

## Evidence on the potential effects of *halal* meat on sleep/wake cycles and mood state profile: A pilot study

<sup>1\*</sup>Carrasco, C., <sup>2</sup>González-Mateos, A., <sup>1</sup>Gómez, M. A. and <sup>1</sup>Rodríguez, A. B.

<sup>1</sup>Neuroimmunophysiology and Chrononutrition Research Group, Department of Physiology, University of Extremadura, Avenida de Elvas s/n, CP 06006, Badajoz, Spain

<sup>2</sup>Cell Biology and Communication Research Group, Department of Physiology, University of Extremadura, E-10003, Caceres, Spain

### Article history

Received:

17 March 2021

Received in revised form:

20 August 2021

Accepted:

14 October 2021

### Keywords

*halal meat,*  
*sleep/wake cycle,*  
*mood state profile,*  
*tryptophan,*  
*serotonin,*  
*melatonin*

### Abstract

Nowadays, *halal* meat is attracting consumers as a healthier product. However, little is known about its nutritional content and possible health effects. A comparative analysis of the protein and amino acid contents between *halal* and non-*halal* meats (beef and lamb) was carried out in the present work. Additionally, a pilot study was also simultaneously performed to analyse the impact of their consumption on sleep/wake cycles and mood state profile in general population. Participants ( $n = 25$ ) were asked to exclusively consume *halal* meats (1,000 g of gross weight/week) for 30 days. Objective and subjective sleep qualities, mood state profile, and levels of the urinary metabolites of serotonin and melatonin were assessed at baseline and post-intervention. Significantly higher contents of proteins and some amino acids were observed in *halal*-lamb and *halal*-beef, respectively. The consumption of *halal* meats for 30 days caused a slight improvement in men's diurnal activity and mood state profile, as well as in women's subjective sleep quality. Significantly higher urine levels of serotonin were also reported, particularly in men. *Halal* meat consumption seems to have a positive impact on sleep/wake cycles and mood state profile, likely due to the higher protein and amino acid contents. Additional scientific research is needed to support consumer trends in the coming years.

© All Rights Reserved

### Introduction

Nowadays, there is a great deal of controversy regarding the dietary recommendation to limit the consumption of red and processed meat due to its possible harmful effects on human health. However, a recent systematic review and meta-analysis of cohort studies conducted by Zeraatkar *et al.* (2019) concluded that the association between red and processed meat consumption with all-cause mortality and adverse cardiometabolic outcomes was very small, and the evidence was of low certainty. For this reason, Johnston *et al.* (2019) recommended adults to continue with the current unprocessed and processed red meat consumption. It is important to remember that red meat (*e.g.*, beef and lamb) is one of the most important dietary sources of many nutrients, including proteins of high biological value, fatty acids, vitamins, and trace elements. There is growing evidence about the impact of red meat consumption in certain physiological processes and conditions, notably sleeping. Among the nutrients underlying

these effects are essential amino acids such as tryptophan, which may act through its conversion to serotonin and melatonin. These molecules are well known to play an important role on the perception of pain, sleep/wake rhythm, mood, or food intake (Richard *et al.*, 2009; Yurcheshen *et al.*, 2015; Lana *et al.*, 2019).

In recent years, the global *halal* industry has become a rising market, which is mainly conceived to meet the Islamic dietary needs of the 1.8 billion Muslims consumers worldwide. Excluding Islamic finance, it is expected to grow to \$3.2 trillion by 2024, directly impacting key sectors such as clothing, tourism, media and recreation, pharmaceutical, cosmetic, and especially food (DinarStandard, 2020). *Halal* (lawful) food encompasses all the alimentary products that meet the Islamic dietary regulations as prescribed by the Islamic primary scripture, the Holy Qur'an, and therefore are free from contact with any *haram* (unlawful) substance (Riaz and Chaundry, 2004; Campbell *et al.*, 2011). Notable among these is the *halal* meat. It is produced from animals

\*Corresponding author.  
 Email: ccarom@unex.es

considered as unprohibited/unlawful by Islam (*e.g.*, veal, beef, mutton, lamb, chicken, *etc.*), and humanely neck-slaughtered (*i.e.*, full draining of blood) in accordance with the Islamic law, in which the stunning of animals before slaughter is prohibited in the majority of Muslim countries. The term *halal* also involves the animal's welfare throughout the complete production chain, including management and handling of animals either at the farm, during transportation, and at slaughter points. Overall, the production of *halal* meat maintains the quality and wholesomeness of the final product, which increases consumer satisfaction and food safety (Aghwan *et al.*, 2016).

Nowadays, *halal* meat is attracting consumers all over the world, independently of their religious beliefs. In Europe, *halal*-labelled meat and meat products are currently more available in supermarkets and fast-food restaurants (Lever and Miele, 2012). Among the factors influencing this food trend is the growing interest of consumers in quality products considered more natural and eco-ethical than conventional ones (ITC, 2015). However, little is known about the possible health effects of these meat products. For this reason, the aims of the present work were (1) to quantify the protein and amino acid contents of *halal* meats, and (2) to analyse the impact of *halal* meat consumption on physiological variables such as sleep and mood.

## Materials and methods

### Meat samples

In order to carry out the comparative analysis of the protein and amino acid contents of the different types of meats studied (*i.e.*, beef and lamb), two types of samples were used: *halal* and non-*halal* meat. *Halal* meat samples came from Merino lambs and Limousin crossed beefs (aged  $\approx$  3 - 4 and 12 - 14 months, respectively). Both types of animals were raised in extensive farming conditions, fed with organic raw materials (fodder or grass, depending on their availability), and without being subjected to antibiotic or hormonal treatments. The afternoon before their slaughter, the animals were transported to a slaughterhouse specialised in the production of *halal* meat (Golden Worldwide Trade S.L., Olivenza, Spain), under conditions of respecting animal welfare. There, they remained stabled until next morning, when they were slaughtered under fasting conditions. Within the 24 h after the slaughter, the

meat samples were obtained from the longissimus dorsi muscle of the left medial canal (lumbar portion, 5 - 10<sup>th</sup> vertebra), and transported to the laboratory under refrigeration conditions; there, samples were identified and stored (-20°C) until further analyses. As regards to non-*halal* meat, samples with similar characteristics to those of *halal* meats in terms of age, race, and anatomical parts were also supplied by the abovementioned slaughterhouse, subsequently identified and stored (-20°C) until further analyses. Conventional meat is characterised by being produced in intensive livestock farms, where animal's feed is based on grass and fodder. Since it is considered that this type of meat is consumed by the majority of the population, it served as a control for the comparative analysis with *halal* meats.

### Protein and amino acid contents

The assessment of protein and amino acid contents in meat samples (*halal* and non-*halal*) were conducted in triplicate. Protein content (%) was determined using the Kjeldahl method (AOAC, 2000; ref. 992.15). A total of 16 amino acids (*i.e.*, essential and non-essential) were determined including alanine, arginine, aspartate, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine.

### Participants

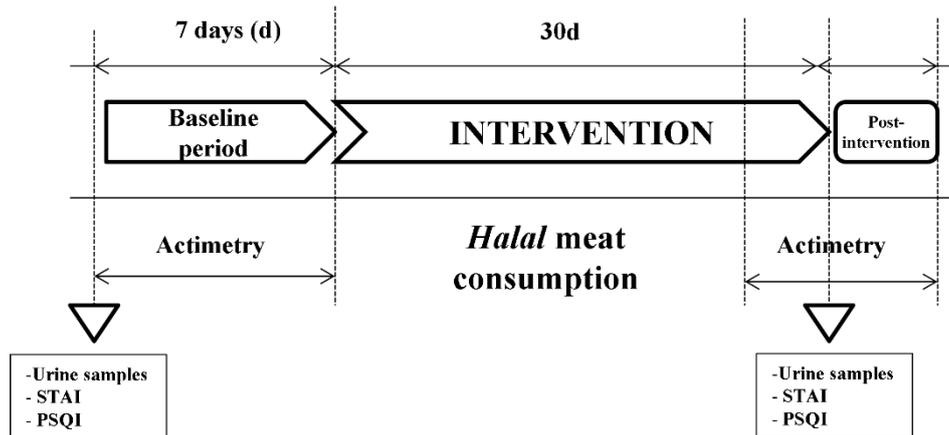
Recruitment of volunteers was based on word of mouth based on the inclusion criteria: males and females aged 35 - 65 and meat eaters. Participants were excluded if they were regular consumers of *halal* meats, had diagnosed endocrine/metabolic or digestive conditions, had taken vitamin supplementation or antibiotic treatment in the previous two months, and if they were not willing to complete the study. Additionally, because their diets were not standardised, all participants were advised not to change their lifestyles (*i.e.*, diet, physical activity, and sleeping habits), and not to take nutritional supplements (*i.e.*, vitamins or carotenoids) or antibiotic treatment during the trial. Compliance was strictly monitored in case of any occurrence of adverse events during the study. All adults gave written consent for participation after being informed. The study was approved by two ethical committees (University of Extremadura and Extremadura Health Service) following the updates of the Declaration of

Helsinki, amended by the 64<sup>th</sup> World Medical Association General Assembly (WMA, 2013).

### Procedures

Data from this pilot study analysing the effect of one-month *halal* meat consumption on sleep and mood in general population were collected from February to August 2018 in the city of Badajoz (Spain). Participants were assessed prior to the intervention to obtain their baseline values for the

outcome measures as follows: daily activity and objective sleep quality were assessed during a seven-day period (baseline week); at the end of the baseline week (day 7), participants completed the questionnaires and urine samples were collected. After one-month consuming *halal* meat, participants were assessed again (post-intervention) for the study variables at two time points: (1) immediately post-intervention (day 30) and (2) two-days post-intervention (day +2) (Figure 1).



**Figure 1.** Pilot study timeline. PSQI: Pittsburgh Sleep Quality Index; STAI: State-Trait Anxiety Inventory.

### Intervention

Participants were asked to exclusively consume *halal* meats for 30 days. They were supplied a serving of 1,000 g of gross weight of *halal* meats (*i.e.*, 500 g of each lamb and beef) once a week, corresponding to approximately 300 - 400 g of the net quantity of each type of meat (~ 600 - 700 g of the total portion; the recommended amount for an adult).

### Outcome measures

To study the effects of the consumption of *halal* meats on sleep/wake cycles including melatonin and serotonin levels, and mood state profile, specific analyses were performed.

### Sleep/wake cycles

To evaluate daytime activity and objective sleep quality, an individualised actigraphic monitoring was performed. For this purpose, each participant was asked to wear on their wrist an actimeter (Kronowise<sup>®</sup>, University of Murcia, Spain); this device logged the temporal patterns of activity and rest at two time points: (1) seven days prior to the trial to obtain the baseline measurement, and (2) a five-day period, including the last three days of the trial plus the two days post-intervention. At both

points in the study, average scores of the evaluation periods were obtained. Data were then analysed with the Circadianware<sup>®</sup> software (Department of Information and Communication Engineering, University of Murcia, Murcia, Spain) to obtain the following variables: percentage of day and night activities, duration of sleep (minutes), and depth of sleep. Additionally, subjective sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) (Buysse *et al.*, 1989).

### Urine sample collection

First-void morning urine was collected at baseline (day 7), and at the end of the trial (day 30). The samples were stored at -80°C until biochemical assay.

### 5-hydroxyindolacetic acid

5-hydroxy indoleacetic acid (5-HIAA) is the main metabolite of serotonin. To determine the levels of this neurotransmitter, urinary 5-HIAA was analysed using a commercial kit based on the enzyme-linked immunosorbent assay (ELISA) (DRG Diagnostics, Marburg, Germany) and following the manufacturer's instructions. Later, the variation of the analyte in the urine dilution was adjusted by

expressing data as a ratio of 5-HIAA/creatinine in urine. The quantitative determination of creatinine was determined using the commercial kit based on the Jaffé reaction, following the manufacturer's instructions (Chemelex, S.A., Barcelona, Spain).

#### 6-sulphatoxy-melatonin

Melatonin is metabolised in the liver to 6-hydroxymelatonin, and later, to 6-sulphatoxymelatonin (aMT6-s), which is excreted in the urine. Thus, the urinary concentration of aMT6-s correlates with the total level of melatonin in the blood. For this reason, urinary aMT6-s was analysed using a commercial kit based on ELISA (DRG Diagnostics, Marburg, Germany), and following the manufacturer's instructions. Values were expressed as aMT6-s/creatinine in urine, as described above.

#### Mood state profile

To assess the possible effect of the consumption of *halal* meats on mood, participants were asked to complete the Spanish version of the State-Trait Anxiety Inventory (STAI) (Spielberger *et al.*, 1982).

#### Statistical analysis

Values are expressed as mean  $\pm$  standard deviation (SD). Data were assessed for normality using Kolmogorov-Smirnov test. To analyse significant differences in the protein and amino acid contents between *halal* and non-*halal* meat samples, a comparative statistical analysis was carried out using parametric tests (Student's *t*-test of unpaired samples) or non-parametric (Mann-Whitney U test), as appropriate. Regarding the pilot study, results were statistically compared to detect a possible significant impact of *halal* meat consumption on sex groups. Values of actimetry, urinary metabolites, and mood state profile were individually processed and expressed as the ratio between final and baseline values (fold change) to facilitate the processing and interpretation of results. To minimise the effect of the interpersonal variability, values were normalised and expressed with respect to the baseline value established at 1. The Student's *t*-test, One-way analysis of variance (ANOVA), followed by Bonferroni multiple comparisons test were later applied. Statistical analyses were carried out using the GraphPad Prism® (version 5.0, 2007; GraphPad Software, Inc., San Diego, CA). Statistical significance was established at  $p < 0.05$ .

## Results

#### Protein and amino acid contents

The comparative analysis of the protein and amino acid contents between *halal* and non-*halal* meat samples (Table 1) revealed that the protein content was significantly higher in *halal* lamb (*h-L*) than non-*halal* lamb (*nh-L*) samples ( $24.94 \pm 0.35$  vs  $14.32 \pm 1.04$ ,  $p = 0.005$ ), in contrast to what was observed for beef meat samples (non-*halal* beef (*nh-B*) =  $34.16 \pm 0.45$  vs *halal* beef (*h-B*) =  $25.68 \pm 1.35$ ,  $p = 0.004$ ). However, the content of the essential amino acids namely histidine ( $24.63 \pm 0.88$  vs  $23.08 \pm 0.05$ ,  $p = 0.011$ ), leucine ( $18.06 \pm 0.83$  vs  $16.43 \pm 0.02$ ,  $p < 0.001$ ), and tryptophan ( $2.35 \pm 0.30$  vs  $1.90 \pm 0.23$ ,  $p = 0.017$ ), as well as the non-essential ones such as arginine ( $19.73 \pm 0.94$ ,  $p = 0.031$ ) and tyrosine ( $8.58 \pm 0.35$  vs  $8.12 \pm 0.11$ ,  $p = 0.004$ ) was significantly higher in *h-B* than *nh-B*. With regard to *h-B*, overall content of amino acids was significantly lower than found in *nh-B* (Table 1).

#### Intervention

The main findings of the pilot study of *halal* meat consumption are presented below. A total of 25 participants of both sexes (12 males and 13 females, Caucasian ethnicity) were initially enrolled in the pilot study; however, five of them (two males and three females) withdrew due to personal, diverse, and unforeseen reasons during the experimental period. It should be mentioned that no adverse events were reported.

#### Sleep/wake cycles

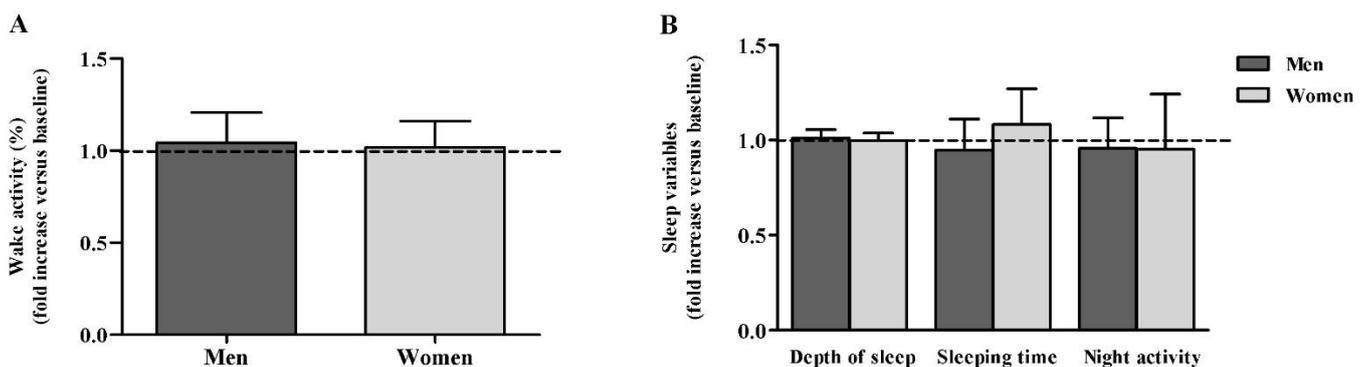
Actigraphic results showed that the consumption of *halal* meats did not cause any change in the daily activity (Figure 2A) in both men and women. Likewise, no significant variations were observed in the sleep variables post-intervention. Slight positive trends were reported for the variables "daily activity" in men ( $1.044 \pm 0.163$ ,  $p = 0.419$ ) and "sleep time" in women ( $1.082 \pm 0.189$ ,  $p = 0.465$ ) (Figure 2B).

On the other hand, the overall mean score for PSQI indicated a low subjective sleep quality ( $\geq 5$  points) as well as a great variability among the participants. When compared with the baseline values, no significant differences were observed after consuming *halal* meats, although a slight improvement in the sleep quality was detected in

**Table 1.** Comparison of protein (mg/mL) and amino acid (essential and non-essential; g/kg) contents of *halal* and non-*halal* meat samples.

		Meat sample					
		<i>nh-B</i>	<i>h-B</i>	<i>p</i> value	<i>nh-L</i>	<i>h-L</i>	<i>p</i> value
<b>Protein</b>		34.16 ± 0.45	25.68 ± 1.35**	0.004	14.32 ± 1.04	24.94 ± 0.35**	0.005
<b>Amino acid</b>							
<b>Essential</b>	Histidine	23.08 ± 0.05	24.63 ± 0.88*	0.011	21.76 ± 1.25	16.99 ± 0.39**	0.004
	Isoleucine	11.23 ± 0.97	11.67 ± 0.62	0.376	12.61 ± 0.10	10.33 ± 0.60***	< 0.001
	Leucine	16.43 ± 0.02	18.06 ± 0.83***	< 0.001	19.55 ± 0.08	15.83 ± 0.79***	< 0.001
	Lysine	44.46 ± 0.45	28.17 ± 1.94***	< 0.001	45.86 ± 1.06	34.67 ± 2.44***	< 0.001
	Methionine	7.54 ± 0.78	7.95 ± 0.02	0.230	8.56 ± 0.25	6.77 ± 0.41***	< 0.001
	Phenylalanine	10.25 ± 0.30	10.52 ± 1.38	0.654	12.05 ± 0.22	11.29 ± 0.66*	0.023
	Threonine	20.47 ± 1.78	20.25 ± 1.12	0.806	23.52 ± 0.36	19.09 ± 1.02***	< 0.001
	Tryptophan	1.90 ± 0.23	2.35 ± 0.30*	0.017	2.18 ± 0.24	2.25 ± 0.26	0.614
	Valine	14.00 ± 1.22	14.19 ± 0.55	0.735	15.40 ± 0.16	13.06 ± 1.02***	< 0.001
<b>Non-essential</b>	Alanine	12.62 ± 0.99	12.53 ± 0.49	0.838	14.25 ± 0.45	11.31 ± 0.46***	< 0.001
	Arginine	18.26 ± 1.08	19.73 ± 0.94*	0.031	19.71 ± 0.60	16.44 ± 0.63***	< 0.001
	Aspartic acid	14.69 ± 1.74	15.05 ± 1.47	0.709	16.37 ± 0.71	12.68 ± 0.19***	< 0.001
	Glutamic acid	28.77 ± 1.07	30.49 ± 1.76	0.069	34.92 ± 0.10	25.93 ± 0.67***	< 0.001
	Glycine	10.62 ± 0.55	10.18 ± 0.40	0.139	8.43 ± 1.56	8.74 ± 0.83	0.679
	Serine	9.61 ± 0.76	9.57 ± 0.43	0.903	10.88 ± 0.23	8.56 ± 0.32***	< 0.001
	Tyrosine	8.12 ± 0.11	8.58 ± 0.35**	0.004	9.58 ± 0.02	8.12 ± 0.27*	0.011

*nh-B*: non-*halal* beef; *h-B*: *halal* beef; *nh-L*: non-*halal* lamb; *h-L*: *halal* lamb. Values are mean ± standard deviation (SD). Statistical significance as compared to non-*halal* meat samples (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).



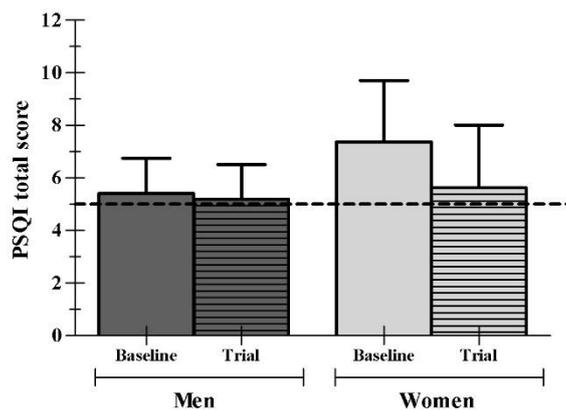
**Figure 2.** Daytime activity and objective sleep quality, expressed as mean ± SD, in men and women. (A) daytime activity (percentage); (B) depth of sleep, sleep time (minute), and night activity (percentage).

women (baseline:  $7.375 \pm 2.326$  vs intervention:  $5.625 \pm 2.387$ ,  $p = 0.197$ ) (Figure 3).

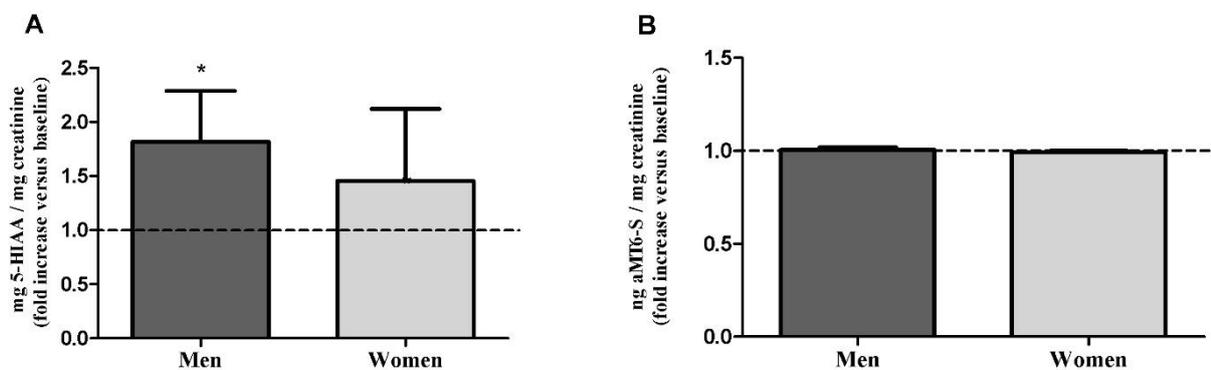
#### Determination of 5-HIAA and aMT6-s

As can be seen in Figure 4A, results showed an increase in the participants' urine levels of 5-HIAA

when compared with baseline values, which was statistically significant in men ( $1.818 \pm 0.470$ ,  $p = 0.036$ ). However, no significant changes were observed in the aMT6-s levels in either group (Figure 4B).



**Figure 3.** Pittsburgh Sleep Quality Index (PSQI) total score, expressed as mean  $\pm$  SD, in men and women.

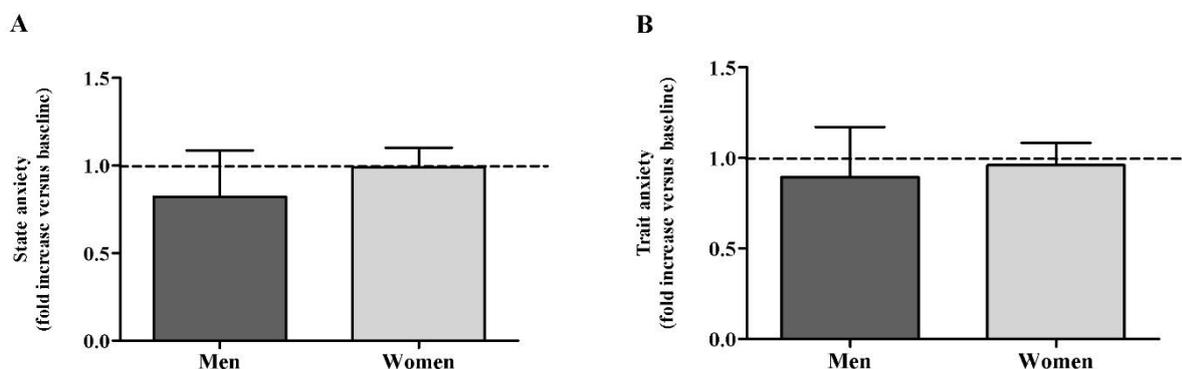


**Figure 4.** Urinary levels of (A) 5-hydroxy indoleacetic acid (mg 5-HIAA/mg creatinine) and (B) 6-sulphatoxymelatonin (ng aMT6-s/mg creatinine) expressed as mean  $\pm$  SD, in men and women. \* $p < 0.05$  indicates statistically significant differences as compared to baseline.

#### Mood state profile

The results about the mood state profile are shown in Figure 5. Neither S/A (Figure 5A) nor T/A (Figure 5B) were significantly affected by the consumption of *halal* meats for 30 days. However, it

should be mentioned that a slight decrease in the mean global score was particularly evident in men when compared with baseline values (S/A:  $0.824 \pm 0.264$ ,  $p = 0.098$ ; T/A:  $0.893 \pm 0.278$ ,  $p = 0.510$ ).



**Figure 5.** State-Trait Anxiety Inventory (STAI) scores expressed as mean  $\pm$  SD, in men and women: (A) state anxiety; (B) trait anxiety.

## Discussion

Nowadays, there is an increasing global interest in consuming products considered more natural and eco-ethical than conventional ones. This food trend is having an impact in the different food sectors, particularly in the meat industry. In this context, *halal* meat is gaining attraction. In a similar way as organic meat, *halal* meat is produced from grass-fed animals, which are not treated with antibiotics or growth hormones (ITC, 2015). Thus, it is expected to hold higher nutritional quality than conventional meat, and consequently, can provide significant health benefits. For this reason, the present work was designed to determine the nutritional properties of *halal* meats, and to analyse the possible physiological effects of its consumption.

After analysing the nutritional results, significant higher contents of proteins and amino acids – particularly those related to sleep-wake cycles such as histidine, tyrosine, and tryptophan – were observed in *h-L* and *h-B*, respectively, when compared with non-*halal* meat samples. It is well known that animal diets could influence the nutritional profile of the final meat product. Previous studies have pointed out the influence of different diets on the amount of proteins in both beef and lamb (Mapiye *et al.*, 2013; Costa *et al.*, 2018; Lima *et al.*, 2018), as well as amino acid content. Among other factors, the amount of dry matter ingested and the efficiency in the use of nitrogen of the products consumed may be implicated (Fraser *et al.*, 2000). In addition, protein from pasture is more readily digestible in the rumen when compared with that available in grains and concentrated diets. Consequently, higher levels of amino acids in beef were associated with a pasture diet when compared to grain. Particularly, lush pasture is a rich source of readily degradable protein and a potential source of tryptophan. Therefore, it is likely that feeding livestock with organic raw materials (*i.e.*, fodder or grass) has had a positive impact on the content of proteins and amino acids of *halal* meat samples in comparison to non-*halal* ones, which were produced from animals fed with feeding stuffs. Given these differences, the possible effect on sleep-wake cycles was analysed as described below.

There is limited evidence on the effects of dietary energy and macronutrients – especially protein intake – on sleep variables (Peuhkuri *et al.*, 2012). Some studies have shown that dietary protein

intake is positively associated with sleep duration, quality, and patterns (Zadeh and Begum, 2011; Grandner *et al.*, 2013; Zhou *et al.*, 2016). Based on our findings on the protein content of the *h-L* meat, it would be expected that the intervention had a positive effect on the regulation of the sleep-wake cycle. However, the consumption of *halal* meat for 30 days did not significantly change both objective and subjective sleeps of the participants, with only a slight improvement in the mean PSQI score of women. Among other factors, this may be because of the differential effect of protein intake in men and women by reason of body mass, as well as by the so-called net protein utilisation coefficient, that is, the percentage of dietary protein retained (FAO, 2013). Additionally, O'Connor *et al.* (2018) observed that the global PSQI score improved with the adoption of a Mediterranean pattern. However, they concluded that there were no significant variations in sleep indexes as a consequence of the intake of different amounts of unprocessed lean red meat. On their part, Lana *et al.* (2019) reported that a high consumption of meat (128 g/day of white, red, and/or processed meat) is associated with negative changes in the duration and quality of sleep in older adults.

On the other hand, it should not be forgotten that the effect of proteins on the sleep/wake cycle will depend on the content of certain amino acids. Thus, tryptophan is well known to influence the induction and maintenance of sleep – as precursor of serotonin and melatonin (Yurcheshen *et al.*, 2015) – and others such as histidine and tyrosine are involved in the modulation of wakefulness (Monti and Monti, 2007). Consequently, the consumption of *h-B* meat rich in these amino acids might have been responsible for the slight improvement reported post-intervention in men with regard to the wake activity. Surprisingly, increasing tryptophan intake only induced a slight improvement in subjective sleep in women. It should be mentioned that dietary tryptophan is absorbed in the intestine, where a part becomes free tryptophan, and the rest is transported into the brain from the circulating albumin bound. This transport can be affected by the competition of other free amino acids such as leucine and tyrosine (Jansman *et al.*, 2002), which were reported to be significantly higher in *h-B* than *nh-B*. Other authors pointed out that the intake of certain foods rich in tryptophan such as vegetables, fruit, cereals, fish, and white meat has a positive effect on the induction and maintenance of sleep, and therefore, on the quality of sleep (Yurcheshen *et al.*,

2015), unlike foods such as pasta, cheese, and processed meats (Van Lee *et al.*, 2017). Given these differences, more studies are needed to analyse how the different components of the dietary matrix can interact with the tryptophan transport, and therefore, with its effect on the sleep-wake cycle.

The consumption of tryptophan rich *h*-B was also reported to increase urine levels of the metabolite serotonin 5-HIAA, particularly in men, which might have been responsible for the slight increase observed in both daytime activity and mood state profile in men. Contrary to what might be expected, no significant changes were observed in the levels of the metabolite of melatonin aMT6-s. Our hypothesis is that the catabolism of melatonin may occur by ways other than those indicated above; consequently, the increase in serotonin content was not mirrored by an increase in aMT6-s. These results contrast with those obtained in our previous studies about the intake of a nutraceutical made from Valle del Jerte cherries (Garrido *et al.*, 2012; 2013). This product, rich in tryptophan, serotonin, and melatonin, was reported to induce an increase in the urinary levels of 5-HIAA and aMT6-s, with the consequent improvement of the sleep variables in the study participants. According to Tan *et al.* (2014), the melatonin content of foods such as beef, lamb, pork, chicken, and fish is comparable to that found in other food sources, which makes us suspect that its consumption might have potential health effects. For this reason, the analysis of serotonin and melatonin in *halal* meats must be addressed in future studies, which could lead to the elucidation of its content and possible effects on the study variables.

To the best of our knowledge, the present work could be the first to analyse the health effects of the consumption of *halal* meats. However, the potential limitations of the present work should be taken into account for further research. Since the sample size and the intervention time might have influenced the detection of significant effects of the *halal* meats, increasing both experimental factors could help to clarify the long-term effect of this food trend on the health of a statistically representative sample. Furthermore, given the characteristics of this pilot trial, it could be possible that the detection of significant variations could have been influenced by the fact that some participants have not been strictly following the study instructions about not to change their lifestyles.

## Conclusion

*Halal* meat consumption for 30 days seemed to have a positive impact on sleep/wake cycles and mood state profile in general population. This potential effect could be due to the higher protein and amino acid contents reported in *halal* meat when compared with non-*halal* one. From our point of view, growing interest of consumers for this and other trendy products must be accompanied by scientific evidence supporting consumer trends, and consequently, the changes in a future food market, in which food production is required to be more eco-ethical and healthier.

## Acknowledgement

The authors would like to thank Research Support Service of University of Extremadura for its excellent technical assistance in the determination of amino acid content; Golden Worldwide Trade S.L. for meat samples; and all the participants who have collaborated in the present work. The present work was financially supported by Regional Government of Extremadura-ERDF funds (grant no.: BBB021-GR18040 and post-doctoral grant no.: PO17027), and Golden Worldwide Trade S.L. under research agreement with the University of Extremadura (ref. no.: 141/18). Neither the government agency nor the sponsoring company played a role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

## References

- Aghwan, Z. A., Bello, A. U., Abubakar, A. A., Imlan, J. C. and Sazili, A. Q. 2016. Efficient *halal* bleeding, animal handling, and welfare: A holistic approach for meat quality. *Meat Science* 121: 420-428.
- Association of Official Analytical Chemist (AOAC). 2000. Official methods of analysis of the AOAC international. 17<sup>th</sup> ed. United States: AOAC.
- Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R. and Kupfer, D. J. 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Research* 28: 193-213.

- Campbell, H., Murcott, A. and MacKenzie, A. 2011. *Kosher in New York City, halal in Aquitaine: challenging the relationship between neoliberalism and food auditing*. *Agriculture and Human Values* 28: 67-79.
- Costa, J. B., Oliveira, R. L., Silva, T. M., Barbosa, A. M., Borja, M. S., de Pellegrini, C. B., ... and Bezerra, L. R. 2018. Fatty acid, physicochemical composition and sensory attributes of meat from lambs fed diets containing licuri cake. *PLoS One* 13(11): article ID e0206863.
- DinarStandard. 2020. State of the global Islamic economy report 2019/20 - driving the Islamic economy revolution 4.0. Retrieved on February 11, 2020 from Salaam Gateway website: <https://cdn.salaamgateway.com/special-coverage/sgie19-20/full-report.pdf>
- Food and Agriculture Organization (FAO). 2013. Dietary protein quality evaluation in human nutrition. Retrieved on April 30, 2020 from FAO website: <http://www.fao.org/3/a-i3124e.pdf>.
- Fraser, M. D., Fychan, R. and Jones, R. 2000. Voluntary intake, digestibility and nitrogen utilization by sheep fed ensiled forage legumes. *Grass and Forage Science* 55(3): 271-279.
- Garrido, M., Espino, J., González-Gómez, D., Lozano, M., Barriga, C., Paredes, S. D. and Rodríguez, A. B. (2012). The consumption of a Jerte Valley cherry product in humans enhances mood, and increases 5-hydroxyindoleacetic acid but reduces cortisol levels in urine. *Experimental Gerontology* 47(8): 573-580.
- Garrido, M., González-Gómez, D., Lozano, M., Barriga, C., Paredes, S. D. and Rodríguez, A. B. 2013. A Jerte Valley cherry product provides beneficial effects on sleep quality. Influence on aging. *Journal of Nutrition, Health and Aging* 17(6): 553-560.
- Grandner, M. A., Jackson, N., Gerstner, J. R. and Knutson, K. L. 2013. Dietary nutrients associated with short and long sleep duration. Data from a nationally representative sample. *Appetite* 64: 71-80.
- International Trade Centre (ITC). 2015. Chapter 1 - from niche to mainstream – *halal* goes global. Geneva: ITC.
- Jansman, A. J. M., Smink, W., Van Leeuwen, P. and Rademacher, M. 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. *Animal Feed Science and Technology* 98: 49-60.
- Johnston, B. C., Zeraatkar, D., Han, M. A., Vernooij, R. W. M., Valli, C., El Dib, R., ... and Guyatt, G. H. 2019. Unprocessed red meat and processed meat consumption: Dietary guideline recommendations from the Nutritional Recommendations (NutriRECS) Consortium. *Annals of Internal Medicine* 171: 756-764.
- Lana, A., Struijk, E. A., Arias-Fernandez, L., Graciani, A., Mesas, A. E., Rodriguez-Artalejo, F. and Lopez-Garcia, E. 2019. Habitual meat consumption and changes in sleep duration and quality in older adults. *Aging and Disease* 10: 267-277.
- Lever, J. and Miele, M. 2012. The growth of halal meat markets in Europe: An exploration of the supply side theory of religion. *Journal of Rural Studies* 28: 528-537.
- Lima, A. G., Oliveira, R. L., Silva, T. M., Barbosa, A. M., Nascimento, T. V., Oliveira, V. S., ... and Bezerra, L. R. 2018. Feeding sunflower cake from biodiesel production to Santa Ines lambs: Physicochemical composition, fatty acid profile and sensory attributes of meat. *PLoS One* 13(1): article ID e0188648.
- Mapiye, C., Aalhus, J. L., Turner, T. D., Rolland, D. C., Basarab, J. A., Baron, V. S., ... and Dugan, M. E. R. 2013. Effects of feeding flaxseed or sunflower-seed in high-forage diets on beef production, quality and fatty acid composition. *Meat Science* 95: 98-109.
- Monti, J. and Monti, D. 2007. The involvement of dopamine in the modulation of sleep and waking. *Sleep Medicine Reviews* 11(2): 113-133.
- O'Connor, L. E., Paddon-Jones, D., Wright, A. J. and Campbell, W. W. 2018. A Mediterranean-style eating pattern with lean, unprocessed red meat has cardiometabolic benefits for adults who are overweight or obese in a randomized, crossover, controlled feeding trial. *The American Journal of Clinical Nutrition* 108(1): 33-40.

- Peuhkuri, K., Sihvola, N. and Korpela, R. 2012. Diet promotes sleep duration and quality. *Nutrition Research* 32: 309-319.
- Riaz, M. N. and Chaudry, M. M. 2004. *Halal* food production. United States: CRC Press.
- Richard, D. M., Dawes, M. A., Mathias, C. W., Acheson, A., Hill-Kapturczak, N. and Dougherty, D. M. 2009. L-tryptophan: basic metabolic functions, behavioral research and therapeutic indications. *International Journal of Tryptophan Research* 2: 45-60.
- Spielberger, C., Gorsuch, R. and Lushene, R. 1982. STAI, manual for the state-trait anxiety inventory (self-evaluation questionnaire). In Buela-Casal, G., Guillén-Riquelme, A. and Seisdedos, N. (eds). Madrid: TEA Ediciones.
- Tan, D. X., Zanghi, B. M., Manchester, L. C. and Reiter, R. J. 2014. Melatonin identified in meats and other food stuffs: potentially nutritional impact. *Journal of Pineal Research* 57(2): 213-218.
- Van Lee, L., Chia, A. R., Loy, S. L., Colega, M., Tham, E., Cai, S., ... and Chong, M. F. F. 2017. Sleep and dietary patterns in pregnancy: findings from the GUSTO cohort. *International Journal of Environmental Research and Public Health* 14(11): article no. 1409.
- World Medical Association (WMA). 2013. World Medical Association declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* 310: 2191-2194.
- Yurcheshen, M., Seehuus, M. and Pigeon, W. 2015. Updates on nutraceutical sleep therapeutics and investigational research. *Evidence-Based Complementary and Alternative Medicine* 2015: article ID 105256.
- Zadeh, S. S. and Begum, K. 2011. Comparison of nutrient intake by sleep status in selected adults in Mysore, India. *Nutrition Research and Practice* 5(3): 230-235.
- Zeraatkar, D., Guyatt, G. H., Alonso-Coello, P., Bala, M. M., Rabassa, M., Han, M. A., ... and Dib, R. E. 2019. Red and processed meat consumption and risk for all-cause mortality and cardiometabolic outcomes. *Annals of Internal Medicine* 172: 511-512.
- Zhou, J., Kim, J. E., Armstrong, C. L., Chen, N. and Campbell, W. W. 2016. Higher-protein diets improve indexes of sleep in energy-restricted overweight and obese adults: results from 2 randomized controlled trials. *The American Journal of Clinical Nutrition* 103(3): 766-774.