Contribution of healthy and unhealthy primary school meals to greenhouse gas emissions in England: linking nutritional data and greenhouse gas emission data of diets

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Abstract

Background/Objective—School meals represent the largest sector in Government food procurement in the UK. This paper aims to quantify, simultaneously, the nutritional quality and carbon footprint of meals provided by primary schools in England.

Methods—The School Food Trust conducted the “Primary School Food Survey 2009” (PSFS) in a nationally representative sample of 139 primary schools in England. The survey included 6,690 students who consumed school lunches and 3,488 students who brought packed lunches. We estimated the total greenhouse gas emissions (GHGEs) per Kg of the food items contributing to those lunches based on the results of a systematic review of life cycle analyses.

Results—In both school lunches and packed lunches the “meat, fish and alternatives” group contributed the largest share of GHGEs. The mean GHGE value per school lunch was estimated to be 0.72 (95% uncertainty interval 0.52-1.34) KgCO\textsubscript{2}e and per packed lunch as 0.70 (0.58-0.94) KgCO2e. The total GHGEs due to primary school meals in England per year is 578.1 million KgCO2e (455 million-892 million).

Conclusion—If all children achieved a healthy meal defined by having a low level of salt, free sugars and saturated fat the total GHGEs from primary school meals would be 441.2 million KgCO2e (384 – 1,192), saving 136.9 million KgCO2e compared to the current total emissions from primary school meals. This paper demonstrates that changes in the primary school food sector can have an impact on UK greenhouse gas emissions.

Keywords

Nutrition; Non-communicable Disease; Prevention; School Health; Health Promoting Schools; Climate Change; Environmental Impact; Food; UK
Introduction

Climate change affects health through multiple pathways such as less clean air, less safe drinking water, availability of foods and extreme heat (1). Increasing global temperatures are expected to impact on yields of food crops and increase global food insecurity (2), which could have a bigger impact on health(3). Hence, there is strong public health incentive for mitigating climate change (4, 5).

The UK Climate Change Act 2008 sets a target to cut the total annual greenhouse gas emissions (GHGEs) by 80% by 2050, compared with 1990 levels (6). UK food production and consumption is responsible for 18—30% of total GHGE (6).

Different food groups have different carbon footprints, with livestock (particularly sheep and cows) and dairy footprints orders of magnitude higher than cereals, fruit and vegetables (7). Therefore, changing the food that we eat can have a substantial impact on GHGE in the UK (8). It is not clear, however, whether such changes would lead to healthier diets (9–11).

The Government report ‘Food 2030’ highlights that providing healthy and sustainable diets is an overarching objective and the government should provide stewardship by adopting these principles for food provided in the government sector (12). The public sector spends about £ 2.4 billion per year for food and catering services in schools, hospitals, armed forces, prisons and government agencies. The highest percentage of public sector spending for food is on school dinners (29%) (13). This study examines the GHGE and nutritional quality of primary school meals. This is an area of food provision which has received a great deal of attention recently due to the new School Food Plan (14) and recent changes around free school meals. The UK Government introduced a policy on universal free school meals for children in reception, year 1 and year 2 in state-funded schools in England from September 2014 (15). It is expected that the uptake of primary school meals will increase significantly with the new policy, which will impact on the total GHGE of primary school meals and the nutritional quality of meals eaten by children.

The nutritional quality of primary school meals in England has been assessed previously (16, 17). These studies have used food composition tables to estimate nutrients in school foods consumed in a representative sample. For the analyses in this paper, we have added measures of GHGE to this dataset and developed a novel method to estimate the uncertainty around GHGE of primary school meals (both school lunches and packed lunches), based on uncertainty about the parameter estimates for GHGE for different food groups.

Research questions

This study aims to answer the following three research questions:

1: What is the contribution of primary school meals to the total greenhouse gas emissions (GHGEs) in England?

2: Are school lunches associated with more or less GHGEs than packed lunches?

3: Are healthy primary school meals less GHG intensive than unhealthy meals?
Methods

Data set

The “Primary School Food Survey (PSFS)” data set, a survey conducted in 139 primary schools in England (18), was used in this study. The survey involved 6,690 students who ate school lunches and 3,488 students who brought packed lunches. This survey was commissioned and funded by the Children’s Food Trust (formerly the School Food Trust). It involved selecting a random sample of 290 primary schools (from the Department for Children, Schools and Families database) stratified according to region, school type and postcode. Of the 290 schools, 50% agreed to take part and six schools withdrew later. Reasons given by schools to refuse participation are listed in the appendix 4 of the PSFS full technical report (18). Individual level meal data were collected by trained researchers. They visited each school for five days in a week and collected details of 10 pupils having a school lunch from different service points or rooms and five children per day having packed lunches in the same schools.

Nutritional data on primary school meals

Nutritional information for all the foods served to the school lunch tray or brought in the packed lunch box by each participating student on that day was recorded. This dataset included the name of the food item, a unique food code, weight of the serving and nutritional information for key macro and micro nutrients. Nutritional analysis of the foods was estimated with reference to the Food Standards Agency Nutrient Databank (19). In the PSFS dataset there were 1,556 unique food codes consumed by students.

GHGE data on primary school meals

We conducted a systematic review to estimate the GHGEs associated with the production of 100g of different food groups (20). See supplementary material for this EJCN paper for details [add Ref]. This systematic review involved searching peer-reviewed and grey literature from 1995-2012 on GHGEs associated with the production and consumption of food items (estimated by Life Cycle Analysis). The overall environmental burden of products is reported in Global Warming Potential (GWP) a cumulative value for main greenhouse gases such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). This systematic review provides GHGE values for food items listed in a commonly used Food Frequency Questionnaire (FFQ) (21). If the food item was not available in the GHGE dataset the average value for that group was used (e.g. for apricot the average value of all fruits was used). The systematic review does not provide GHGE values directly for composite food items (i.e. foods that are made from more than one primary ingredient, such as pizza or chicken curry). The GHGE values of composite foods were estimated by allocating GHGE values of ingredients using a recipe for that food item. Recipes were sourced initially from the McCance and Widdowson (22) series of food composition tables (which contribute to the UK Nutrient Databank), supplemented with standardised searches of Google. This approach was adopted from a previous study conducted by Scarborough et al (8).
Categorisation of foods

We categorised all of the meals in the PSFS by the five food categories in the eatwell plate (23), thereby allowing an assessment of the main sources of GHGE in the primary school food sector. These five groups are: bread, rice, potatoes, pasta and other starchy foods, fruits and vegetables, meat, fish, eggs, beans and other non-dairy sources of protein, milk and dairy foods and foods and drinks high in fat and/or sugar. Each food code in the PSFS was allocated in to the relevant category of the eatwell plate using the National Food Guide and its food group classification (24), which provides a list to classify food in to the five food groups of the eatwell plate. The composite foods were split between the different eatwell plate categories by estimating the proportion of weight for each raw ingredient, using data from the recipes identified using the method described earlier.

Defining healthy and unhealthy meals

Our primary measure used to identify healthy primary school meals was achievement of target levels of salt, saturated fat and free sugar. This measure takes account of three nutrients that the WHO considers to be related to non-communicable diseases (10, 25). Since this measure does not account for micronutrient quality of foods (which previous research has suggested should be considered if sustainability of diets is addressed (26)), we also use a secondary measure which considers both macro and micronutrients (achieving any 7 or more standards from the 14 nutrient based standards set for primary school meals) (16). The PSFS report provides reference values for a primary school meal for these 14 nutrients - energy, protein, carbohydrate, non-milk extrinsic sugars, fats, saturated fatty acids, fibre, sodium, vitamin A, vitamin C, folate, calcium, iron and zinc (16). There is no accepted, gold standard method to categorise “healthy” and “unhealthy” meals and any definition will have practical challenges when assigning meals in to a specific category.

Statistical analysis and estimation of uncertainty

The average GHGEs per school and packed lunch was estimated by aggregating all the items served per child on to their lunch tray on that day and these values were used to estimate the total GHGEs from primary school meals in England, per year. We estimated the GHGEs and nutritional quality of school meals served. The leftover amounts were not considered in this analysis.

The number and proportion of meals which met nutrient based criteria are presented for both packed lunches and school lunches. Mean GHGE value for healthy meals and unhealthy meals were estimated for both packed lunches and school lunches and compared using t-tests.

There are two main types of uncertainties associated with this analysis: 1) uncertainty due to sampling error (introduced by the representative sample from the PSFS), 2) the uncertainty due to parameters we used to estimate nutritional values and GHGE values. Standard statistical analyses estimate the uncertainty that is due to sampling error.

A novel method was developed to estimate the uncertainty due to GHGE values. For point estimates of results, the GHGE value used is the mean of the GHGE values reported for each
food group in the literature, as identified by the systematic review. But for the uncertainty analysis a Monte Carlo simulation with 1000 iterations (27) was conducted, where the individual GHGE parameters identified from the systematic review are allowed to vary randomly from the selection identified in the literature.

**Results**

**The contribution of primary school meals to GHGEs (overall and by eatwell food group)**

In the PSFS dataset, there were 1,556 unique food items consumed by 6,690 students. In total 38,148 food items were consumed of which 11,906 of them were composite food items. In packed lunches there were 943 unique food items consumed and in total 17,272 food items were consumed by 3,481 students. Sample was equally distributed among age groups and sex did not have a significant impact on the mean GHGEs per meal. The GHGE values of individual school meals were normally distributed with a long tail. For school lunches the interquartile range was 0.40 - 0.80 and for packed lunches it was 0.46 – 0.86 KgCO$_2$e (Table 1).

In both, school lunches and packed lunches the contribution of the meat, fish and alternatives category to GHGEs is much higher than its contribution by weight. Around 62% of the weight of packed lunches was from fruit and vegetables (primarily due to the large serving sizes of fruit juices, which make up 44% of the weight of packed lunches) (Figure 1).

The mean GHGE value per school lunch was estimated as 0.72 KgCO$_2$e (Table 2). Of the 4.2 million primary school children in England around 39% take school lunches (16). There are 190 school days per year in England. Therefore, the annual GHGE from primary school lunch is approximately 224 million KgCO$_2$e per year. The mean GHGE value per packed lunch is 0.70 KgCO$_2$e and 61% of primary school children take packed lunches on 190 school days per year. The total GHGEs of packed lunches was estimated as 354 million KgCO$_2$e per year.

Table 2 shows that for both school lunches and packed lunches around 90% of the uncertainty around the mean GHGE value is due to the uncertainty around GHGE parameters. Uncertainty intervals around the estimate of total GHGEs from school lunches (including packed lunches) is 455 million – 892 million KgCO$_2$e per year.

Table 3 shows that when healthy meals are defined by salt, saturated fat and sugar levels, the mean GHGE of healthy school lunches was 0.54 (0.47 – 1.46) KgCO2e and the mean GHGEs of unhealthy school lunches was 0.81 (0.57 – 1.44) KgCO2e. Similarly the mean GHGEs of healthy packed lunches (0.39 KgCO2e) was lower than the mean GHGEs of unhealthy packed lunches (0.72 KgCO2e).

Previous studies have highlighted the importance of considering micro-nutrient levels when looking at opportunities to reduce the GHGE from meals (26). The Primary School Food nutrient based standards had values for 14 macro and micro-nutrients (16). The PSFS report used the achievement of 7-10 nutrients as an indicator to measure the improvement of school meals over time. There were 4,312 school lunches (64.5%) which achieved at least seven...
nutrient based standards (out of 14) and therefore we used this definition to create a second method to classify meals as healthy.

Table 3 also shows results of comparing the mean GHGE of healthy meals vs non-healthy meals for both school lunches and packed lunches and the uncertainty when micro-nutrients are considered to define healthy meals. The mean GHGE for healthy school lunches was 0.79 KgCO$_2$e (0.56 – 1.44) compared to 0.59 KgCO$_2$e (0.42 – 1.04) for unhealthy lunches. Among packed lunches the mean GHGE of healthy lunches (0.83 KgCO$_2$e) was higher than the mean GHGE of unhealthy lunches (0.60 KgCO$_2$e).

Reducing GHGEs from meals could help to reduce nutrients such as saturated fat, salt and sugar. But as shown in Table 4 these healthy meals with lower GHGEs have less fibre and micronutrients - iron, calcium, zinc and folate. Under the second definition, “healthy meals” have higher levels of fibre and micronutrients, but the GHGE is also increased.

Discussion

This study found that the total GHGEs due to primary school meals in England is 578.1 million KgCO$_2$e a year, equivalent to more than 578,000 economy class return air journeys between London and New York (28). School lunches have a marginally higher GHGE mean value than packed lunches, but the overall emission is higher from packed lunches as more primary school children take packed lunches.

The study also investigated whether healthier school meals were associated with less or more GHGEs. When we used a common definition to define healthy meals (lower levels of salt, saturated fat and sugar) results suggest that healthy school lunches and packed lunches are low in GHGEs, but are also lower in fibre and some micronutrient levels.

This difference in GHGE in healthy and unhealthy meals could be due to several reasons. For example it could be associated with the amount of food items included in the meal and therefore with the total energy of the meal. To test this, we estimated the GHGE per set amount of energy in a primary school meal. The mid-point of the energy reference range for primary school meals is 530Kcal. We estimated the GHGE for a 530Kcal meal in both healthy and unhealthy groups.

When GHGE per 530 Kcal is estimated in healthy and unhealthy school meals classified according to salt, sugar and saturated fats, the healthier meals has more GHGEs.

We also used a secondary method to define healthy and unhealthy meals using seven out of 14 nutrient standards. When GHGE per 530Kcal is estimated in healthy and unhealthy school meals classified according to seven out of 14 standards, still “healthy meals had a higher GHGE value (0.85) compared to unhealthy meals (0.77 KgCO2e). Now the difference is 0.08 compared to the previous difference of 0.2 KgCO2e. But there is no change in the previous conclusion of comparison between healthy and unhealthy school lunches, based on this definition. It is not straight forward to provide a reason for the difference in GHGE values in healthy and unhealthy meals.
If all students adopted a healthy school lunch defined by low salt, sugar and saturated fat (with a mean GHGE value of 0.54 KgCO\textsubscript{2}e), the total GHGE from primary school meals would be 441.2 million KgCO\textsubscript{2}e, saving 136.9 million KgCO\textsubscript{2}e compared to the current total emissions from primary school meals. This is equivalent to avoiding more than 139,000 economy class return air journeys between London and New York (28).

This study also showed that 106 million KgCO\textsubscript{2}e (30% of the GHGEs associated with packed lunches) are from fruit juices. These could be removed by replacing fruit juice with tap water, which has also been recommended as a priority in another government policy- the Government Buying Standards for Food and Catering Services published by the Public Health England (29).

There are significant differences in dietary patterns due to religious and cultural reasons. It is argued that cultural backgrounds should be given a central attention if we try to promote healthier and sustainable food choices in multi-ethnic communities (30). These are important lessons for the UK which has a diverse community with immigrants from different parts of the world.

**Comparison with other literature**

No previous studies have estimated the carbon footprint of the primary school food sector, in any country. However, a number of studies have estimated the total carbon footprint of the food system in the UK (8, 31, 32). These studies have estimated that total GHGE due to food consumption in the UK is around 95MtCO\textsubscript{2}e/year (33). The total emissions from primary school lunches in England, as estimated in this paper represent about 0.6% of that, which is to be expected since primary school children represent 6.5% of the population in the UK and the school lunch only represents a third of their food intake in 190 days in a year.

The literature suggests that policy options should aim to reduce GHGE intensive meat meals to achieve both environmental goals and health benefits (34). Healthy meals are often considered to be low GHGE intensive (35). There are mixed findings on whether moving to a healthier diet or moving towards recommended dietary guidelines would reduce GHGEs (36). The previous literature has also reported that sometimes, despite containing large amounts of plant-based foods, high nutritional quality diets don’t always have the lowest GHGE values (37). Tom et al 2015 found that reducing caloric intake levels and shifting current US diet to recommended food patterns based on the US Dietary Guidelines would increases energy use by 38% and GHGEs by 6% (38). The difference in GHGE in healthy and unhealthy meals could be impacted by several factors such as the type of food items and energy levels in the meals and therefore it is important to consider all these aspects, when making recommendations for sustainable healthy diets.

Previous studies have also highlighted that changing diets to make them sustainable could compromise the micro-nutrient levels(26). A paper from Horgan et al showed that it is very difficult to meet all dietary recommendations, especially for fibre and sodium; reducing GHGE makes it even more difficult, using adult diets from the UK National Diet and Nutrition Survey (39). Our results also support these findings and challenge the statement of “healthy meals are always environmentally friendly”. Therefore this analysis supports
findings from other papers which suggest that the association between GHGE reduction and nutritional quality is complex and may not always be synergistic (9, 10, 39).

**Strengths and limitations**

For this study a method that has not been used before was used to address the uncertainty due to GHGE parameters of food. Use of GHGE values of food to create databases combining GHGE values and nutrition information is a relatively new discipline. Therefore researchers need to develop methods in order to incorporate uncertainty in final results appropriately.

The GHGE estimates are from a systematic review, which included papers from a wide variety of sources, countries and different production systems.

We have included the GHGE of all primary school food items served to trays and we did not consider leftover food. Disposal of food waste also contributes to the GHGE and therefore we underestimate the GHGE associated with primary school meals. The PSFS collected data between February and April 2009 and therefore this dataset does not allow us to assess any seasonal variations in school meals.

This study is based on actual meals consumed by school children and differs from many other published studies which examine computer generated meals. This study provides valuable insights into the GHGEs of consumed meals and could be useful for policy decisions regarding food provision in the public sector. We have established methods to define healthy diets or healthy foods, but not individual meals. A systematic review by Marshall et al shows that there are more than 80 different diet quality indices for children and they have used food only standards, nutrient only standards or combination of both of them to assess the “healthiness” of diets (40). They require data obtained from 24 hour dietary recalls or food frequency questionnaires. In this dataset, we have data only for a single meal of the day, per child and it is difficult to draw conclusions about “healthiness of diets” using these existing tools.

**Conclusion**

This paper demonstrates that changes in the primary school food sector can have an impact on UK greenhouse gas emissions, and that meals that are lower in salt, saturated fat and sugar are associated with fewer greenhouse gas emissions. However, the results suggest that lowering greenhouse gas emissions in school meals may compromise fibre levels and micronutrient quality. These methods can be used to estimate the greenhouse gas emissions of other government food procurement sectors including hospitals, prisons and the military.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgements**

We thank the Children’s Food Trust (formerly the School Food Trust) for making data available for this analysis.
Funding:

Kremlin Wickramasinghe and Nick Townsend are supported by a grant from the British Heart Foundation (006/P&C/CORE/2013/OXFSTATS). Mike Rayner and Peter Scarborough are supported by a programme grant from the British Heart Foundation (021/P&C/Core/2010/HPRG). Michael Goldacre was funded in part by Public Health England. The views expressed are the authors’ and do not necessarily reflect the views of the funding bodies.

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Figure 1. Distribution of weight and GHGE by eatwell food groups- primary school meals

1a: Distribution of weight by food group, school lunches

1b: Distribution of GHGE by food group, school lunches

1c: Distribution of weight by food group, packed lunches

1d: Distribution of GHGE by food group, packed lunches
|                          | School lunches | Packed lunches |
|--------------------------|----------------|----------------|
| **No of meals**          | 6,690          | 3,481          |
| **Non-composite items**  |                |                |
| No of non-composite food items in each eatwell food group |                |                |
| bread, other cereals and potatoes | 7,415          | 361            |
| milk and dairy           | 1,686          | 1,284          |
| meat, fish and alternatives | 2,253          | 213            |
| fruit and vegetables     | 9,601          | 5,311          |
| fatty and sugary foods   | 1,829          | 77             |
| water                    | 3,456          | 513            |
| **Composite food items** |                |                |
|                          | 11,906         | 9,485          |
| Percent by weight of composite food by eatwell food group |                |                |
| bread, other cereals and potatoes | 24.8 %         | 37.2%          |
| milk and dairy           | 24.2 %         | 6.3%           |
| meat, fish and alternatives | 19.2 %         | 23.0%          |
| fruit and vegetables     | 20.6 %         | 17.2%          |
| fatty and sugary foods   | 11.2 %         | 16.3%          |
| **Mean GHGE value (KgCO₂e)** | 0.72          | 0.70           |
| **Standard Deviation (SD)** | 0.60          | 0.42           |
| **Range – Mean GHGE value (KgCO₂e)** | 0.04 - 5.59   | 0.34 - 10.99   |
| **Interquartile range (IQR) of GHGE (KgCO₂e)** | 0.40 - 0.80   | 0.46 - 0.86    |
Table 2
GHGE values (KgCO$_2$e), mean per lunch for school lunches and packed lunches

|                  | Mean GHGE (KgCO$_2$e) | 95% uncertainty due to sampling error only | 95% uncertainty due to GHG parameter variance only | 95% uncertainty interval based on both sampling error and GHG parameter variance |
|------------------|------------------------|-------------------------------------------|-------------------------------------------------|--------------------------------------------------------------------------------|
| School lunches   | 0.72                   | 0.71 – .74                                | 0.53 – 1.26                                      | 0.52 – 1.34                                                                    |
| Packed lunches   | 0.70                   | 0.69 – 0.72                               | 0.59 – 0.90                                      | 0.58 – 0.94                                                                    |
### Table 3

GHGE of healthy meals vs non-healthy meals

|                      | Healthy meals defined by salt, saturated fat and sugar levels only | Healthy meals were defined by seven out of 14 nutrient based standards |
|----------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|
|                      | Number of meals | Mean GHGE (kg CO₂e) | 95% uncertainty due to sampling error only | 95% uncertainty due to GHG parameter variance only | 95% uncertainty interval based on sampling and GHG parameter variance | Number of meals | Mean GHGE (kg CO₂e) | 95% uncertainty due to sampling error only | 95% uncertainty due to GHG parameter variance only | 95% uncertainty interval based on sampling and GHG parameter variance |
| School lunches       |                |                    |                                            |                                                |                                                         |                |                    |                                            |                                                |                                                         |
| Unhealthy            | 4,552          | 0.81               | 0.79 - 0.83                               | 0.89 - 1.39                                   | 0.57 - 1.44                                           | 2379           | 0.59               | 0.58 - 0.61                               | 0.44 - 0.98                                   | 0.42 - 1.04                                           |
| Healthy              | 2,130          | 0.54               | 0.52 - 0.96                               | 0.44 - 1.02                                   | 0.47 - 1.46                                           | 4312           | 0.79               | 0.78 - 0.81                               | 0.59 - 1.40                                   | 0.56 - 1.44                                           |
|                      |                |                    |                                            |                                                |                                                         |                |                    |                                            |                                                |                                                         |
| P<0.001              |                |                    |                                            |                                                |                                                         |                |                    |                                            |                                                |                                                         |
| Packed lunches       |                |                    |                                            |                                                |                                                         |                |                    |                                            |                                                |                                                         |
| Unhealthy            | 3290           | 0.72               | 0.70 - 0.73                               | 0.60 - 0.92                                   | 0.59 - 0.96                                           | 1307           | 0.60               | 0.59 - 0.61                               | 0.51 - 0.76                                   | 0.50 - 0.78                                           |
| Healthy              | 191            | 0.39               | 0.35 - 0.42                               | 0.32 - 0.50                                   | 0.31 - 0.52                                           | 1974           | 0.83               | 0.80 - 0.86                               | 0.69 - 1.08                                   | 0.67 - 1.15                                           |
|                      |                |                    |                                            |                                                |                                                         |                |                    |                                            |                                                |                                                         |
| P<0.001              |                |                    |                                            |                                                |                                                         |                |                    |                                            |                                                |                                                         |

*p values are based on uncertainty from sampling error only.
Table 4
Levels of positive food elements in healthy and unhealthy meals, based on two different definitions of ‘healthy’.

|                | Standard “Healthy” defined by Saturated fats, salt and sugar | “Healthy” defined by 7 out of 14 nutrients |
|----------------|-------------------------------------------------------------|------------------------------------------------|
|                | Healthy          | Unhealthy          | P*      | Healthy          | Unhealthy          | P*      |
|                | Mean  | SE    | Mean  | SE    | P<0.001 | Mean  | SE    | Mean  | SE    | P<0.001 |
| Fibre (g)      | 4.2   | 2.93  | 0.14  | 4.13  | 0.04    | P<0.001 | 5.44  | 0.06  | 3.01  | 0.03    | P<0.001 |
| Iron (mg)      | 3     | 1.55  | 0.58  | 2.79  | 0.21    | P<0.001 | 3.45  | 0.34  | 2.16  | 0.02    | P<0.001 |
| Calcium (mg)   | 193   | 142.28| 4.84  | 294.97| 2.54    | P<0.001 | 343.42| 4.15  | 243.20| 2.64    | P<0.001 |
| Zinc (mg)      | 2.5   | 1.07  | 0.04  | 2.10  | 0.02    | P<0.001 | 2.56  | 0.03  | 1.64  | 0.67    | P<0.001 |
| Folate (µg)    | 53    | 33.35 | 1.63  | 55.28 | 0.62    | P<0.001 | 71.61 | 1.03  | 40.69 | 0.52    | P<0.001 |
| Energy (kcal)  | 504-557| 331.87| 2.15  | 558.20| 2.40    | P<0.001 | 515.20| 2.73  | 432.58| 3.41    | P<0.001 |