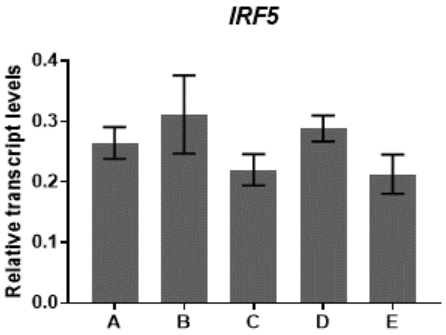
1.0
0.5
0.0
A
B
C
D
E


IRF5
Relative transcript levels
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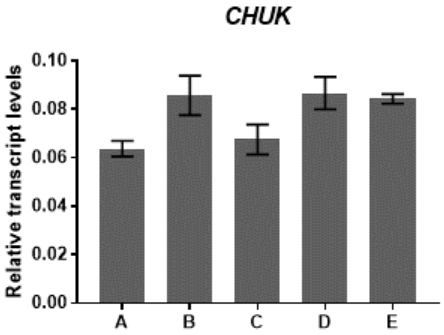
0.3
0.2
0.1
0.0
A
B
C
D
E


CHUK
Relative transcript levels
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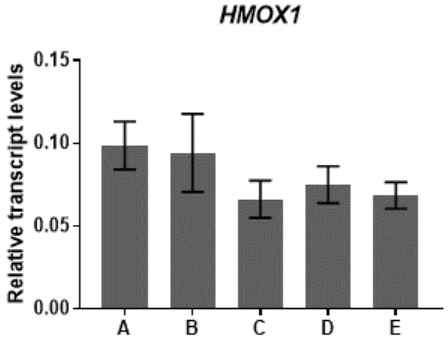
0.08
0.06
0.04
0.02
0.00
A
B
C
D
E


HMOX1
Relative transcript levels
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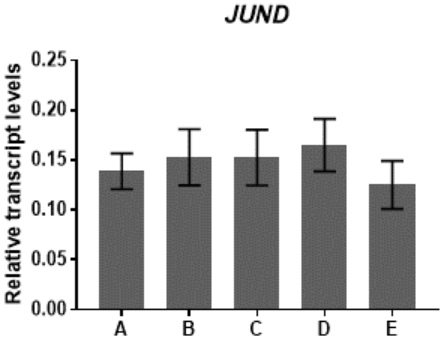
0.10
0.05
0.00
A
B
C
D
E


JUND
Relative transcript levels
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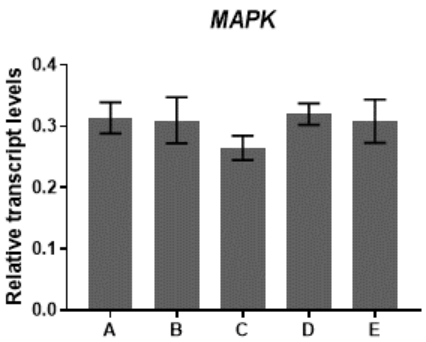
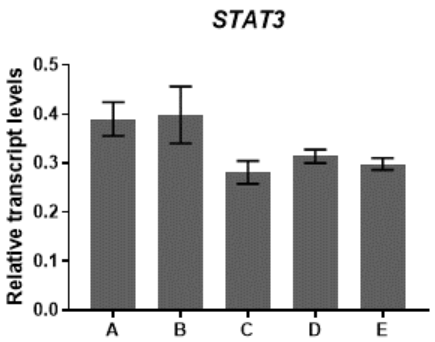
0.20
0.15
0.10
0.05
0.00
A
B
C
D
E


MAPK
Relative transcript levels
0.4
0.3
0.2
0.1
0.0
A
B
C
D
E


STAT3
Relative transcript levels
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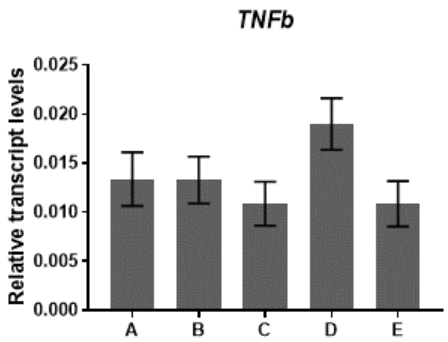
0.4
0.3
0.2
0.1
0.0
A
B
C
D
E


TNFb
Relative transcript levels
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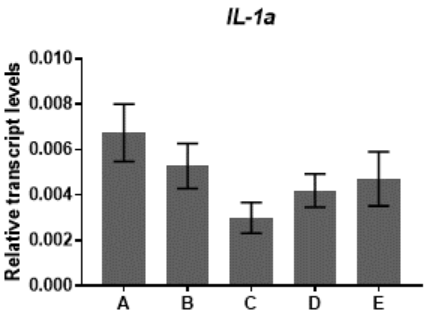
0.020
0.015
0.010
0.005
0.000
A
B
C
D
E


IL-1a
Relative transcript levels
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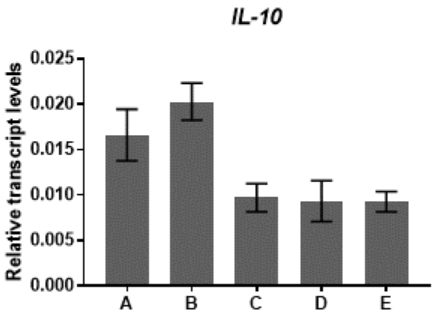
0.008
0.006
0.004
0.002
0.000
A
B
C
D
E


IL-10
Relative transcript levels
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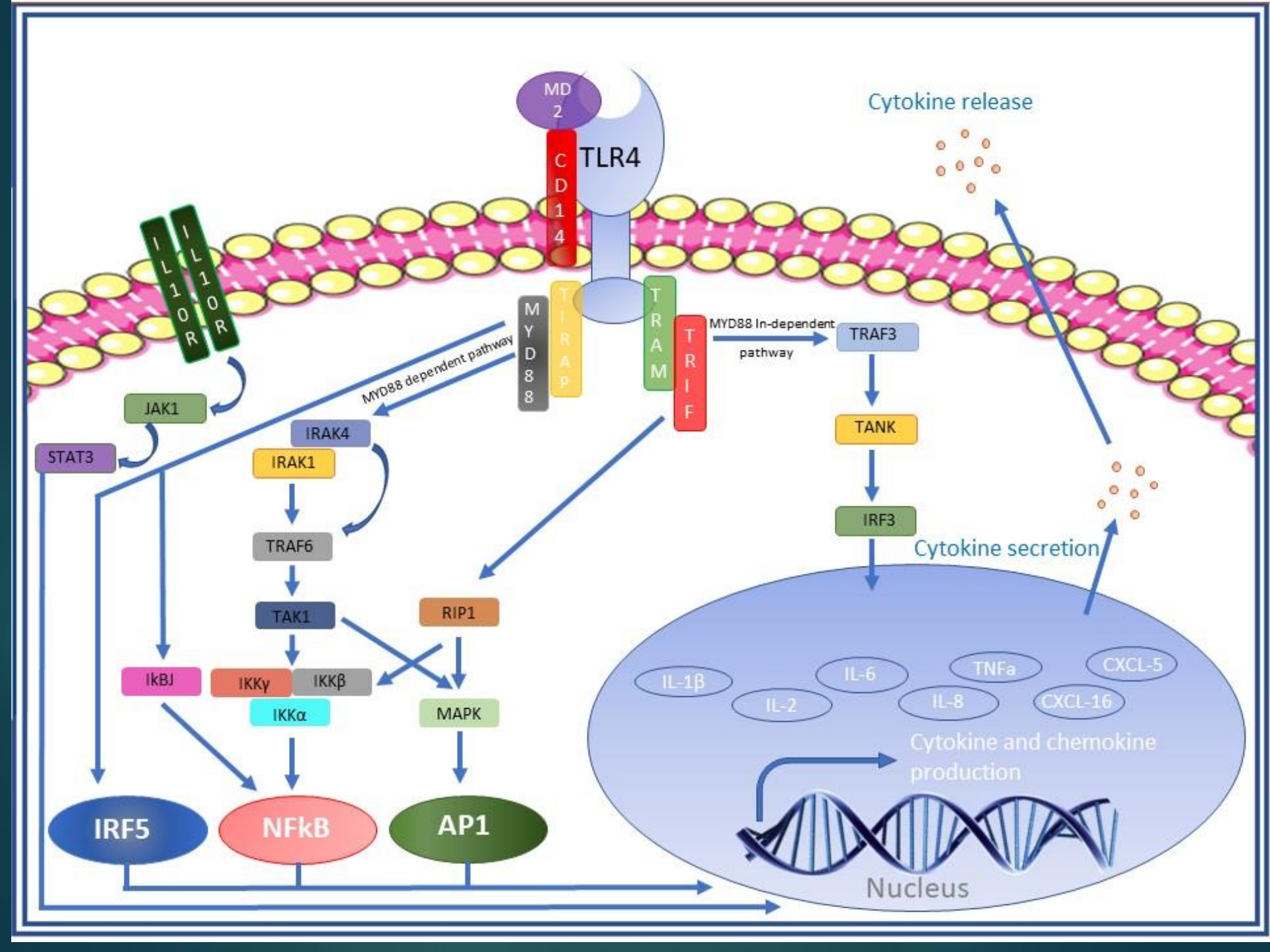
0.020
0.015
0.010
0.005
0.000
A
B
C
D
E


MD 2
TLR4
CD14
Cytokine release
IL10R
IL10R
MYD88
TIRAP
TRAM
TRIF
MYD88 In-dependent pathway
TRAF3
MYD88 dependent pathway
JAK1
STAT3
IRAK4
IRAK1
TRAF6
TANK
IRF3
Cytokine secretion
TAK1
RIP1
IkBJ
IKKγ
IKKβ
IKKα
MAPK
IL-1β
IL-6
TNFa
CXCL-5
IL-2
IL-8
CXCL-16
Cytokine and chemokine production
IRF5
NFkB
AP1
Nucleus