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# ARTICLE IN PRESS

Animal xxx (2020) xxx



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# Effect of ambient temperature on the productive and carcass traits of growing rabbits divergently selected for body fat content

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# ABSTRACT

Rabbits are particularly sensitive to heat stress which can affect productive performance, with rabbit breed/line possibly playing a role on the response to this condition. The study aimed at evaluating the effect of different ambient temperatures on the live performance and carcass traits of growing rabbits divergently selected for total body fat content. The two genetic lines (Lean and Fat) were selected based on the total body fat content estimated by computer tomography during five generations. From birth to slaughter (13 weeks of age), the rabbits were housed in two rooms where the temperature was controlled with air conditioners; in the control room the average ambient temperature was 20 °C and in the high temperature room it was 28 °C. After weaning (35 d), 60 Lean and 60 Fat rabbits/ room were housed by two in wire-mesh cages and fed ad libitum with commercial pellets. The BW and feed intake (FI) were measured at 5, 7, 9, 11 and 13 weeks of age to calculate the daily weight gain (DWG) and feed conversion ratio (FCR). Mortality was recorded daily. At the end of the experiment, rabbits were slaughtered and carcass traits were measured. Mortality was independent of temperature and line. The temperature significantly influenced the FI, DWG, BW and the fat deposits: they were lower at higher ambient temperature. The effect of temperature differed according to the rabbits' total body fat content. At control temperature, the FI (165 vs 155 g/day; P < 0.05) and FCR (4.67 vs 4.31; P < 0.05) were higher in Fat rabbits, which also had more perirenal (36.2 vs 23.1 g; P < 0.05) and scapular fat (10.8 vs 7.1 g; P < 0.05). At high temperature, no differences in fat depots (14.5 vs 9.8 g; 5.3 vs 3.5 g) were found between the two lines. It can be concluded that temperature × genetic line interaction had an important role in productive and carcass traits, as the effect of temperature differs between Lean and Fat rabbits.

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# **Implications**

Livestock production is being greatly affected by global warming. Among the farmed species, rabbits are particularly sensitive to high environmental temperatures which generally have detrimental effects on their productive performance. Therefore, a better understanding of the effects of the high ambient temperature on rabbits is fundamental to ensure both animal welfare as well as production efficiency and quality. This study confirmed that a high ambient temperature has a great influence on productive and carcass traits of rabbits and also demonstrated that Lean and Fat rabbits respond differently to heat stress condition.

# Introduction

Nowadays, livestock production is greatly affected by global warming. This includes areas with hot climates and areas with temperate climate zones, with hot summers. Rabbits are more sensitive to heat stress than most farm animal species because they have few sweat glands and their body is covered with hair (Maya-Soriano et al., 2015).

Outside the comfort zone of rabbits, which is around 21 °C (Marai et al., 2002), the efficiency of energy utilisation for production decreases. Several papers were published about the effect of environmental temperature on productive performances (Chiericato et al., 1993 and 1997; Marai et al., 1999; Zeferino et al., 2011; Ali and Abdel-Wareth, 2014; Szendrő et al., 2018). The most important heat stress effect is the decrease in feed intake (**FI**) and increase in water consumption. The FI decline occurs at about 22 °C (Szendrő et al., 2018) and could range between 3 and 38%. Contradictory results were reported on feed conversion ratio (**FCR**): Chiericato et al. (1997), Marai et al. (1999)

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# ARTICLE IN PRESS

Z. Matics, Z. Gerencsér, R. Kasza et al. Animal xxx (2020) xxx

and Zeferino et al. (2011) found no significant changes, Ali and Abdel-Wareth (2014) reported worsening results, whereas Chiericato et al. (1993) published improving results. Limited results have been published up to now on the effect of the heat stress on carcass traits. These results show that when rabbits are farmed at high temperatures, considered outside their comfort zone they consume less feed, thus, when they are slaughtered at the same age, the weight of the gastrointestinal part is reduced (Chiericato et al., 1993) the dressing out percentage (**DoP**) increased (Chiericato et al., 1993; Zeferino et al., 2013) but the fat depots reduced (Marai et al., 1999; Zeferino et al., 2013).

To the author's knowledge no past experiments evaluated the productive performance and carcass traits of divergently selected rabbit lines reared under different ambient temperature.

Based on the above-mentioned premises, the aim of the study was to investigate the effect of ambient temperature on growing rabbits divergently selected for total body fat content. The productive performance and carcass traits were examined in the fifth generation, focusing on the ambient temperature × genetic line interactions.

# Material and methods

# Experimental design

The trial was conducted at the Kaposvár University (Hungary) using Pannon Ka growing rabbits, which were selected over 5 generations for low (Lean) and high (Fat) total body fat content as estimated by computer tomography (Kasza et al., 2016). Divergent selection process was carried out under conventional farm conditions on 15-25 °C ambient temperature. In the present trial, the rabbits (30 does and their litters per line) were housed in two rooms (in flat-deck cages;  $86 \times 38 \times 30$  cm) where the temperature was controlled with air conditioning (Fujitsu Air conditioning-system, ARYG30LMLE, Fujitsu General Limited, Suenaga, Takatsu-ku, Kawasaki, Japan). The average ambient temperature was 20 °C in the control room and 28 °C in the other room. During the experiment, the ambient temperature and the relative humidity were continuously registered every 30 min with an EBI 300 USB data collector (ebro Electronic GmbH, Ingolstadt) in both rooms. Starting from birth, the rabbits were housed at control (20 °C) or high (28 °C) ambient temperatures and, after weaning (5 weeks of age (wk)), they continued to be reared in the same room until 13 wk, of age. Temperature-humidity index was calculated based on Marai et al. (2002) as followings:

$$THI = db^{o}C - [(0.31-0.31 \text{ RH}) (db^{o}C - 14.4)]$$

where db°C dry bulb temperature in Celsius and RH = relative humidity percentage/ 100. The calculated average temperature-humidity indexes were 18.5 and 25.4 in the control and high temperature rooms, respectively.

At weaning, a total of 240 rabbits (1 male and 1 female rabbits from each litter), 60 per line (Fat and Lean) and temperature room (20 and 28 °C), were randomly housed in wire-mesh cages ( $40 \times 38 \times 30$  cm; 2 rabbits/cage). They were fed *ad libitum* with commercial pellets (5–9 wk.: digestible energy: 9.94 MJ/kg, CP: 15.7%, crude fibre: 19%; 9–13 wk.: digestible energy: 10.6 MJ/kg, CP: 16.3%, crude fibre: 17.7%), and water was freely available from nipple drinkers. The lighting schedule was 16 h light and 8 h dark in both rooms.

# Production and carcass traits

Body weight (BW; individual) and FI (per cage) of the rabbits were measured at 5, 7, 9, 11 and 13 wk of age to calculate the daily weight gain (**DWG**; individual) and FCR (per cage, as the FI per cage divided with the sum of DWG of the 2 rabbits in the cage). Mortality was monitored daily. At the age of 13 wk., rabbits (n = 227) were transported to a slaughterhouse (transport with fasting length was 4 h) and

slaughtered by cutting the carotid arteries and jugular veins after electrical stunning. Body weight at slaughter, hot carcass (HC), chilled carcass (CC, after 24 h chilling in a ventilated room at 4 °C), head, set of organs (heart + lungs + thymus + trachea + oesophagus), liver, kidneys and reference carcass (RC; corresponding to the CC minus the set of organs, liver and kidneys) were weighed. From the RC (which included meat, bones and fat depots), the perirenal and scapular fat was removed, carcasses were cut between the 7th and 8th thoracic vertebrae and between the 6th and 7th lumbar vertebrae to obtain the fore, mid and hind parts of the carcass, which were also weighed separately. The Longissimus thoracis et lumborum (LTL) muscle (both sides) of the mid part was dissected, as well as the meat of the hind legs (HL) through deboning. The CC yield (DoP) was calculated as CC weight divided by slaughter weight × 100. Percentage of carcass parts, perirenal and scapular fats were computed to the RC weight. The meat/bone ratio of the carcass was predicted with the meat/bone ratio of the HL as it represents a reasonable estimation (Hernández et al., 1996).

# Statistical analysis of results

Statistical analyses were performed using SAS Version 9.4 package (SAS Institute Inc., 2014). BW and carcass traits of the present experiment were tested using Multi-way ANOVA. DWG, FI and FCR were evaluated considering repeated measures (weeks) analyses for these traits, taking the covariance among repeated measures into account. Temperature, line and temperature  $\times$  line interaction were considered as fixed factors. Group means were compared by means of Tukey test. A  $\chi^2$  test was performed on kits mortality to detect the differences among treatments.

### **Results**

# Productive performance

The rabbits had been reared at control and high temperature rooms since their birth and, as a consequence, a different BW of rabbits at weaning (5 wk) was found (Table 1). Overall, the ambient temperature and line significantly affected the productive performance of growing rabbits. Specifically, BW of rabbits remained affected throughout the experiment by both temperature (P < 0.001) and line (P < 0.05), while the temperature × line interaction was found at 5, 7, 9 and 11 wk. (P < 0.05). The significant temperature × line interaction (P < 0.01) showed that the BW of rabbits differed only at 28 °C temperature between lines, i.e., rabbits of the Fat line reared at higher temperature had lower BW than the Lean line.

Higher DWG was measured in rabbits reared at 20 °C compared to those at 28 °C (P < 0.001; Table 1). The differences in DWG between control and high temperature were 20.6 and 21.2% in Lean and Fat groups, respectively. The Line did not affect the above-mentioned trait.

Temperature and line significantly affected also the FI during the whole experimental period (P < 0.001 and P < 0.01, respectively). Temperature  $\times$  line interactions were also detected (P < 0.05): Rabbits housed at 28 °C ate less than those at 20 °C, and Fat rabbits ate more than Lean rabbits only when reared at 20 °C.

Concerning the FCR, significant temperature and line effects were manifested (P < 0.001): at 28 °C the FCR was lower, thus better, that at 20 °C. Furthermore, Fat rabbits had a worse FCR than Lean ones. Temperature  $\times$  line interaction (P < 0.05) showed that rabbits from Lean line used the feed more efficiently than Fat line at 20 °C, while at higher temperature no differences between lines were found.

The mortality of growing rabbits was independent of temperature and line (P > 0.05): it was 5.0 and 5.0% at 20 °C, 3.3 and 8.3% at 28 °C in Lean and Fat lines, respectively The causes of mortality were not examined.

Z. Matics, Z. Gerencsér, R. Kasza et al. Animal xxx (2020) xxx

Table 1 Effect of ambient temperature on the productive performance of growing rabbits of Lean and Fat genetic lines.

Temperature (T)											
Line (L)	20 °C		28 °C		SE	P-values					
	Lean <sup>1</sup>	Fat <sup>2</sup>	Lean <sup>1</sup>	Fat <sup>2</sup>		T	L	$T \times L$			
BW, g											
n	60	60	60	60							
5 weeks of age	868 <sup>a</sup>	858 <sup>a</sup>	737 <sup>b</sup>	687 <sup>c</sup>	5.86	< 0.001	0.001	0.001			
7 weeks of age	1530 <sup>a</sup>	1538 <sup>a</sup>	1311 <sup>b</sup>	1250 <sup>c</sup>	9.92	< 0.001	0.014	0.001			
9 weeks of age	2050 <sup>a</sup>	2048 <sup>a</sup>	1770 <sup>b</sup>	1694 <sup>c</sup>	13.1	< 0.001	0.014	0.021			
11 weeks of age	2547 <sup>a</sup>	2549 <sup>a</sup>	2111 <sup>b</sup>	2002 <sup>c</sup>	18.7	< 0.001	0.004	0.003			
13 weeks of age	2879	2835	2333	2246	21.3	< 0.001	0.001	0.273			
Weight gain, g/day											
n	60	60	60	60							
5-13 weeks of age	35.9	35.3	28.5	27.8	0.29	< 0.001	0.132	0.591			
Feed intake, g/day											
n	30	30	30	30							
5-13 weeks of age	155 <sup>b</sup>	165 <sup>a</sup>	111 <sup>c</sup>	112 <sup>c</sup>	2.39	< 0.001	0.001	0.010			
Feed conversion ratio											
n	30	30	30	30							
5-13 weeks of age	4.31 <sup>b</sup>	4.67 <sup>a</sup>	3.89 <sup>c</sup>	3.99 <sup>c</sup>	0.03	< 0.001	< 0.001	0.013			

 $<sup>\</sup>overline{a,b,c}$  In case of interaction, values within a row with different superscripts differ significantly at P < 0.05.

# Carcass traits

The results depicted in Table 2 showed that slaughter weight, weights of HC, CC and RC, as well as the weight and percentages of perirenal and scapular fat were affected by both the ambient temperature (P < 0.001) and the rabbit line (P < 0.01). In general, Lean rabbits showed lower fat depots and higher carcass weights compared to Fat ones. Moreover, rabbits farmed at 28 °C had the lowest carcass weights and they were the leanest. The above-mentioned effects, however, did not influence the DoP which was similar in all rabbit groups (average DoP: 61% CC). For the perirenal (P = 0.05) and scapular fat (P < 0.01) contents, an interaction was also observed: the differences in fat deposits between Lean and Fat lines were larger at control than at warm

The ambient temperature and line also affected the organs to CC ratios: liver (P < 0.01) and kidneys (P < 0.01) ratios were lower in rabbits reared at 28 °C than at 20 °C, and Fat line showed higher set of organs (P < 0.01) and liver (P < 0.05) ratios to CC compared to Lean ones.

Table 2 Effect of ambient temperature on the carcass traits of growing rabbits of Lean and Fat genetic lines.

Temperature (T)										
Line (L)	20 °C		28 °C		SE	P-values				
	Lean <sup>1</sup>	Fat <sup>2</sup>	Lean <sup>1</sup>	Fat <sup>2</sup>		T	L	$T \times L$		
n	57	57	58	55	-	_	-	-		
Slaughter weight, g	2883	2838	2319	2238	22.4	< 0.001	0.006	0.431		
Hot carcass weight, g	1781	1748	1 447	1384	13.5	< 0.001	0.001	0.287		
Chilled carcass weight, g	1754	1722	1 425	1362	13.4	< 0.001	0.001	0.278		
Reference carcass weight, g	1470	1433	1192	1131	11.5	< 0.001	0.001	0.342		
Perirenal fat, g	23.1 <sup>b</sup>	36.2°	9.8 <sup>a</sup>	14.5 <sup>a</sup>	0.31	< 0.001	< 0.001	0.050		
Scapular fat, g	7.12 <sup>b</sup>	10.8 <sup>c</sup>	3.56 <sup>a</sup>	5.31 <sup>ab</sup>	0.94	< 0.001	< 0.001	0.001		
DoP, <sup>3</sup> % (CC)	60.9	60.8	61.5	60.9	0.20	0.303	0.415	0.588		
Ratio to chilled carcass, %										
Set of organs <sup>4</sup>	1.29	1.39	1.25	1.32	0.01	0.079	0.005	0.579		
Liver	4.52	4.80	3.82	3.92	0.05	0.001	0.036	0.302		
Kidneys	0.91	0.92	0.82	0.81	0.01	0.001	0.735	0.536		
Ratio to reference carcass, %										
Fore part	26.2	26.0	26.1	26.4	0.11	0.463	0.814	0.324		
Mid part	34.3	33.7	33.6	33.4	0.12	0.036	0.122	0.442		
Hind part	37.5	37.0	39.2	38.5	0.20	< 0.001	0.120	0.766		
Perirenal fat	1.56 <sup>b</sup>	2.52 <sup>a</sup>	0.81 <sup>c</sup>	1.27 <sup>b</sup>	0.06	< 0.001	< 0.001	0.006		
Scapular fat	0.48	0.74	0.29	0.46	0.02	< 0.001	< 0.001	0.177		
LTL <sup>5</sup>	12.1	12.4	12.1	12.7	0.10	0.454	0.016	0.461		
HL <sup>6</sup>	26.1	26.1	27.3	27.7	0.14	0.001	0.518	0.369		
M/B <sup>7</sup>	3.03	3.09	2.95	3.03	0.02	0.102	0.084	0.795		

 $<sup>^{</sup>a,b,c}$  In case of interaction, values within a row with different superscripts differ significantly at P < 0.05.

<sup>&</sup>lt;sup>1</sup> Lean: selected for low total fat content during five generations.

<sup>&</sup>lt;sup>2</sup> Fat: selected for high total fat content during five generations.

<sup>&</sup>lt;sup>1</sup> Lean: selected for low total fat content during five generations.

<sup>&</sup>lt;sup>2</sup> Fat: selected for high total fat content during five generations.

<sup>&</sup>lt;sup>3</sup> DoP (CC): dressing out percentage (chilled carcass).

Set of organs: heart + lungs + thymus + trachea + oesophagus.

<sup>&</sup>lt;sup>5</sup> LTL:. Longissimus thoracis et lumborum muscle.

<sup>&</sup>lt;sup>6</sup> HL: hind leg.

<sup>&</sup>lt;sup>7</sup> M/B: meat to bone ratio.

# ARTICLE IN PRESS

Z. Matics, Z. Gerencsér, R. Kasza et al. Animal xxx (2020) xxx

Ratios to the RC were mostly affected by the ambient temperature: at 28 °C, the mid part (P < 0.05), perirenal fat (P < 0.001) and scapular fat (P < 0.001) ratios were the lowest, whereas hind part ratio (P < 0.001) and HL (P < 0.01) were the highest. Diversely, the LTL meat cut and the meat to bone ( $\mathbf{M/B}$ ) ratio were independent from temperature. The rabbit line had only effect on the perirenal (P < 0.001) and scapular (P < 0.001) fat ratios, and LTL cut (P < 0.05) which showed the highest values in Fat rabbits.

### Discussion

Although, lower BW was found in Fat rabbits at high temperature, no differences were found at control temperature. It may be explained by the pre-weaning period, because the DWGs of the lines were similar during the growing period, but BW was already different at the age of 5 wk.

Despite results showed that farming temperature significantly affected the BW of rabbits from weaning, the ambient temperature had no direct effect on kits till the age of 3 wk. (data not shown), because their thermoneutral zone is higher than that of weaned rabbits. In previous studies it was observed that high temperature had a negative effect on milk yield (e.g. Fernández-Carmona et al., 1995; Bakr et al., 2015; Szendrő et al., 2018), thus, kits consumed less milk and their weight gain was lower (Szendrő et al., 2018).

The weight of kits at weaning was also different when the effect of season was investigated (Chiericato et al., 1993). Similarly to our results at 20 °C ambient temperature, Milisits and Lévai (2002) did not find any difference between BW of rabbit lines divergently selected for body fat content by TOBEC. Guo et al. (2011) also described no difference between BW of chicken lines divergently selected for abdominal fatness; however, Baéza et al. (2015) reported higher BW in the fat line chickens.

DWG differed along the whole fattening period. Similar differences were observed by Chiericato et al. (1993) and Zeferino et al. (2011), whereas Cervera et al. (1997) found a low decline.

Compared to control temperature, at high ambient temperature, the FI decreased by 28 and 32% in Lean and Fat lines, respectively, thus confirming the trend from previous results (Chiericato et al., 1993; Zeferino et al., 2011). Heat stress enhances the secretion of leptin and adiponectin. Leptin stimulates the hypothalamic–pituitary–adrenal axis and results in a reduced FI, whereas adiponectin regulates the feeding behaviour (Slimen et al., 2016). From the results of the productive performance of rabbits, it seems that the higher FI of Fat rabbits at control temperature was used to build fat deposits but not for growth, as difference was not found between the DWG of the two lines. The effect of temperature was similar in Lean and Fat rabbits, as no significant temperature  $\times$  line interaction was observed in DWG.

Coherently with the present study, Szendrő et al. (2012) observed that rabbits with lower muscle but higher fat content consume more feed: the energy requirement for fat deposition is higher than the energy requirements for protein (muscle) (Whittemore, 1980; National Research Council [NRC], 1998), and therefore the fat rabbits consumed more feed to achieve the same DWG. Baéza et al. (2015) also confirmed the higher FI in fat chicken line compared to Lean one. In the present study, difference in FI between Lean and Fat rabbits was only found at control temperature, whereas the FI of the lines did not differ at 28 °C, likely due to the heat stress condition.

The lower FI seems to be the main reason for the lower DWG. However, since the difference in DWG under different temperatures was smaller than in FI, the impact of higher temperature on growth performances cannot be explained solely by the decrease in FI (Slimen et al., 2016).

The combination of the effects of temperature on DWG and FI results in a significant temperature effect on the FCR, which was more favourable in rabbits farmed at high ambient temperature. Opposite results were published by Chiericato et al. (1993), whereas no significant differences were observed by Chiericato et al. (1997) and Zeferino et al. (2011). These contrasting results could depend on several factors, such

as the use of different rabbit genetic lines, likely with different precocity, or the influence of the environmental humidity, often not measured, even though strongly affecting the perceived temperature. The significant differences observed for FCR between the Lean and Fat lines at control temperature were explained by their higher FI and similar DWG. These results confirm previous studies on rabbits (Szendrő et al., 2012) and on chickens (Baéza et al., 2015).

The most important effect of the divergent selection on total body fat content was the change in fat deposits; the differences in amount and percentage of perirenal and scapular fat between Lean and Fat lines were significant. Milisits and Lévai (2002) and Milisits et al. (2003) selected growing rabbits for high or low body fat and similarly to our results reached differences in the ratio of abdominal and scapular fat. Recently, Martínez-Álvaro et al. (2016, 2018a and 2018b) selected rabbits divergently for intramuscular fat content. The phenotypic difference between lines in generation 7 was 0.39 g of intramuscular fat per 100 g longissimus dorsi muscle on a fresh basis (Martínez-Álvaro et al., 2016). At the same time, the perirenal, scapular and dissectible fat weights were higher in the high-fat line than in the low-fat line (Martínez-Álvaro et al., 2018b) which is in line with our results when selecting rabbits for total body fat content. Jiang et al. (2017) used an independent up-selection for intramuscular fat content in one chicken line and a balanced selection with up-selection for intramuscular fat and down-selection for abdominal fat percentage in the other line. The intramuscular fat content increased in both lines, while the abdominal fat only increased in the first line. As a result of the 60-generation divergent selection for fatness of mice, the body fat content was 5.5 times higher in Fat than in Lean line (22% vs 4%; Bünger et al., 2003). Compared to rabbits selected for low HL muscle mass, rabbits selected for high HL muscle mass had a lower percentage of perirenal (1.90% vs 2.40%; P < 0.05) and scapular fat (0.49% vs 1.07%; P < 0.001) to reference carcass (Szendrő et al., 2012). When rabbits were selected for higher body fat content, higher fat percentage of abdominal wall was measured compared to rabbits selected for lower body fat content (8.75% vs 11.8%; *P* < 0.05; Szendrő et al., 2016). All results show the efficiency of selection for body fat or intramuscular fat content and a correlation between the two fat deposits.

The differences found for perirenal and scapular fat amounts at warm temperature were more or less half of the differences observed at control temperature. Many authors found lower differences in amount of fat depots; however, they investigated the effect of season with a daily fluctuating temperature (Chiericato et al., 1993; Marai et al., 1999; Zeferino et al., 2013). In broilers under heat stress, slight decrease was reported in abdominal fat proportion (1.57% vs 1.35%; Lu et al., 2007).

On the one hand, higher amount of perirenal and scapular fat implies that rabbits exploited feed nutrients to build fat deposits which, at the slaughterhouse, also resulted in offal. On the another hand, rabbit may benefit from a higher fat reserve, their production level could improve ensuring also a longer life span (Pascual et al., 2013). Moreover, a higher intramuscular fat content often corresponds to a higher meat tenderness, juiciness and flavour, and this is particularly true for lean (Wood et al., 2008) and white meats (Hernández et al., 2000). Therefore, lower or higher body fat content can either be advantageous or disadvantageous, depending on the production purpose.

The DoP was independent from ambient temperature. Conversely, Chiericato et al. (1993) and Zeferino et al. (2013) found that high temperatures increased DoP. The reason for the better DoP when rabbits are farmed at high temperatures is the lower FI, which results in a smaller incidence of the gastrointestinal tract and content (Chiericato et al., 1993). However, in the present study, no differences were observed in this sense. Concerning carcass traits, the fact that the LTL meat cut as well as the M/B ratio were independent from temperature confirmed previous results (Zeferino et al., 2013). Our results are also in agreement with the results published by Martínez-Álvaro et al. (2016) who found that the difference in M/B between lines with high

# ARTICLE IN PRESS

Z. Matics, Z. Gerencsér, R. Kasza et al. Animal xxx (2020) xxx

and low intramuscular fat content cannot be considered relevant. When rabbits were divergently selected for high and low volume of HL muscle, the increase of the hind part cut, HL and meat percentage on the HL lead to the decrease of perirenal and scapular fat percentage (Szendrő et al., 2012). It seems that this relationship is not true when rabbits are selected for body fat content.

The present study showed that higher temperature primarily affects FI, but also directly and indirectly other productive performances. The effect of high temperature was prevalent, especially because it started affecting rabbits from their birth. At control temperature, higher FI was measured in the Fat than in the Lean line which shows the effect of selection on the FI. However, due to temperature × line interaction, the difference in FI between the two lines disappeared at higher temperature. At the same time, interaction was observed in fat deposits: differences were found for perirenal and scapular fat between lines at control temperature, but the difference between lines decreased at high temperature. The temperature × line interaction plays a role in FI and body fat content, so the effect of temperature resulted different on Lean and Fat rabbits.

# **Ethics approval**

The investigation was not a permission needed activity, all animals were handled according to the principles stated in the European directive 86 609/EEC regarding the protection of animals used for experimental and other scientific purposes (EC, 2010).

# Data and model availability statement

None of the data were deposited in an official repository.

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# **Author contributions**

Zsolt Matics: Methodology, Supervision, Writing – original draft, Writing – review & editing. Zsolt Gerencsér: Data curation, Investigation, Visualization, Writing – review & editing. Rozália Kasza: Investigation. Katalin Terhes: Investigation. István Nagy: Formal analysis, Software, Validation, Writing – review & editing. István Radnai: Resources. Antonella Dalle Zotte: Formal analysis, Writing – review & editing. Marco Cullere: Formal analysis, Writing – review & editing. Zsolt Szendrő: Conceptualization, Funding acquisition, Supervision, Writing – original draft.

# **Declaration of interest**

The authors state no conflict of interest.

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Z. Matics, Z. Gerencsér, R. Kasza et al. Animal xxx (2020) xxx

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