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REVIEW ARTICLE

Physiological Effects Associated with Quinoa Consumption and Implications for Research Involving Humans: a Review

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Abstract Quinoa is a pseudo-grain consumed as a dietary staple in South America. In recent years, consumer demand for quinoa in the developed world has grown steadily. Its perceived health benefits have been cited as a driving force behind this trend, but there are very few human studies investigating the impact of quinoa consumption. The aim of this review was to identify physiological effects of quinoa consumption with potential for human health. A critical evaluation of animal model studies was conducted. The quality of identified studies was assessed using a methodological quality assessment tool and summative conclusions were drawn to guide the direction of future human research. The majority of studies were of fair quality. Purported physiological effects of quinoa consumption included decreased weight gain, improved lipid profile and improved capacity to respond to oxidative stress. These physiological effects were attributed to the presence of saponins, protein and 20-hydroxyecdysone in the quinoa seed. The implications of these findings are that human studies should investigate the impact of quinoa consumption on weight gain and lipid levels. The role of quinoa as an antioxidant is still unclear and requires further elucidation in animal models.

Keywords Quinoa · Animal · Weight gain · Lipids · Antioxidant effects · Saponins

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Abbreviations

DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
MQA	Methodological quality assessment
QI	Quality index
RQ	Respiratory quotient

Introduction

Across the globe, cereals form an integral part of the human diet, with an estimated 35 % of daily dietary energy derived from this source [1]. Specifically, cereals encompass grains, such as wheat and barley as well as pseudo-grains such as quinoa and buckwheat [2]. Inclusion of the whole grain form of cereals in the diet is associated with health benefits such as a reduction in the risk of developing cardiovascular disease and diabetes [2]. These properties have contributed to the establishment of dietary guidelines that encourage the regular consumption of whole grains in the diet [3, 4].

As a consequence of the health benefits that whole grains offer, research efforts have begun to concentrate on specific grains and the role they could play in human nutrition. Quinoa is an example of a pseudo-grain that has been grown in the Andes and used for human consumption and livestock feed for thousands of years [5]. The leading producers of quinoa are Peru and Bolivia [6], however there is emerging global interest to produce quinoa as an alternative food crop [5]. Desirable agronomic properties in conjunction with higher prices induced by increased demand have been the drivers of this emerging interest [7, 8].

As global awareness continues to grow, research efforts exploring the possible health benefits associated with quinoa

consumption become more valuable. Unique health imparting properties increase the marketability of a food and are of interest to manufacturers to pursue. As an example, quinoa protein, unlike most other grains, is not limited by the amino acid lysine creating a point of differentiation and potential health [9–11]. *In vitro* experiments have shown that the digestibility of starch from quinoa is similar to pasta and lower than white bread, while the antioxidant potential is similar to wheat and superior to other so-called ancient grains such as amaranth [12, 13].

Reviews synthesising the literature surrounding quinoa have focussed on the nutrient composition [14, 7], as well as the functional potential of quinoa in the human diet [5, 7, 14]. Recently, it has been suggested that conducting systematic reviews of preclinical studies, such as animal studies, is a valuable tool for establishing the likelihood of mechanistic understanding being translated into human research applications [15]. In particular, evaluating the validity of the methods underpinning these studies and the results that are generated can determine hypotheses for future human studies. This is relevant to quinoa as it is becoming an increasingly popular food, but its human health benefits are relatively poorly researched. The primary aim of this review was to identify physiological effects from quinoa consumption, which have potential for human health benefits. The implications for research involving humans are discussed.

Method

A systematic review of the scientific literature was conducted according to published standards. Since animal studies were the focus, the quality appraisal approach defined by Downs and Black [16] and adjusted for use among animal studies by Ainge et al. [17] was applied.

Inclusion and Exclusion Criteria

The eligibility criteria were determined prior to the commencement of the search so as to minimise any bias in inclusion and exclusion of studies. All animal studies that investigated the impact of quinoa consumption on physiological outcomes were considered for inclusion. Included papers were limited to original research published since 1975 in peer reviewed journals and published in the English language. Studies were excluded if they did not include quinoa as part of an experimental diet. Previously conducted reviews were also excluded from this systematic review.

Search Terms and Strategy

"Quinoa", "animal", "health" and "feeding" formed the search terms. Combinations of these terms were joined with the Boolean operator 'AND' to identify relevant articles. The search encompassed the time period from 1975 onwards (40 year period) and involved seeking relevant articles from the following electronic databases: Agricola, Cambridge Journals Online, Cochrane Library, CINAHL, MEDLINE, PubMed, SAGE Journals Online, ScienceDirect, Scopus, SPORTDiscus, Springer Link, Web of Science and Wiley Online. The same set of search terms were used in each database during the search phase, performed in February 2015.

Initially, the title of the article was examined for inclusion. Articles, which appeared to be of relevance, were further reviewed through their abstract to determine if they met the eligibility criteria. The full text of articles whose abstract met the criteria was then saved and analysed to ensure the article met the inclusion criteria. The reference lists of articles included for review were also examined for relevant articles. These were assessed using the same eligibility criteria.

Data Extraction

Of the studies that met the inclusion criteria, the following information was extracted into a summary table; animal species utilised, animal age, sample size, duration of the experiment, the control and intervention diet/s, quinoa content in the intervention diet/s, main findings and the quality of the article. The sample size reported in the summary table was restricted to animals that were fed either the control or intervention diet/s and was not necessarily equal to the sample size for the overall experiment. Studies that presented significant findings in graphs without an explicit presentation of the effect size in a table (or in text) had their result summarised in the summary table as being significantly different to their respective control.

Methodological Quality Assessment

The methodological design and validity of included studies were assessed by using a modified version of the Quality Index (QI), developed by Downs and Black [16] and adjusted for use among animal studies by Ainge et al. [17]. This modified tool, known as the methodological quality assessment (MQA), was refined further for this systematic review to include all animal studies, rather than just studies utilising rats (Fig. 1). The MQA provides a quantitative measure of study quality, enabling an assessment of the rigour of individual studies to be made.

Of the 19 review questions, 12 assess the reporting quality, six the internal validity and one the power of the studies. A 'yes' or 'no' response was reported as a one or zero for each question respectively, with the total score determined by summing together the answers to each of the 19 equally weighted questions. There were two possible ways for a study to fulfill the criteria regarding power. Either an explicit power calculation was provided within the paper, or the study identified a

Reporting

General

1. Were the hypothesis/aims/objectives of the study clearly described within the introduction?
2. Were the main outcomes to be measured clearly described in the introduction or methods section?

Animal characteristics

3. Was animal species/strain identified?
4. Was the animal age at commencement of the study or at conception specified?
5. Have the animal weights at commencement or at conception of the study been specified?
6. Have the animal starting numbers, including litter number and sizes been specified?
7. Have the housing details been specified?

Design and outcomes

8. Were the interventions of interest clearly described?
9. Were the main findings of the study clearly described?
10. Were estimates of the random variability in the data for the main outcomes provided?
11. Have all important adverse events that may be consequences of the intervention be reported?
12. Have the actual probability values been reported for the main outcomes except where probability value is less than 0.0001?

Internal validity

Bias

13. Was an attempt made to blind those measuring the main outcomes of the intervention?
14. Were the statistical tests used to assess the main outcomes appropriate?
15. Were the main outcomes measures used accurate (valid and reliable)?

Confounding

16. Was it stated in the text that the animals were randomised to intervention groups?
17. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?
18. Were loses of animals explained?

Power

19. Was the paper of sufficient power to detect a clinical important effect where the probability value for a difference being due to chance is less than 5%?

Fig. 1 Methodological quality assessment questions [17], modified from Downs and Black [16] quality index

significant effect of the treatment with respect to the primary outcome. Reporting and internal validity scores were determined separately and reported [17]. In a similar manner to previous work [18], individual study quality was categorised into four discrete quality levels based on the overall score: excellent (17–19), good (14–16), fair (10–13) and poor (less than 10). Furthermore, responses to individual quality questions across the included studies were summed in order to show general strengths and weaknesses across the literature.

Results

The systematic search of the scientific databases resulted in the identification of 888 articles for analysis. After eliminating articles that did not fit the eligibility criteria, a total of 17 articles were included in the final review. Hand searching of the reference lists of the included articles yielded two additional articles (Fig. 2.) After the application of the eligibility criteria, one of these articles was appropriate to include in the review. Therefore the combination of electronic and hand searching resulted in 18 articles being included for review.

The results from the MQA as well as the quality of the included studies were summarised in descending order (Table 1). The overall scores ranged from 6 (poor) to 14 (good), with the average total score being 10.9 (fair) [19, 20]. The vast majority of studies (12) were classified as fair quality. Four were classified as being of poor quality, two as good and none as excellent quality. A summary of the reporting and internal validity scores for each study is also provided in Table 1. Generally, the scores achieved in the reporting component of the MQA were superior to the scores generated for the internal validity component across all the studies. Furthermore, the low internal validity scores were generally responsible for the low overall scores generated among all the studies. An overview of the responses to the MQA questions across the body of literature is depicted in

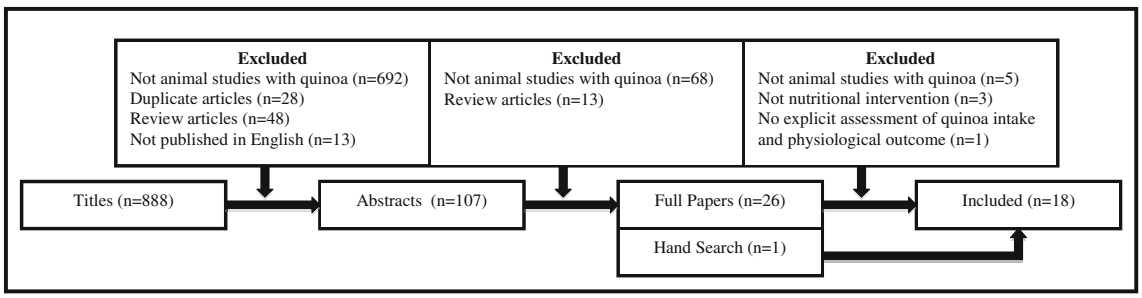

Fig. 2 Flow chart of literature screening process, with combinations of "quinoa", "animal", "health" and "feeding" identifying a total of 888 titles that would then be screened based on their titles, abstracts and full text

Table 1 A summary of the reporting, internal validity, total methodological quality assessment scores and study quality (excellent, good, fair or poor) attained by each study as well as the average for these components across the body of literature

Reference	Quality	Reporting score (n/12)	Reporting (%)	Internal validity score (n/7)	Internal validity (%)	Total score (n/19)
[20]	Good	9	75	5	71	14
[19]	Good	11	92	3	43	14
[21]	Fair	9	75	4	57	13
[22]	Fair	10	83	3	43	13
[23]	Fair	9	75	3	43	12
[24]	Fair	9	75	3	43	12
[11]	Fair	8	67	3	43	11
[25]	Fair	8	67	3	43	11
[26]	Fair	8	67	3	43	11
[27]	Fair	8	67	3	43	11
[28]	Fair	9	75	2	29	11
[29]	Fair	9	75	1	14	10
[30]	Fair	7	58	3	43	10
[31]	Fair	8	67	2	29	10
[10]	Poor	7	58	2	29	9
[32]	Poor	7	58	2	29	9
[33]	Poor	8	67	1	14	9
[9]	Poor	5	42	1	14	6
Average	Fair	8.3	69	2.6	37	10.9

Table 2. Reporting factors that were poorly assessed included adverse impacts that could result from the intervention as well as exact probability values. A lack of blinding and randomisation as well as inadequate adjustment for confounding factors and an absence of explanations for the loss of animals were consistently noted across the majority of studies reviewed, reflecting a poor level of internal validity across the literature. A summary of the animal species, animal age, sample size, duration of study, control and intervention diet, quinoa concentration in the diet as well as the main findings of each included study is depicted in Table 3. The majority of studies were performed in rats (11), while mice, chickens and piglets were also used to conduct experiments.

Physiological outcomes that were comparatively assessed between animals consuming quinoa and a control diet included weight gain and metabolic outcomes (16 studies), lipid profiles (6 studies) and antioxidant effects (2 studies). Several studies examined a combination of these outcomes, thus explaining the discrepancy between the number of studies included in the review (18) and the number of studies showing physiological outcomes (24).

Of the studies pertaining to weight gain, two were of good quality, 10 of fair and four of poor quality. The vast majority of studies showed a positive association between quinoa consumption and decreased weight gain among animals. The largest effect was a comparative decrease of 89 % between the control and quinoa group [32]. The studies that showed a comparative increase (of up to 10 %) in weight gain among animals fed quinoa were unable to show statistically significant increases. A general trend among the studies investigating weight gain was for relative differences in weight gain between the quinoa and control group to narrow as study quality declined.

Three studies investigating weight gain also analysed the concentration of hormones involved in the regulation of appetite. The consumption of quinoa in the diet was associated with a decrease in the concentration of plasma leptin by between 14 and 35 % [31, 22]. Postprandial ghrelin and cholecystokinin differences among the quinoa group were respectively 5.4 % lower and 45.5 % higher than levels among the control group [28]. In addition, one of these studies investigated differences in the release of cytokines (such as monocyte chemoattractant protein-1, interleukin-1β and plasminogen activator inhibitor-1) from adipose tissue (adipokines) among mice fed high fat diets [22]. The addition of quinoa to the diet decreased the mass of adipose tissue and significantly reduced the expression of inflammatory adipokines [22].

Six studies, all of fair quality, investigated the impact of quinoa consumption on lipids. Across the body of literature, the consumption of quinoa was associated with decreases in cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The largest decreases in cholesterol, triglycerides and HDL were 25.5, 46.5 and 9.6 %, respectively [27]. It was not possible to accurately quantify the relative decreases in LDL levels because none of the studies reported the level of this biomarker in a tabular format. However, it appeared that as the concentration of quinoa in the diet rose above 50 g/kg so too did the efficacy of

Table 2 A summary of the number and proportion of positive (yes) responses to each MQA[a] question for the 18 studies that were reviewed

	Reporting quality													Internal validity (indication of bias, confounding & power)					
Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Positive response	14	15	17	10	10	15	17	15	17	18	0	1	0	15	15	5	0	3	9
Proportion of positive responses (%)	78	83	94	56	56	83	94	83	94	100	0	6	0	83	83	28	0	17	50

[a] Methodological quality assessment

Table 3 Summary of all studies reviewed

Reference	Animal species	Animal age at start	Sample size (n)	Trial length	Control diet	Intervention diet	Quinoa in diet (g/kg)	Main outcome measure	Main findings	Quality[1]
[20]	Male broilers (ASA Chick A/S)	6 days	525	31 days	Regular broiler feed	Regular broiler feed with raw or processed quinoa	100, 200, 400	Weight gain	Control group gain – 1323 g. Weight gain (with increasing raw quinoa content) 1247 g ($p>0.05$), 1065 g ($p<0.05$) and 765 g ($p<0.05$). Weight gain (with increasing processed quinoa content) 1232 g ($p>0.05$), 1079 g ($p>0.05$) and 875 g ($p<0.05$).	Good
		0 days	960	39 days	Regular broiler feed	Regular broiler feed with raw or processed quinoa	50, 150		Control group gain after 20 days – 627 g. Weight gain (group eating 150 g/kg processed quinoa) 593 g ($p<0.05$) after 20 days. Weight gain did not differ between groups at 39 days ($p>0.05$).	
[19]	Landrace Yorkshire Duroc cross-bred piglets	28 days	400	28 days	Basal diet without quinoa	Basal diet with South American or Denmark quinoa hull meal	0.1, 0.3, 0.5	Weight gain	Control group gain – 294 g/day. Quinoa groups gained 280–307 g/day ($p=0.41$). Jejunum epithelial conductance of control group – 22mS/cm^2. In quinoa groups, conductance was 24–25mS/cm^2 ($p=0.04$).	Good
[21]	Wistar rats	60 days	64	30 days	Rodent chow (Nuvilab®)	Nuvilab® with hydrolysed quinoa	2	Weight gain	Sedentary control group gain – 60.2 g, exercised control group gain – 94.2 g. Weight gain, (among quinoa fed groups) sedentary – 16.5 g ($p<0.05$) and exercised – 60.0 g ($p<0.05$)	Fair
								Lipids	Sedentary control group triglycerides – 92.9 mg/dL, exercised control group – 63.1 mg/dL. Triglycerides (among quinoa fed groups) sedentary – 73.9 mg/dL ($p<0.05$) and exercised – 60.9 mg/dL ($p>0.05$). Non-significant difference in cholesterol between control and quinoa group ($p>0.05$).	
[22]	C57BL/6 J mice	6 weeks	36	3 weeks	1. Low fat (LF) diet 2. High fat (HF) diet	High fat diet with added quinoa extract (HFQ)	Not stated	Weight gain Lipids	LF group gain – 3.0 g. HF group and HFQ group gain 5.1 g ($p<0.001$) and 5.6 g ($p<0.001$) respectively. HF group epididymal adipose tissue (EAT) – 28.8 mg/g body weight. HFQ EAT – 21.7 mg/g body weight ($p<0.01$). HF group plasma leptin – 6.0 ng/ml. HFQ group plasma leptin – 3.9 ng/ml ($p<0.05$). Plasma adiponectin and expression of mRNA for SREBP-1c[2] and PAI-1 were lower in HFQ compared to LF group ($p<0.05$). Expression of mRNA for LPL[3], PPAR-γ, PEPCK, Leptin, TLR4, MCP1, CD68, GILZ, OST and PAI-1 were lower in the HFQ group and mRNA expression for UCP2[4] and UCP3 were higher in HFQ group compared to the HF group (all $p<0.05$). LF and HF group triglycerides – 0.50 g/l and 0.53 g/l. HFQ group triglycerides – 0.51 g/l ($p>0.05$). LF and HF group plasma cholesterol – 1.25 g/l and 1.33 g/l. HFQ group plasma cholesterol – 1.35 g/l ($p>0.05$).	Fair
[23]	Male Wistar rats	Not stated	24	5 weeks	Corn or corn with 31 % fructose	Quinoa or quinoa with 31 % fructose	310	Antioxidant activity	The quinoa group had lower liver GPX[5] and CAT, lower CAT in the testis and higher GPX in the spleen (all $p<0.05$) compared to the corn control.	Fair

Table 3 (continued)

Reference	Animal species	Animal age at start	Sample size (n)	Trial length	Control diet	Intervention diet	Quinoa in diet (g/kg)	Main outcome measure	Main findings	Quality[1]
									The quinoa with fructose group showed lower MDA[6] levels compared to the corn with fructose group ($p<0.01$).	
[24]	Male Wistar rats	Not stated	24	5 weeks	Corn or corn with 31 % fructose	Quinoa or quinoa with 31 % fructose	310	Lipids	Cholesterol, triglycerides and LDL of the quinoa group were significantly lower ($p<0.05$, $p<0.05$, $p<0.008$, respectively) than levels in the corn control group.	Fair
[11]	Male Sprague–Dawley rats	Not stated	15	4 weeks	Casein	1. Quinoa flour 2. Cooked quinoa	680	Weight gain	Control group gain – 57 g. Weight gain for the quinoa flour group – 43 g ($p>0.05$) and for cooked quinoa group – 89 g ($p<0.01$). Control group protein efficiency ratio (PER) – 2.67. PER for quinoa flour group – 2.09 ($p<0.01$) and 2.71 ($p>0.05$) for cooked quinoa group.	Fair
[25]	Male broiler chicks	3 days	90	28 days	Maize diet (13.2 % protein)	Raw or polished quinoa (13.2 % protein)	953.5	Weight gain	After 14 days, control group gain – 76 g. Weight gain in raw and polished quinoa group 64.2 and 67.6 g, respectively (both $p<0.05$).	Fair
			90	28 days	Maize diet (18 % protein)	Raw or polished quinoa (18 % protein)	835		After 21 days, control group gain – 486.9 g. Weight gain in raw and polished quinoa group 118.6 and 210.1 g respectively (both $p<0.05$).	
			120	14 days	Maize diet (13.3 % protein)	Raw, polished or washed quinoa (13.3 % protein)	962.5		After 7 days, control group gain – 87.5 g. Weight gain in raw, polished and washed quinoa group 53.0 g ($p<0.05$), 54.9 g ($p<0.05$) and 92.9 g ($p>0.05$) respectively.	
			120	31 days	Maize diet (23 % protein)	Raw, polished or washed quinoa (23 % protein)	800		After 31 days, control group gain – 891.4 g. Weight gain in raw, polished and washed quinoa group 160.4, 383.3 and 737.6 g (all $p<0.05$) respectively.	
[26]	Male Wistar-ST rats	4 weeks	10	13 days	Diet free of quinoa	Control diet with methanolic quinoa extract	11	Weight gain	Control group gain – 14.5 g. Quinoa group gain – 15.1 g ($p>0.05$).	Fair
								Antioxidant activity	Control and quinoa group serum α-Tocopherol – 8.5 μg/ml and 5.6 μg/ml ($p<0.05$) respectively. Control group serum and liver MDA 2.0 nmol/mL and 33.3 nmol/g respectively. Quinoa group serum and liver MDA 3.0 nmol/mL and 40.3 nmol/g (both $p<0.05$) respectively. No differences in serum or liver GPX ($p>0.05$).	
[27]	Male Crj: CD-1 (ICR) mice	7 weeks	18	4 weeks	0.5 % cholesterol, 20 % casein	Control diet with casein substituted for a quinoa protein extract	25, 50	Weight gain	Control group gain – 11.28 g. Weight gain (with increasing quinoa extract) 12.02 and 10.78 g ($p>0.05$).	Fair
								Lipids	Plasma cholesterol (0 to 5 % quinoa) 268.2 mg/dl, 199.9 mg/dl ($p<0.05$), 204.5 mg/dl ($p<0.05$). Liver cholesterol (0 to 5 %) quinoa 10.31 mg/dl, 8.16 mg/dl ($p>0.05$), 6.30 mg/dl ($p<0.05$). Plasma triglycerides (0 to 5 % quinoa) 84.5 mg/dl, 55.4 mg/dl, 45.2 mg/dl ($p>0.05$). Liver triglycerides (0 to 5 % quinoa) 14.06 mg/g, 10.36 mg/g, 9.24 mg/g ($p>0.05$). Daily faecal bile acid (0 to 5 % quinoa) 125.8, 212.3 ($p<0.05$), 202.5 μg/50 g body weight ($p<0.05$). Expression of HMG-CoA[7] reductase was significantly lower ($p<0.05$) in the quinoa groups than the control group.	

Table 3 (continued)

Reference	Animal species	Animal age at start	Sample size (n)	Trial length	Control diet	Intervention diet	Quinoa in diet (g/kg)	Main outcome measure	Main findings	Quality[1]
[28]	Male Wistar rats (albino strain)	Not stated	16	15 days	Casein	Quinoa in place of casein	200	Weight gain	No difference in weight gain between control and quinoa group ($p>0.05$). Control group and quinoa group postprandial CCK[8] levels 8.63 and 12.56 ng/ml ($p<0.01$), respectively. No differences in fasting CCK, ghrelin and leptin and postprandial ghrelin and leptin between groups ($p>0.05$).	Fair
								Lipids	Cholesterol in the quinoa group was significantly lower ($p<0.01$) than the control group.	
[29]	Wistar rats	Not stated	40	14 days	Milled and cooked wheat cereal	Bitter, washed bitter or sweet quinoa	862, 866, 873	Weight gain	The control group gained more weight than the bitter, washed bitter and sweet quinoa groups (no statistics provided).	Fair
[30]	Y DY commercial cross piglets	8 weeks	144	5 weeks	Maize and wheat meal	Maize and wheat meal with quinoa	50, 100	Weight gain	Control group gain – 294 g/day. Weight gain (with increasing quinoa content), 285 g/day and 248 g/day (both $p>0.05$).	Fair
[31]	Male C57BL/6 J mice	6 weeks	Not stated	3 weeks	High fat (HF) diet	High fat quinoa (HFQ) diet	2.8	Weight gain	Over a 24-h period, the respiratory quotient and glucose oxidation of the HFQ group was higher than the control group (both $p<0.05$). Control and HFQ plasma leptin – 4.2 and 3.6 ng/ml ($p>0.05$), respectively.	Fair
								Lipids	Control and HFQ plasma triglycerides – 0.62 and 0.68 g/L ($p>0.05$), respectively. Over a 24-h period, HFQ faecal lipid content was higher than control group ($p<0.05$).	
[10]	Rats	Not stated	20	4 weeks	Corn starch with casein	Dehulled quinoa	641	Weight gain	Control and quinoa group gain – 130 and 126 g ($p>0.05$), respectively. Control and quinoa group protein efficiency ratio – 3.5 and 3.8 ($p<0.05$) respectively.	Poor
[32]	Male Hooded-Lister rats	32 days	8	10 days	Basal diet with casein	Basal diet with quinoa	758	Weight gain	Control and quinoa group gain – 11.0 and 1.2 g/day respectively (no statistics provided).	Poor
[33]	Male Sprague–Dawley rats	Not stated	10	9 days	Maize starch with casein	Maize starch with quinoa	Not stated	Weight gain	The quality of protein from quinoa was poorer than the protein from the control diet (no statistics provided).	Poor
[9]	Male Sprague–Dawley rats	Not stated	Not stated	9 days	Maize starch with casein	Maize starch with quinoa	Not stated	Weight gain	Gain (in increasing order) was control group, washed quinoa group and raw quinoa group (no statistics provided).	Poor

[1] The quality of the studies (excellent, good, fair or poor) was based on the Methodological Quality Assessment score: excellent (17–19), good (14–16), fair (10–13) and poor (less than 10)

[2] *SREBP-1c* sterol regulatory element-binding proteins, *PAI-1* plasminogen activator inhibitor-1

[3] *LPL* lipoprotein lipase, *PPAR-γ* peroxisome proliferator-activated receptor-γ, *PEPCK* phosphoenolpyruvate carboxykinase, *TLR4* toll-like receptor 4, *MCP-1* monocyte chemoattractant protein-1, *CD68* cluster of differentiation 68, *GILZ* glucocorticoid-induced leucine zipper, *OST* osteopontin

[4] *UCP2* uncoupling protein 2, *UCP3* uncoupling protein 3

[5] *GPX* glutathione peroxidase, *CAT* catalase

[6] *MDA* malondialdehyde

[7] *HMG-CoA reductase* 3-hydroxy-3-methylglutaryl coenzyme A

[8] *CCK* cholecystokinin

reductions in cholesterol, HDL and LDL. This apparent relationship between dose and effect did not appear to persist for decreases in triglyceride levels.

Finally, the two studies investigating the antioxidant effects of quinoa were both of fair quality. These studies measured the concentration of antioxidant compounds such as glutathione peroxidase, catalase and superoxide dismutase as well as markers of oxidative damage such as malondialdehyde. The expression of these antioxidant compounds showed a vast degree of variability between organs and between animals subjected to varying degrees of oxidative stress. Measures of lipid peroxidation between the two studies were in complete contrast. The inclusion of quinoa in the diet resulted in a decrease in lipid peroxidation by between 29.6 and 66.1 %, but also a 21 to 50 % increase in peroxidation compared to the control group [23, 26].

Discussion

Among the included animal model studies, weight gain, lipid profiles and antioxidant responses were the main physiological outcomes affected by quinoa consumption. However, the body of literature supporting these effects showed wide variation in terms of rigour and quality. The value of conducting a defined quality assessment for evidence-based review was demonstrated here. Specifically, the MQA tool showed that the quality of animal studies could be improved by incorporating design aspects such as blinding, randomisation and power calculations. These methodological tools would help minimise the impact of bias, including improved reporting on study design and corresponding MQA score.

Effects on Weight Gain

Animal feeding experiments investigating quinoa as a potential food source have identified the presence of saponins, which have been implicated in the reduction of weight gain and feed consumption among animals [25]. However, there is potential for saponins to play a role in human nutrition, particularly in developed countries, where over nutrition is more widespread than under nutrition.

Across the body of literature, it appeared that the presence of saponins in quinoa was connected to decreased weight gain. This association was replicated in rats, mice and chickens and was achieved using a range of different dietary concentrations of quinoa. However, it was not replicated in two piglet studies [19, 30], with speculation that the concentration of saponins in the diet was too low to induce a significant change in weight gain. More generally, it became apparent that as the methodological quality of the studies decreased, so too did the detection of differences in weight gain between treatment and control groups.

Despite the underlying weight loss effect, the magnitude of the effect varied across studies, possibly due to the different concentration of saponins present in quinoa seeds. Each variety of quinoa has a slightly different composition of saponins and each study used processing techniques to prepare the intervention diet, which may have resulted in the loss of saponin fractions. Evidence of these contrasting effects was seen in the two good quality studies where saponins appeared to inhibit weight gain among chickens, but had no effect among piglets [19, 20]. Both studies used large sample sizes, randomisation and employed a similar time period for the intervention to be performed. However, the saponin content was markedly lower in the latter study with piglets.

It was postulated that the mechanism through which saponins operate revolves around their ability to interfere with intestinal function [29]. Studies in an Ussing chamber showed that the presence of saponins derived from quinoa resulted in an increased conductance of pig jejunum [19]. This finding suggests that there was an increase in the permeability of the intestinal lining, resulting in a decreased capacity to actively absorb nutrients for animal growth and development.

The bitter taste of saponins has been implicated in reducing the palatability of certain quinoa varieties. This was shown to decrease food intake and was given as an additional explanation for the incidence of decreased weight gain. A further rationale for the decreased food intake may be due to changes in the expression of gut hormones upon the consumption of quinoa [20, 21, 28, 29]. In particular, post-prandial cholecystokinin levels were elevated after the consumption of quinoa, resulting in a feeling of satiety [28]. Although most commercially available quinoa has been processed to remove the bitter tasting saponins, the presence of protein, dietary fibre and phenolics within the seed may be capable of inducing feelings of satiety, assisting in the reduction of food intake and weight gain.

The ability of quinoa to induce decreased weight gain was unable to be replicated among mice fed a high fat diet with added quinoa [22]. Despite the null finding, the mice fed quinoa showed a slight decrease in adipose tissue mass as well as a decrease in the expression of lipid storage genes such as lipoprotein lipase and peroxisome proliferator-activated receptor-γ [22]. The quinoa extract used in this study was rich in the naturally occurring steroid hormone, 20-hydroxyecdysone. This compound is structurally similar to Vitamin D, which has been shown to affect lipid accumulation in adipose tissue [22]. It was postulated that Vitamin D receptors formed suitable binding sites for 20-hydroxyecdysone, enabling it to influence the expression of genes responsible for lipid storage, however this mechanism requires further elucidation.

A recent follow up study suggested that the presence of 20-hydroxyecdysone in quinoa was responsible for an increase in glucose oxidation and respiratory quotient (RQ) among mice

[31]. However, the explanation for the change in the RQ appears to be counterintuitive. It was suggested that this was indicative of a decrease in fat oxidation and decreased rate of *de novo* lipogenesis [31]. These both seem unlikely since levels of lipid oxidation among the quinoa and the control diet did not differ [31] and furthermore, increased, rather than decreased *de novo* lipogensis from carbohydrate would lead to an increase in the RQ value [34].

A high fat diet fed to mice was shown to increase the expression of inflammatory cytokines released from adipose tissue [22]. This agrees with findings among overweight and obese individuals that display elevated levels of inflammation due to the release of cytokines from adipose tissue [35]. The addition of a quinoa extract rich in 20-hydroxyecdysone to the high fat diet reversed the expression of inflammatory cytokines to levels associated with a low fat diet. This effect may be due to a decrease in adipose tissue mass among the quinoa group and therefore less capacity to release adipokines. It may also be due to the action of 20-hydroxyecdysone and its metabolites binding membrane receptors and as such influencing signal transduction and the expression of adipokines. Future research should aim to identify the underlying cause, which is likely to involve a complex interplay between these factors.

The concentration of quinoa needed to induce weight loss effects in a human cohort must be explored in order to determine if the amount needed to achieve these effects is attainable in the context of a regular diet. In addition, further studies investigating the action of quinoa on weight gain should control the energy density by using isoenergetic diets or calculate average energy intake by measuring the quantity of food consumed in order to ascertain the effect of quinoa on weight gain independent of energy intake. Identifying the potential for quinoa to influence weight gain is of such interest due to the unacceptably high incidence of overweight and obesity; estimated to be 39 and 13 % of the global population respectively [36]. This represents a significant public health burden, particularly since overweight and obesity are known risk factors for a chronic diseases such as cardiovascular disease, Type 2 diabetes and some cancers [36].

Effects on Lipid Profile

The studies investigating lipids were all of fair quality, and showed similarities in terms of their weaknesses. Baseline measures were not explicitly reported, which is a basic limitation of the findings. It could be argued that baseline measures among the animals would not show significant variability due to the similarity in the ages and species of animals. However, providing baseline measures would enable a comparison of changes in lipid biomarkers between intervention and treatment diets to be performed. This would be more informative than a comparison of levels at the completion of the study.

Despite this limitation, it was shown that the inclusion of quinoa in the diet had a significant effect on cholesterol levels in as little as 15 days [28]. A similar acute cholesterol lowering effect has been previously reported among humans consuming β-glucan, where favourable outcomes were noted in as little as 2 weeks [37]. It was proposed that proteins present within the quinoa seed facilitated a reduction in the reabsorption of bile acids and a reduction in hepatic cholesterol synthesis. This was supported by findings that bile acid excretion was elevated and the expression of hepatic HMG-CoA reductase was decreased among mice fed a quinoa diet [27]. This is a similar mechanism to that indicated in other food components such as β-glucans [38], which are effective at decreasing cholesterol [37].

The presence of 20-hydroxyecdysone in the outer casing of the quinoa seed has also shown potential lipid lowering properties. In particular, it was implicated in causing modifications to lipid absorption, which caused significantly higher levels of lipids to be excreted in the faeces of mice fed a high fat diet supplemented with quinoa [31]. Additionally, the cholesterol lowering properties of quinoa were sustained when hypercholesterolemia [27] and oxidative stress [24] were induced through the addition of cholesterol and fructose to the diet respectively. Collectively, this suggests that quinoa may play an active role in the metabolism of cholesterol.

Based on the literature, it appears that the cholesterol lowering properties of quinoa only become significant when at least 2.5 % of the diet (2.5 g per 100 g) contains quinoa [27]. In contrast, there is very little evidence to suggest that the concentration of quinoa has an obvious impact on triglyceride levels. It appears that significant changes in triglycerides are not observed until quinoa is consumed in the diet for at least 30 days [21]. A greater understanding of the process occurring is therefore necessary before firm conclusions can be drawn regarding quinoa and the impact on triglycerides.

None of the included studies were able to demonstrate that quinoa had a significant impact on HDL, while only one study showed that a diet containing quinoa was able to significantly lower LDL levels [24]. Interestingly, this study also had the highest dose of quinoa and was performed over the longest time period. The tentative conclusions of these findings are that consuming quinoa can reduce LDL over a longer time frame. Extending the intervention period (beyond 4 or 5 weeks) may therefore lead to additional improvements in the lipid profile. However, without the guidance of previous work investigating quinoa consumption over a longer duration, it is difficult to determine the optimum intervention period.

Heterogeneity in study design is likely to have played a part in generating the variable outcomes. This heterogeneity included differences in animal species, animal ages, quinoa content in the diet and duration of the intervention period. In addition, it was not clear which bioactive compound/s were

responsible for the underlying effects observed in these studies. Animal studies should further investigate the lipid lowering effects imparted by quinoa and attempt to refine the possible mechanisms that are in operation. It is well established that high cholesterol levels are a risk factor for developing cardiovascular disease [37]. Therefore, food products that can assist in improving the lipid profile in the human body, without radically altering the diet are extremely desirable from a functional and nutritional perspective.

Antioxidant Effects

The antioxidant activity of quinoa has been previously investigated using validated methods such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and Ferric reducing antioxidant power (FRAP) assay [39]. This review identified two animal studies that explored the physiological effect of quinoa consumption on markers of oxidative stress and concentration of antioxidant compounds.

The antioxidant properties of quinoa were most prominent during periods of oxidative stress. Plasma lipid peroxidation was decreased while the expression of antioxidant compounds such as glutathione peroxidase and catalase were elevated in several organs [23]. This suggests that quinoa has the ability to regenerate antioxidant species that can then attack free radicals and therefore protect tissues against oxidative damage. However, these antioxidant properties were less clear when oxidative stress was not intentionally induced in the diet. Since similar analytical methods were used to determine lipid peroxidation, differences in study design are more likely to explain the contrasting results. This includes the use of quinoa extracts that did not possess antioxidant properties, short intervention periods and the use of vitamin supplements in the control diet, which may have acted as antioxidants and nullified any advantageous effects that were generated by consuming quinoa [26].

A limitation of both studies investigating the antioxidant potential of quinoa was the absence of a detailed analysis (identification and quantification) of the main (bioactive) compounds. Quinoa is known to possess compounds with strong antioxidant activity, such as flavonoids and phenolic acids [39], however the presence of these compounds was not assessed in either study despite the phytochemical composition of quinoa known to vary due to genetic and environmental factors. Additionally, there was no attempt to determine the presence of potential *in vivo* metabolites in the blood, urine or faeces of animals, which is crucial in understanding the *in vivo* bioactivity of compounds found in plant foods such as quinoa. As a first step, future studies should determine the presence of bioactive compounds followed by an assessment of the bioactivity of these compounds.

It is well established that the consumption of foods rich in phytochemicals is associated with a decrease in oxidative stress [40] and risk of mortality from cardiovascular disease [41]. However, it is necessary to identify the specific phytochemicals present in the quinoa seed and their relative bioactivity in order to begin to understand the potential physiological benefits that they could impart upon consumption. This will provide a more thorough understanding of their action and could be used to design experiments that test their efficacy in a human population.

Limitations of Review

Throughout the design and completion of this literature review, steps were taken to minimise the level of bias in the generation of the results. Despite these efforts, there are several limitations that have been identified. Firstly, studies were included regardless of their overall quality and as such, possible associations between dietary consumption and physiological effects may have been under or overestimated. This was mitigated to a certain degree by using a quality-rating tool, which provided a transparent guide to ranking studies within the body of literature.

The second limitation refers to the doses consumed by animals in the respective studies. It is difficult to infer the dose that would be appropriate in a human context and whether dose dependency would persist, however, this is the critical issue and needs to be addressed in any future human study. Additionally, this review treats studies that use isolated extracts, processed forms and raw forms of the quinoa seed as equally valid dietary interventions. The weakness of this assumption is that humans eat foods and not food extracts. Therefore, it is difficult to predict the efficacy with which specific compounds present in the quinoa seed would impact human health when consumed as part of the diet. This is a limitation inherent in research exploring the effect of specific compounds or nutrients. However, the underlying aim of this review was to identify potential physiological effects of quinoa. Exploring the efficacy of quinoa in the whole diet would be an appropriate procedure once these initial outcomes are identified.

Recommendations for Future Research

Animal studies provide a valuable tool for exploring the possible mechanisms that food components operate through in delivering a health outcome. These types of studies cannot be used to validate health claims within the regulatory context, but they can be used to inform the design of future human clinical studies. Despite the heterogeneity introduced through the use of differing animal models, doses of quinoa, sample sizes and study time frames, it appears that the consumption of quinoa generates beneficial physiological outcomes among animals.

The process of rating the quality of the individual studies is a prudent technique to identify the underlying rigour with which the physiological effects were achieved. In particular, there appeared to be a lack of blinding and randomisation in the majority of studies, which should be addressed in future work. In addition the reliability of future work could be improved by using larger samples, while the scope could be improved by varying the dose of quinoa used in order to elucidate possible dose-dependent effects.

Based on the findings from this systematic review, human studies that investigate the impact of quinoa with varying levels of saponins on weight gain would be a viable experiment to perform. In addition, human studies could investigate the impact of quinoa consumption on the lipid profile. Despite the potential antioxidant properties shown by quinoa, systematic analytical research using state of the art analytical equipment such as HPLC-ESI-MS and NMR spectroscopy is required to identify and quantify the main bioactive compounds in quinoa before human studies can be justified.

Conclusion

This systematic review of the animal model literature has identified that the consumption of quinoa may lead to comparatively lower weight gain, and improved lipid profile and potential antioxidant effects. These physiological outcomes require further investigation, with a particular focus on elucidating the mechanism through which bioactive compounds, such as saponins, quinoa proteins, polyphenolic compounds and 20-hydroxyecdysone operate to deliver these desirable outcomes.

Despite the limitations of the animal studies that have been performed to date, there is burgeoning interest in quinoa as a food source and a steady uptake of it in the diet. To add further substance to the health properties that quinoa is perceived to possess, rigorously controlled human studies that aim to investigate the three key outcomes identified in this review should be performed. The identification of health benefits in a human population would encourage further investment in quinoa and galvanise public perception that it is a desirable food that could be consumed as part of a balanced diet.

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Conflict of Interest The authors declare that they have no conflict of interest

Compliance with Ethics The procedures performed in seven of the included animal studies were in accordance with the ethical standards of the institution at which the studies were conducted. Eleven of the included studies did not explicitly state that the experimental procedures were in accordance with the ethical standards of the institution that the study was performed at.

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