EFFECTS OF A. ABSITHIUM SUPPLEMENTED FEED ON THE SPECIFIC CELL-MEDIATED RESPONSE IN PIGS FROM A LOW-INPUT FARM

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Institute (Gaziantep University), Place (IZMIR, TURKEY)

Date of the event (20-22 October 2022)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 816172

Raising pigs in extensive system enhances their susceptibility to changes in micro- and macro- climate (incontrolable stresfull factor)

The infectious pressure is increased due to direct, unrestricted contact with the environment.

Parasitic, bacterial and viral diseases cause major losses in swine, thus inducing a high health, welfare and also economic impact.

More and more wide-spreading free-range farming depends on the factors targeting environment protection, plant health, animal health, food safety, and consumer health.

Under immune suppressive circumstances it is important to define and use

imunestimulating/imunomodulating products of vegetal origin

Potentiate the host ability to control infection

Diminishes the allopatic/synthetic drug consumption

Prevents antibiotico-resistance

Objectives

1. Testing tolerance to **oral administration of *Artemisia absithium***
2. Testing the ***in vitro*** **spontaneous and mitogen induced cell-mediated immune responsiveness**
3. Testing the ***in vitro*** **effects of other plant extracts**

Materials and methods

Alcoholic plant extracts were prepared according to the provisions of German pharmacopoeia by the University of Pharmacy, Cluj-Napoca, Romania

Method 1. A new LC-MS method was used to identify 6 polyphenols in WS extracts: epicatechin, catechin, syringic acid, gallic acid, protocatechuic acid and vanilic acid.

Method 2. The MS signal was used only for qualitative analysis based on specific mass spectra of each polyphenol. The MS spectra obtained from a stanYesrd solution of polyphenols were integrated in a mass spectra library.

Dosages of Artemisia absinthium were established based on the literature

Materials and methods

The research was carried out on extensively raised Mangalitza suckling, weaned piglets and sows (n=10 for each group).

The feed supplemented with 5‰ Artemisia absinthium L and granulated was admninistered as Yesily ratio for 7-10 Yesys/group.

Materials and methods

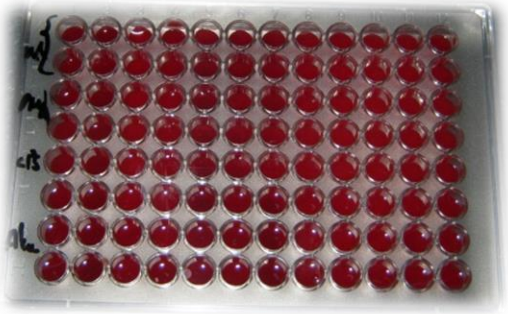
Blood was sampled before and after the end of oral treatment period; then it was mixed with RPMI1640 (1:4, Sigma Aldrich, USA), divided in 200µl aliquots in 96 well-plates and supplemented with alcoholic plant extracts (Calendula officinalis, Satureja hortensis, Allium sativum, Coriandrum sativum, Cucurbita maxima, 1.5 µl/well).

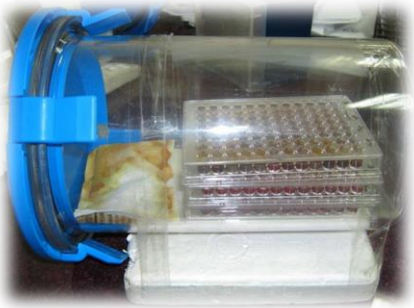





Materials and methods

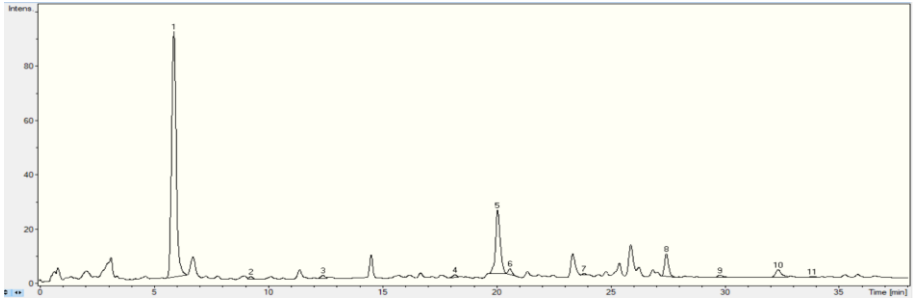
The plates were incubated at 37^{o}C for 48 h, residual glucose was quantified spectrophotometrically (SUMAL PE2, Karl Zeiss, Jena) and stimulation indices (SI %) were calculated.





The groups were compared by Student's t test for statistical significance of the results.

Results and discussions



The MS spectra obtained for poliphenols in Artemisia absinthium
Chlorogenic acid
antidiabetic effect, DNA protective effect, and neuroprotective effect. inhibitory activity against hepatitis B virus (HBV) in vivo and in vitro
antioxidant

Polyphenols (method 1)					
Nr. pe cromatogramă	Compus	Nr.	Identified UV	Identified by qualitatuive MS	Concentration in the extract (µg/ml)
1	Clorogenic acid	4	Yes	Yes	107.150
2	p-cumaric acid	5	Yes	Yes	0.621
3	Ferulic acid	6	Yes	Yes	0.759
4	Vitexine	8	Yes	Yes	1.631
5	Isoquercitrine	11	Yes	Yes	56.754
6	Rutozid	12	Yes	Yes	3.826
7	Quercitrine	15	Yes	Yes	1.113
8	Quercetol	17	Yes	Yes	6.285
9	Luteoline	20	Yes	Yes	1.159
10	Kaempferol	21	Yes	Yes	3.666
11	Apigenine	22	Yes	Yes	0.481

Results and discussions

Polyphenols (method 2)	
Siringic acid	1.85 (µg/mL)
Protocatechuic acid	1.32 (µg/mL)
Vanilic acid	1.98 (µg/mL)

Metoxilate flavones	
Eupatorine	976.53 (ng/mL)
Casticine	15385.14 (ng/mL)
Hispidulin e	3047.92 (ng/mL)

Tocopherols	
Alpha-tocopherol	50.0 (ng/mL)
Gamma-tocopherol	23.8 (ng/mL)
Delta-tocopherol	5.0 (ng/mL)

The extract of Artemisia annua L. has been provides anti-inflammatory, anti-bacterial and anti-microbial properties which can be considered as a promising medicinal component in therapeutic applications.

Results and discussions

Sterols	
Ergosterol	344 (ng/mL)
Stigmasterol	34831 (ng/mL)
Beta-sitosterol	140985 (ng/mL)
Campesterol	3329 (ng/mL)

Sesquiterpen lactones	
Alfa-santonin	450.52 (ng/mL)
Vulgarin	6499.39 (ng/mL)

Vulgarin possesses strong and stable binding efficiency with multidrug resistant (MDR) Acinetobacter baumannii eflux protein (Ab-EP), a known pathogen for one health

Beta-sitosterol exhibited the potential to inhibit the biosynthesis of peptidoglycan and prevent bacteria cell wall formation

Suvaithenamudhan, S.; Ananth, S.; Mariappan, V.; Dhayabaran, V.V.; Parthasarathy, S.; Ganesh, P.S.; Shankar, E.M. In Silico Evaluation of Bioactive Compounds of Artemisia pallens Targeting the Efflux Protein of Multidrug-Resistant Acinetobacter baumannii (LAC-4 Strain). Molecules 2022, 27, 5188

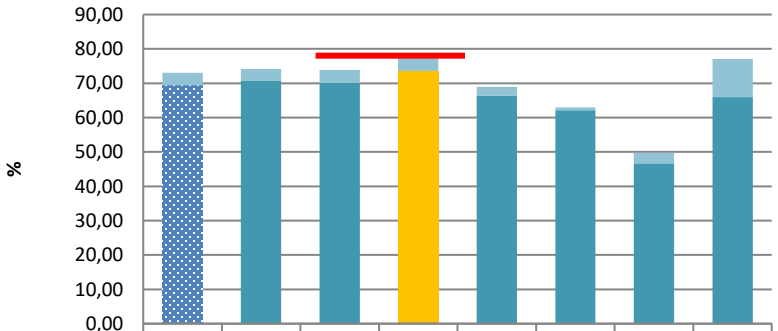
Evangelina IA, Herdiyati Y, Laviana A, Rikmasari R, Zubaedah C, Anisah, Kurnia D. Bio-Mechanism Inhibitory Prediction of β-Sitosterol from Kemangi (Ocimum basilicum L.) as an Inhibitor of MurA Enzyme of Oral Bacteria: In vitro and in silico Study. Adv Appl Bioinform Chem. 2021 Jun 23;14:103-115.

Results and discussions

The oral treatment with the A. absithium supplemented feed significantly ($p<0.05$-0.001) decreased all SI%, the least in suckling piglets.
Only the extract of C.maxima acted stimulating in suckling piglets (53.37±7.13%).

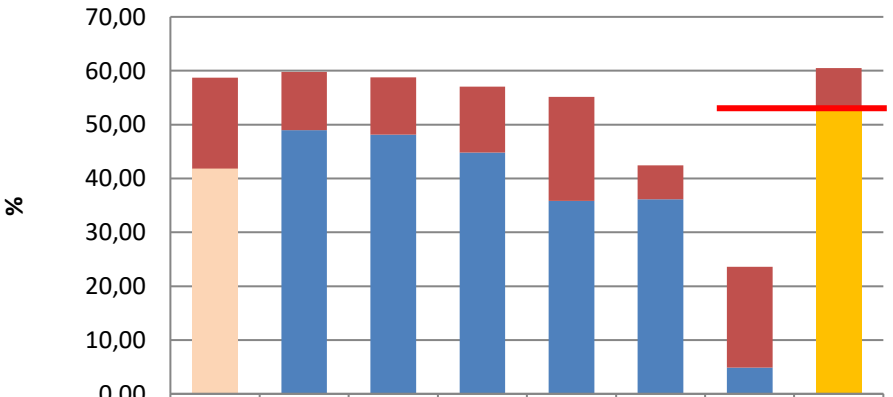
Before

Suckling piglets



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovle
Stdev	3,67	3,36	3,77	4,29	2,60	0,83	3,08	11,07
Med purcei	69,39	70,86	70,10	73,57	66,39	62,17	46,72	66,01

After



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovl
Stdev	16,89	10,82	10,64	12,23	19,27	6,29	18,68	7,13
Med purcei	41,83	48,94	48,13	44,84	35,85	36,14	4,92	53,37

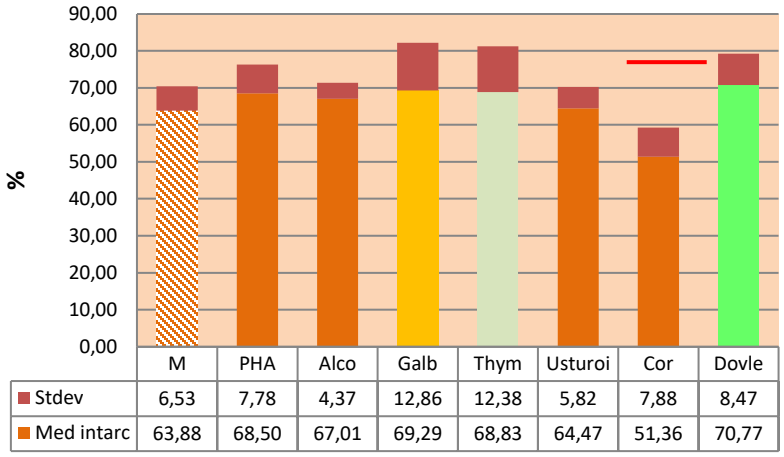
Results and discussions

None of the extracts acted stimulating, the lowest indices being recorded for C. sativum, within the negative range in weaners and sows.

Only the extract of C.maxima acted stimulating in suckling piglets (53.37±7.13%).

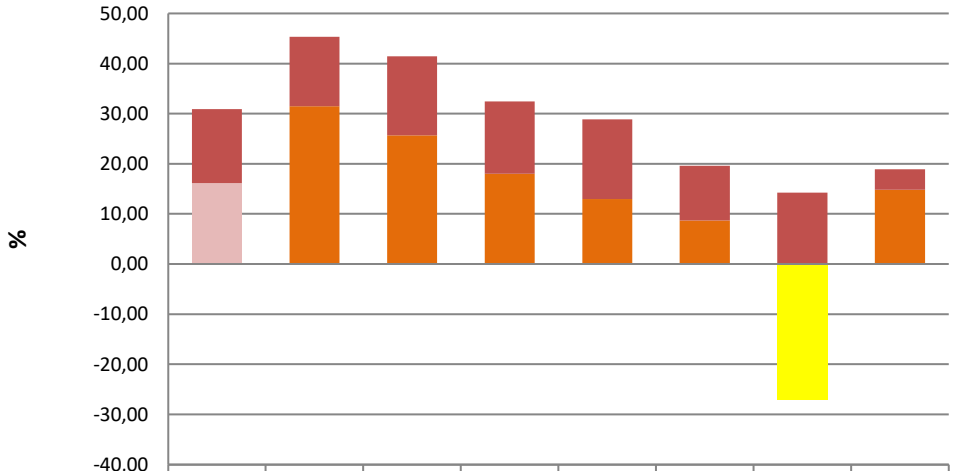
Before

Weaners



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovle
Stdev	6,53	7,78	4,37	12,86	12,38	5,82	7,88	8,47
Med intarc	63,88	68,50	67,01	69,29	68,83	64,47	51,36	70,77

After



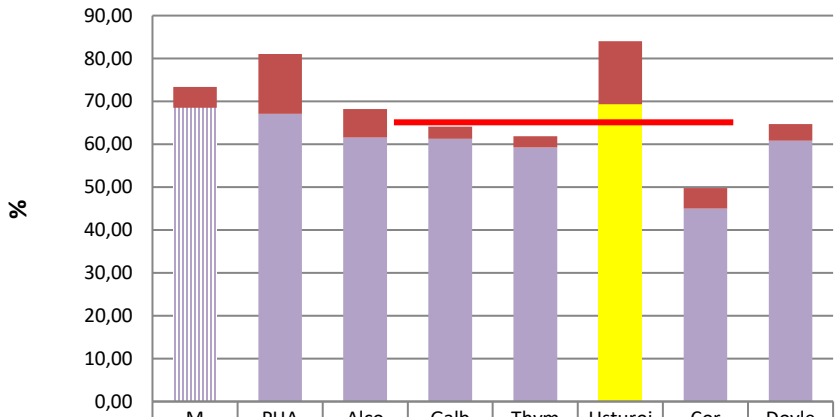
	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovl
Stdev	14,71	13,88	15,76	14,46	15,86	10,95	14,24	4,07
Med intarc	16,18	31,47	25,69	18,00	12,98	8,67	-27,02	14,84

Results and discussions

None of the extracts acted stimulating, the lowest indices being recorded for C. sativum, within the negative range in weaners and sows.

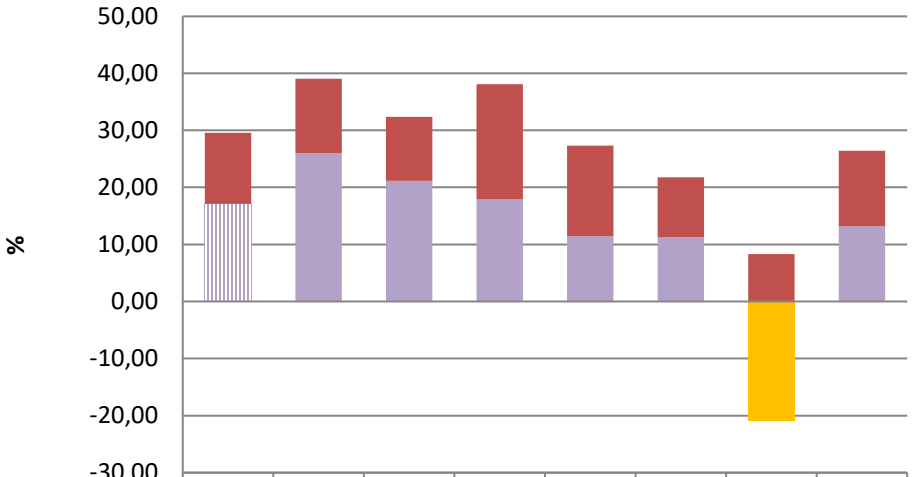
Before

Sows



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovle
Stdev	4,89	14,01	6,67	2,85	2,65	14,73	4,76	3,91
Med scroafe	68,50	67,06	61,54	61,27	59,25	69,31	44,99	60,79

After



	M	PHA	Alco	Galb	Thym	Usturoi	Cor	Dovl
Stdev	12,46	13,08	11,19	20,19	15,90	10,49	8,30	13,25
Med scroafe	17,10	25,96	21,15	17,87	11,42	11,26	-20,98	13,17

Conclusion

The results indicated negative effects of A. absinthium on the specific immune response when administered orally in pigs, suggesting the eventual reconsideration of its administration dosage/protocole.

Acknowledgements

This research was funded by PPILOW projectof the European Union's Horizon 2020 programme, grant agreement 816172.

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