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IMPACT OF HEAT STRESS ON THE MEAT QUALITY OF RABBITS DIVERGENTLY SELECTED FOR TOTAL BODY FAT CONTENT

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ABSTRACT

The present research studied the impact of heat stress condition during farming on the meat quality traits of rabbits divergently selected for total body fat content. A total of 60 10-week old rabbits belonging to the 5th generation of divergent selection were used: they consisted of the 25% of the population with the lowest body fat content (Lean) and the 25% with the highest body fat content (Fat). Lean and Fat rabbits were housed at two different environmental temperatures: 20 °C (Control), and 28 °C (Heat). Therefore, four groups of 15 rabbits each were considered: Control Lean, Control Fat, Heat Lean and Heat Fat. From each carcass, meat was dedicated to the following analytical determinations: proximate composition, heme-iron content, oxidative status and fatty acid (FA) profile. Data were analysed by a two-way ANOVA with environmental temperature (Control, Heat) and divergent selection (Lean, Fat) as fixed effects. Overall, farming temperature and divergent selection, but not their interaction, affected most of the meat quality traits evaluated in the present study. High ambient temperature increased meat heme-iron ($P<0.001$) and water ($P<0.001$) contents, the latter to the detriment of the lipids ($P<0.001$). Heat stressed rabbit exhibited the greatest PUFA ($P<0.001$) and the lowest MUFA ($P<0.001$) fractions. Fat rabbits were characterized by a higher fat ($P<0.001$), ash ($P=0.008$) and heme-iron ($P<0.001$) content compared to Lean rabbits, whereas meat oxidative status displayed the opposite situation ($P=0.037$). Fat rabbits had a FA profile poorer in PUFA ($P<0.001$) and richer in MUFA ($P<0.001$) compared to Lean rabbits, as a result of a significant reduction in the *n*-6 fraction ($P<0.001$). The latter led to a decrease of the *n*-6/*n*-3 ratio ($P<0.001$) in Fat group. Overall, heat stress condition did not impair rabbit meat quality, including its oxidative status. Different body fat contents did not result in specific meat qualitative changes in response to different environmental temperatures.

Key words: Growing rabbits, Divergent selection, Body fat, Heat stress, Meat quality

INTRODUCTION

The rabbit is a homeothermic animal and is very sensitive to high environmental temperatures. It can increase breathing rate and peripheral temperature to favour heat loss, but it only has a few functional sweat glands which represent a strong limit in this capability. The comfort temperature zone of this lagomorph was estimated to be 15-21°C after weaning. When environmental temperatures exceed 25°C, the rabbit starts to adopt strategies to lose as much heat as possible (De Lima *et al.*, 2013). This threshold limit represents a possible issue for rabbit production, especially in tropical areas or, more generally, in climatic zones with hot summers, as it is the case of Mediterranean countries, which are also the biggest rabbit meat producers in Europe. Heat stress is responsible for increased oxidative stress in livestock animals (Belhadj Slimen *et al.*, 2016). Previous findings on rabbits showed that heat stressed animals are characterised by impaired homeostasis which negatively reflects on growth performance, possibly leading to relevant economic loss (Hassan *et al.*, 2016). When assessing meat quality characteristics, heat stress was observed to alter colour profile and increase cooking losses (Zeferino *et al.*, 2013). However, to the best of the authors' knowledge, studies providing a detailed evaluation of meat quality traits of rabbits farmed under heat stress conditions are still rather scarce.

Based on these premises, the present research study aimed to evaluate the impact of heat stress on meat quality attributes of rabbits divergently selected for total body fat content, the latter being a potential source of variation in determining different meat quality responses depending on environmental temperature.

MATERIALS AND METHODS

Animals and experimental design

The rabbits used in the present study belonged to a maternal line (Pannon Ka), which was created at the Kaposvár University (Hungary). For the present trial, a total of 60 10-week old rabbits belonging to the 5^{th} generation of divergent selection were used. Divergent selection was performed according to the total body fat content through Computer Tomography (CT): in each generation, the 25% of the population with the lowest body fat content (Lean) and 25% with the highest body fat content (Fat) were selected and used as breeding stock to breed the next generation. For the present trial, 30 rabbits belonged to the Fat group and the other 30 rabbits to the Lean group. From weaning up to slaughter, Lean and Fat rabbits were farmed at two different environmental temperatures: a lower temperature (20 °C) was used as Control, and a higher temperature (28 °C) was referred to as Heat Stress (Heat). Therefore, four groups of 15 rabbits each were considered in the present trial: Control Lean, Control Fat, Heat Lean and Heat Fat. Rabbits had *ad libitum* access to feed and water throughout the experiment and the photoperiod was set at 16 hours light and 8 hours dark. The day after slaughtering, chilled deboned half carcasses (n=60) were transported in refrigerated conditions to the MAPS Department of the University of Padova (Italy).

Sample preparation and chemical analyses

All the half carcasses were individually coarsely ground by means of a professional meat grinder, equipped with a plate with 5 mm diameter holes. From each carcass, 200 g of meat was sampled and finely ground with a Retsch Grindomix GM 200 (7000 g for 4 s): 20 g was then used to quantify meat heme-iron content (Hornsey, 1956), and the extent of lipid oxidation. The latter was evaluated with a spectrophotometer (Hitachi U-2000; Hitachi, 209 Mannheim, Germany) set at 532 nm, that measured the absorbance of thiobarbituric acid-reactive substances (TBARs) with a 1,1,3,3-tetraethoxypropane calibration curve (Botsoglou *et al.*, 1994). Oxidation products were quantified as malondialdehyde (MDA) equivalents (mg MDA/kg meat). The remaining part of each sample was freeze-dried and used to determine proximate composition (AOAC, 2012) and fatty acid (FA) profile. For the latter, the lipid extraction was performed by Accelerated Solvent Extraction (M-ASE) using the solvent mixture chloroform:methanol 1:2. Samples were subsequently treated according to the method described by Dalle Zotte *et al.* (2018). The results are expressed as % of total detected Fatty Acids Methyl Esters (FAME).

Statistical analysis

Data were analysed by a two-way ANOVA that considered environmental temperature (Temp: Control, Heat) and selection group (Sel: Lean, Fat) as fixed effects (PROC GLM; SAS, 2008). The interaction Temp x Sel was also considered. A Shapiro-Wilk test was performed to evaluate normality. Least square means were obtained using the Bonferroni test and the significance was calculated at $P<0.05$ level.

RESULTS AND DISCUSSION

Results presented in Table 1 show that the single effects of Temp and Sel, but not their interaction, significantly affected most of the studied variables, which is consistent with previous findings on growing rabbits (Zeferino *et al.*, 2013). Heat stress condition decreased meat lipids content ($P<0.001$) in favour of water ($P<0.001$), whereas protein and ash remained unaffected. This also agreed with other studies on rabbits (Chiericato *et al.*, 1996; Dalle Zotte *et al.*, 2016; Zeferino *et al.*, 2013). Under heat stress condition, hyperthermic animals tend to reduce feed intake which is a strategy to reduce

heat production (Belhadj Slimen *et al.*, 2016). Moreover, in mammals heat stress condition seems to lead to a reduced carcass fatness, with different partitions in fat tissues (Baziz *et al.*, 1996). In poultry, it was also observed that heat stress affects fat deposition in a breed-dependent manner, depending on the specific tolerance to changes in environmental temperature (Lu *et al.*, 2007). Despite heat stress conditions typically has a detrimental effect on the *in vivo* oxidative stress, which is mainly due to increased reactive oxygen species production and a decreased antioxidant defence (Belhadj Slimen *et al.*, 2016), in the present study heat stress exerted no effect on meat oxidative status. This could hypothetically be due to an increased mobilization of endogenous antioxidants and antioxidant enzymes to scavenge the produced radicals (Belhadj Slimen *et al.*, 2016), the latter was however not assessed in the present study. Rabbits housed at 28 °C also displayed the highest heme-iron content ($P<0.001$) which could be explained by an overproduction of transition metal ions through an increased rate of iron released from ferritin (Belhadj Slimen *et al.*, 2016). As a result of the divergent selection, rabbits belonging to the Fat group exhibited a higher lipid ($P<0.001$), and ash ($P=0.008$), content compared to Lean rabbits, the latter showing the highest water content ($P<0.001$). These results are coherent with the purpose of the divergent selection in which the two rabbit populations should display different body fat contents, including that of meat (Dalle Zotte *et al.*, 2021). Meat of the Fat group was also richer in heme-iron ($P<0.001$) and, surprisingly, displayed the lowest oxidation degree ($P=0.037$), the latter being potentially due to the modifications occurring to meat FA profile.
Main FA classes were affected by both temperature and divergent selection, but not by their interaction (Table 2). Rabbits housed under heat stress exhibited a reduction in MUFA ($P<0.001$) and an increase in PUFA, both *n*-6 ($P<0.001$) and *n*-3 ($P=0.003$) fractions; conversely, SFA remained unaffected.

Table 1: Effect of environmental temperature (Temp) on the proximate composition (g/100 g meat), heme-iron content (mg/100 g meat) and oxidative status (mg/kg meat) of meat obtained from rabbits in generation 5 divergently selected for low (Lean) or high (Fat) body fat content

Temperature	**Control**		**Heat**		**Prob.**			**MSE**[1]
Selection	**Lean**	**Fat**	**Lean**	**Fat**	**Temp**	**Sel**	**Temp x Sel**	
Rabbits, no.	15	15	15	15				
Water	73.0	71.1	75.2	73.6	<0.001	<0.001	0.772	2.07
Protein	21.7	21.7	21.5	21.9	0.889	0.210	0.108	0.11
Lipids	7.95	10.9	6.11	7.91	<0.001	<0.001	0.264	0.26
Ash	1.25	1.33	1.22	1.33	0.807	0.008	0.614	0.14
Heme-iron	0.42	0.84	0.67	1.03	<0.001	<0.001	0.571	0.28
TBARs	0.14	0.13	0.16	0.14	0.252	0.037	0.583	0.03

[1]Mean square error

Table 2: Effect of heat stress (Heat) on the fatty acids profile (% of total FAME) of meat obtained from rabbits in generation 5 divergently selected for low (Lean) or high (Fat) body fat content

Temperature	**Control**		**Heat**		**Prob.**			**MSE**[1]
Selection	**Lean**	**Fat**	**Lean**	**Fat**	**Temp**	**Sel**	**Temp x Sel**	
Rabbits, no.	15	15	15	15				
SFA	34.2	34.9	34.1	34.8	0.949	0.096	0.973	1.37
MUFA	27.7	32.7	24.3	27.8	<0.001	<0.001	0.288	3.91
PUFA	31.4	27.4	35.3	31.3	<0.001	<0.001	0.966	3.38
n-6	29.1	25.2	32.8	28.8	<0.001	<0.001	0.915	3.26
n-3	2.32	2.19	2.45	2.48	0.003	0.465	0.212	0.25
n-6/*n*-3	12.6	11.5	13.6	11.7	0.130	<0.001	0.268	1.52

[1]Mean square error.

The observed FA profile modifications could be the result of a cellular attempt to modulate specific physiological properties (fluidity and cellular function) of membranes, stimulated by lipid peroxidation due to heat stress condition (Belhadj Slimen *et al.*, 2016). A further possible explanation could be the fact that rabbit diets are rich in MUFA (high presence of alfalfa), thus depressed feed intake due to heat stress should lead to a major effect on the amount of ingested and deposited MUFA (Dalle Zotte, 2002). Five generations of divergent selection allowed appreciation of an increased MUFA ($P<0.001$) proportion of the Fat group to the expense of the PUFA ($P<0.001$) proportion, which could hypothetically explain the lower meat oxidation degree of Fat groups compared to Lean groups. The overall PUFA reduction in the Fat meat is attributable, in particular, to the *n*-6 FA (P<0.001), as the *n*-3 fraction was similar in Lean and Fat groups. As a result, the *n*-6/*n*-3 ratio was lower, thus better in Fat rabbits than Lean rabbits (P<0.001).

CONCLUSIONS

Housing rabbits under heat stress condition did not impair overall meat quality which was often better than that of rabbits housed under normal temperature, especially regarding fat content and FA profile. Moreover, the 5th generation of divergent selection for total body fat content did not alter the capability of rabbits to adapt to heat stress condition during commercial farming.

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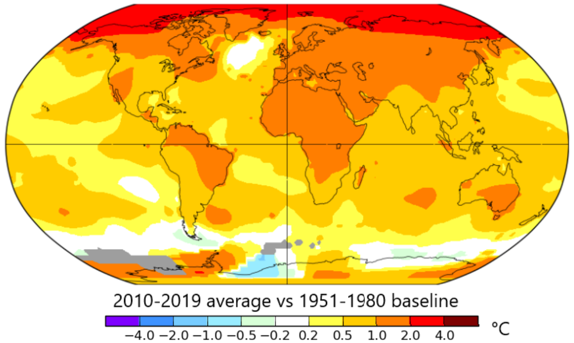

Impact of heat stress on the meat quality of rabbits divergently selected for total body fat content

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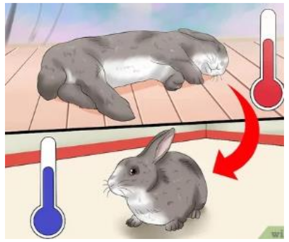
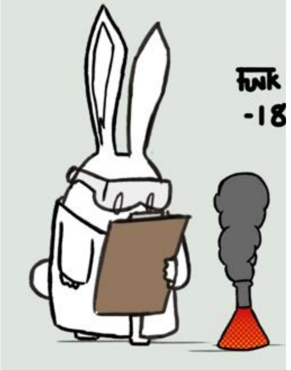
INTRODUCTION



- **Rabbit is very sensitive to T>25 °C:** breathing rate + peripheral temperature increase to allow heat loss but few functional sweat glands
- **Heat stress increases oxidative stress which impairs homeostasis:** possible issue for rabbit production, especially in tropical areas (or in climatic zones with hot summers)

Previous findings:



- **Negative effects** on growth performance and economic revenue (Hassan et al., 2016)
- **Altered** meat colour and increased cooking losses (Zeferino et al., 2013)

What about detailed meat quality characteristics?

AIM

Evaluate the impact of **heat stress** on **meat quality** attributes of **rabbits divergently selected for total body fat content** (potential source of variation in determining different meat quality responses)

- **Experimental design:** 2 body fat contents (Lean and Fat lines) x 2 environmental temperatures (20 and 28 °C)
- **N=60 rabbit does (n=30/line) + litters:** hused in 2 rooms (20 and 28 °C)

- **At weaning (5 wk): n= 240 rabbits** were uniformly distributed into 4 experimental groups (60 rabbits/group):

1. **Lean rabbits housed at 20 °C (Control-Lean)**
2. **Fat rabbits housed at 20 °C (Control-Fat)**
3. **Lean rabbits housed at 28 °C (Heat-Lean)**
4. **Fat rabbits housed at 28 °C (Heat-Fat)**

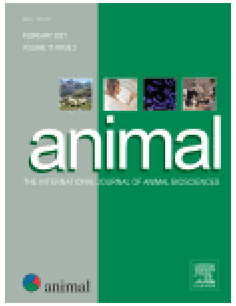
MATERIAL AND METHODS

Experiment layout:

- **Trial duration:** 5-13 weeks of age
- **Housing:** wire-mesh cages (40 × 38 × 30 cm; 2 rabbits/cage)
- **Feed and water:** *ad libitum*
- **Photoperiod:** 16L:8D

Performance and carcass traits:

- **Body weight (individual) and Feed intake (cage):** 5, 7, 9, 11, 13 wk of age
- **DWG (individual) and FCR /cage):** calculated
- **Mortality:** daily check
- **Slaughter:** 13 wk (4h transport/fasting)
- **Carcass dissection:** WRSA guidelines (Blasco & Ouhayoun 1993)



Results about performance and carcass traits: Matics, Zs., Gerencsér, Zs., Kasza, R., Terhes, K., Nagy, I., Radnai, I., Dalle Zotte , A., Cullere, M. & Szendrő, Zs. (2021). **Effect of ambient temperature on the productive and carcass traits of growing rabbits divergently selected for body fat content**. *Animal, 15(2*), 100096.

MATERIAL AND METHODS

Meat quality traits:

- **Chilled deboned half carcasses:** n=60: n=15/experimental group
- **Proximate composition** (*AOAC, 2012*)
- **He-iron content** (*Hornsey, 1956*)
- **Lipid oxidation** (TBARs: *Botsoglou et al., 1994*)
- **Fatty acids profile** (M-ASE, chloroform:methanol 1:2, *Dalle Zotte et al., 2018*)

Statistical analysis:

- **Two-way ANOVA** that considered environmental temperature (Temp: Control, Heat) and selection group (Sel: Lean, Fat) as fixed effects, as well as their interaction (PROC GLM; SAS, 2008)
- **Least square means** were obtained using the Bonferroni test and the **significance** was calculated at **P<0.05 level**

RESULTS

Temperature	Control		Heat		Prob.			MSE[1]
Selection	Lean	Fat	Lean	Fat	Temp	Sel	Temp x Sel	
Rabbits, no.	15	15	15	15				
Water	**73.0**	**71.1**	**75.2**	**73.6**	<0.001	<0.001	0.772	2.07
Protein	21.7	21.7	21.5	21.9	0.889	0.210	0.108	0.11
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Heme iron	**0.42**	**0.84**	**0.67**	**1.03**	<0.001	<0.001	0.571	0.28
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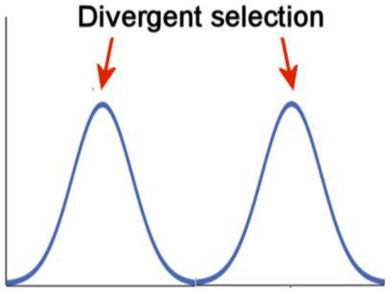
PUFA	**31.4**	**27.4**	**35.3**	**31.3**	<0.001	<0.001	0.966	3.38
***n*-6**	**29.1**	**25.2**	**32.8**	**28.8**	<0.001	<0.001	0.915	3.26
***n*-3**	**2.32**	**2.19**	**2.45**	**2.48**	0.003	0.465	0.212	0.25
***n*-6/*n*-3**	**12.6**	**11.5**	**13.6**	**11.7**	0.130	<0.001	0.268	1.52

[1]Mean squared error

CONCLUSIONS

- **Heat stress: did not impair overall meat quality** which was often better than that of rabbits housed under normal temperature (especially lipids and FA profile)



- **Divergent selection:** for total body fat content **did not alter the capability of rabbits to adapt to heat stress** condition during commercial farming

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Thank you very much for your kind attention

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