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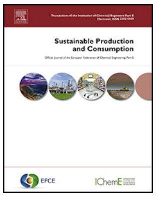

Bovine beta casein polymorphism and environmental sustainability of cheese production: The case of Grana Padano PDO and mozzarella cheese

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ABSTRACT

Several genetic variants of β-casein have been identified but A1 and A2 are the most common. Bovine β-casein variants may play an important role on cheese yield and quality, besides milk production and composition, and, thereby, affect environmental sustainability of cheese production processes.

The aim of the study was to investigate the possible effect on environmental sustainability of cheese production, related to bovine β-casein polymorphism. A Life Cycle Assessment (LCA) was performed, considering Grana Padano PDO and mozzarella cheese, made with either A1A1, A1A2 or A2A2 β-casein milk and using economic and dry matter allocation methods for cheese and co-products. Additionally to characterization, normalization and weighting (endpoints method), were also performed. Results on the environmental impact of 1 kg of packaged cheese showed that, among the β-casein genetic variants, A1A1 seemed to be the most impactful, only due to the lower individual daily milk production of cows belonging to A1A1 group, compared to the cows belonging to A1A2 and A2A2 groups, i.e. 29.6, 37.1 and 34.6 kg of fat and protein corrected milk (FPCM) day cow^{-1}, respectively. Allocation method strongly affected the impacts per kg of cheese product and, consequently, of co-products.

The normalization allowed to understand the relative importance of different impact categories and the result obtained indicated that the notable impact categories of the cheese industry were natural land transformation, aquatic eutrophication and terrestrial acidification. Results of the weighting highlighted that greater damage was given to the ecosystem quality, followed by human health and, finally, resource scarcity. Overall, biggest differences were detected for the two cheeses, rather than for the β-casein genetic variants and the differences in environmental sustainability of cheese made with A1A1, A1A2 and A2A2 milk were mainly due to the different cow milk production, rather than cheese yield. Therefore considering only the technological properties useful for cheese making the selection of milk with A2A2 β-casein may be not so convenient. Normalization and weighting results allow to identify the most impactful categories and so can help decision-makers to determine where to prioritize efforts aimed at reducing cheese environmental impact.

1. Introduction

Bovine milk caseins are formed by four different fractions namely αs1-, αs2-, β- and κ-casein, which are incorporated into large colloidal aggregates known as casein micelles (Daniloski et al., 2022). β-casein is one of the most abundant milk protein fractions and it constitutes up to 45 % of bovine milk total casein, presenting also several genetic variants (Massella et al., 2017). The β-casein, indeed, can be further sub-divided, according to the changes in its amino acid composition, which is encoded by the CSN2 gene, on chromosome 6 (Daniloski et al., 2021).

Currently, several genetic variants of β-casein have been identified: A1, A2, A3, B, C, D, E, F, G, H1, H2 and I, with A1 and A2 variants being the most common (Farrell et al., 2004; Oliveira Mendes et al., 2019).

The difference between the A1 and A2 β-casein genetic variants is a single nucleotide polymorphism at position 8101 of the CSN2 gene, which changes the codon for the amino acid at position 67 from proline (Pro67: A2 β-casein) to histidine (His67: A1 β-casein) in the polypeptide chain (Schettini et al., 2020).

Based on these genetic variants of β-casein, therefore, milk can be classified into different types: bovine milk containing Pro67 is called A2A2 β-casein milk, while A1A2 and A1A1 β-casein milk carry His67 as part of their β-casein structure (Daniloski et al., 2021; Oliveira Mendes et al., 2019).

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The A2 variant originally occurred in ancient European-type and purebred Asian and African cattle, while the A1 variant is the result of a mutation via natural selection (Brooke-Taylor et al., 2017; Kamiński et al., 2007). For this reason, the A2 β-casein variant is renowned as the oldest β-casein proteoform, while the A1 β-casein variant started to predominate in modern European cattle a few thousand years ago (Sebastiani et al., 2020).

The amount, proportion and genetic variants of milk protein fractions, overall, strongly influence milk coagulation properties, therefore playing an important role in dairy processing and, in particular, cheese making (Bittante et al., 2012). Bovine β-casein variants, indeed, may play an important role on cheese yield and quality, besides milk production and composition, and, thereby, affect environmental sustainability of cheese production processes.

Different types of cheese are produced and consumed in Italy: mozzarella can be considered as representative of a soft fresh cheese, while Grana Padano Protected Designation of Origin (PDO) may be regarded as an example of a hard-ripened cheese.

Grana Padano is a cooked, extra-hard, long-ripened cheese, produced in Northern Italy from semi-skimmed raw milk and registered as a PDO cheese (Council Regulation, 2006). This cheese is made with raw milk, from cows milked twice a day, in dairy farms that need to be placed in the production area defined in the Product Specification (European Commission, 2011). Cows ration is based mainly on forages (fresh, hays or silages) and concentrates; at least 50 % DM of the entire daily ration must be produced within the defined production area (Bava et al., 2018). The milk is partly skimmed by natural creaming, in order to decrease the fat content to 2.2 to 2.4 % and, thus, to have the right fat-to-casein ratio, which is important for development of the unique grainy cheese texture; this process a substantial effect on the hygienic quality of the milk, reducing somatic cell count and bacteria (D'Incecco et al., 2015).

After 9 months of ripening, the cheeses are inspected by officials of the *Consorzio per la Tutela del Formaggio Grana Padano DOP*, for verifying the absence of inner structural defects and then marked with the Grana Padano quality label (Masotti et al., 2010).

Milk coagulation properties are of particular importance in the preparation of PDO cheeses; such cheeses, indeed, are produced using traditional processing methods, and cheese quality relies on milk coagulation properties (Bittante et al., 2012).

Mozzarella is an unripened, near-white color, smooth elastic cheese, characterized by a long-stranded, parallel oriented, fibrous protein structure (FAO, 2006). It is manufactured though a *pasta filata* processing, where the curd of a suitable pH is heated, kneaded, and stretched until it is smooth and free from lumps. After that, the warm curd is cut and moulded and then firmed by cooling. This cheese can be produced as two main types: high moisture content mozzarella (56–65 % moisture content) or low moisture content mozzarella (45–54 % moisture content; Jafarzadeh et al., 2019). Generally, mozzarella is made using cow or buffalo milk, or mixtures of the 2; the present study focused specifically on high moisture cow milk mozzarella.

To the best of our knowledge, only few studies of the international bibliography provide environmental impact assessment of these two cheeses (e.g. Bava et al., 2018; Famiglietti et al., 2019; Dalla Riva et al., 2017). Particularly, for mozzarella cheese, international bibliography mainly focused on buffalo milk mozzarella production chain, rather than on mozzarella produced with cow milk (e.g. Berlese et al., 2019; Oliveira et al., 2021).

In addition, β-casein genetic variants, particularly A1 and A2 have received much attention from scientific community mainly because of their influence on human health, principally gastrointestinal physiology and digestive discomfort, rather than milk technological properties (Brooke-Taylor et al., 2017).

Fewer studies of the international bibliography performed the optional steps of normalization and weighting, in the life cycle impact assessment, even though it can help decision-makers prioritize which environmental impacts to address (McClelland et al., 2018).

With regard to the above, the present study could fill the knowledge-gap of environmental impact related to the use of milk containing different β-casein genetic variants, given that A2A2 milk is gaining huge market shares very quickly, arousing discussions and controversy, because it's not easy to understand how its qualities are real and how they are the result of intelligent marketing campaigns. Therefore, the aim of the study was to investigate a possible effect on the environmental sustainability of cheese production, related to bovine β-casein polymorphism. A Life Cycle Assessment (LCA), indeed, was performed, considering two different cheese production processes, Grana Padano PDO and mozzarella cheese, made with either A1A1, A1A2 or A2A2 β-casein milk. Although not required steps in life cycle impact assessment, normalization and weighting (endpoints method), were also performed, in order to ease the interpretation of results obtained. These two additional steps aimed to understand the relative importance of different impact categories and to explain damage at the three areas of protection (human health, ecosystem quality and resource scarcity), for normalization and weighting, respectively.

2. Material and methods

An experimental trial was performed in 2019, at regional experimental dairy farm located at Carpaneta (MN). During the trial, three groups of lactating Holstein-Friesian cows were raised under the same conditions. A number of 45 lactating cows were involved in the experiment, clustered into 3 different groups, depending on their β-casein genetic variants: A1A1 (11 cows), A1A2 (16 cows), A2A2 (18 cows).

At the farm, the cheese making of milk, separated for the experimental groups, was directly made, following Grana Padano PDO or mozzarella cheese productive process.

During the experimental test, therefore, it was possible to collect primary data concerning animal husbandry (animals diet, housing, manure management, etc.), milk (quantity and quality) and cheese production (inputs necessary for cheese making, etc.).

The trial involved two periods of two weeks each belonging either to winter and summer season.

2.1. Life cycle assessment

An evaluation of the environmental sustainability of milk production was performed through LCA method, structured following ISO 14040-compliant and ISO 14044-compliant LCA methodology (ISO 14040, 2006, ISO, 2018), providing international standards for conducting LCA.

2.1.1. Goal and scope definition

The goal of this LCA study was to quantify the environmental sustainability of Grana Padano PDO and mozzarella cheese production, considering A1A1, A1A2 and A2A2 β-casein genetic variants in milk.

2.1.2. Allocation, functional units and system boundaries

One kilogram of packaged cheese was considered as functional unit (FU). Several allocations were calculated while parsing the LCA. Particularly, at farm level, the allocation was performed between milk and meat, using a physical method (IDF International Dairy Federation, 2015). Among fresh cheese and by-products, the environmental impact was allocated, considering either their dry matter (DM) content (IDF International Dairy Federation, 2015) and their economic values. For Grana Padano PDO, among the different cheese products (PDO cheese first quality, PDO cheese second quality, non PDO grated cheese), only an economic allocation, based on market prices was carried out, since DM content is supposed to be the same.

System boundaries considered were from cradle to cheese factory gate. Inputs (e.g. fuel, lubricants, electricity, organic and mineral fertilizers, pesticides, off farm feeds and bedding, plastics and water, cleaners, rennet) and outputs (e.g. emissions to the air, to the soil and into the water, milk, meat, cheese and their by-products) involved in the productive processes were considered (Fig. 1).

The production process was sub-divided into different subsystems: milk production, cheese making and ripening (only for Grana Padano). Emissions related to the packaging of cheese were also considered, for both Grana Padano PDO and mozzarella cheese (Fig. 1).

2.1.3. Life cycle inventory (LCI)

Primary data, collected at regional experimental dairy farm at Carpaneta (MN), during the experimental trial, were used as much as possible. Primary data collected at an industrial cheese factory located in Mantova province were also used. Secondary data from databases (Ecoinvent V3, 2015 and Agri-footprint databases) and tertiary data from the international bibliography (Dalla Riva et al., 2017) were also used.

The present study focused specifically on high moisture cow milk mozzarella. Mozzarella cheese yield was calculated through Barbano (1984) formula, as follows:

$$Y_{mozzarella} = (0.85 \times \%fat + \%cas - 0.1) \times 1.13/(1–M\%/100)$$

where M = moisture; considering a M content of 62.5 % (Dalla Riva et al., 2017).

Whey yield was calculated as: 100-cheese yield%.

Grana Padano PDO cheese yield was calculated through Cassandro et al. (2016) formula, as well as cream and whey yields, as follows:

$$Y_{Grana\ Padano\ PDO(at\ 6\ months\ ripening)} = \left[2.83329 + 0.9877 \times \%Fat_{SM} + 0.179 \times (\%Protein_{SM})^2 + Eff_{RCT} + Eff_{a30}\right] \times (100 - Y_{cream})$$

where Eff_{RCT} and Eff_{a30} were calculated on the basis of data collected (RCT and a30, Table 3) and on the basis of values reported in Cassandro et al. (2008), about genetic parameters of milk coagulation properties and milk production and quality traits typical of the Italian Holstein-Friesian cattle breed:

$$Eff_{RCT} = [(Y_{cheeseB} \times Eff_{MCPmax} \times 0.5)/(\max{}_{RCT} - \min{}_{RCT})] \times [-(RCT_B - RCT)]$$

$$Eff_{a30} = [(Y_{cheeseB} \times Eff_{MCPmax} \times 0.5)/(\max{}_{a30} - \min{}_{a30})] \times (a30_B – a30)$$

$$Y_{cream} = [\%Fat_{FM} – (\%Casein_{FM} \times F:C)]/(\%Fat_{cream}/100)$$

where % Fat_{FM} and $\%Casein_{FM}$ are fat and casein content of full milk, respectively; $\%Fat_{cream}$ is the percentage of fat in the cream, assumed as 25 % (Cassandro et al., 2016) and F:C was assumed to be 1.00, as suggested by Grana Padano Consortium (2011).

$$Y_{whey} = 100 - Y_{cheese} - Y_{cream}$$

The main data concerning cheese making are summarised in Table 1. Lysozime was used only for Grana Padano PDO production, it was used in small quantities (2 g/100 kg of milk) but it was not considered due to the lack of data.

The main data concerning cheese and by products characteristics are summarised in Table 2.

2.1.4. Calculation of the on-farm emissions

At barn level, were calculated all the emissions (in air, water and soil) related to the milk production.

Enteric methane emissions were calculated, starting from chemical composition of the feed rations, using the equation suggested by Moraes et al. (2014).

Methane and dinitrogen monoxide (N_2O) emissions from manure storage were estimated using the Tier 2 method of the Intergovernmental Panel on Climate Change (Dong et al., 2006). N_2O losses from fertilizer application were estimated through the Tier 2 and Tier 1 methods of IPCC (De Klein et al., 2006).

More detailed information concerning the estimation of the on-farm emissions are reported in Gislon et al. (2020a).

2.1.5. Life cycle impact assessment (LCIA)

After classification, characterization was performed through ReCiPe Midpoint (H) V1.10/Europe Recipe H, in order to evaluate the environmental impact of cheese production, therefore characterization factors of this method were used for all the impact categories. The LCIA was performed by using the software SimaPro V 8.3.

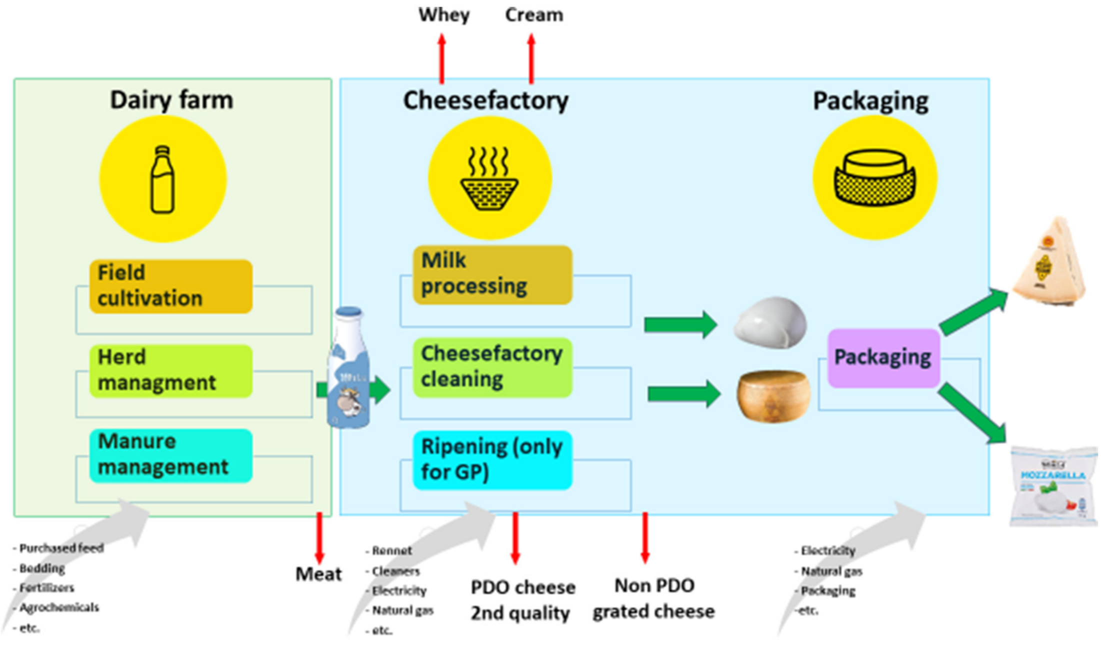

Fig. 1. System boundaries considered for the life cycle assessment.

Table 1
Main inventory data related to the cheese making.

	Unit	Grana Padano PDO	Mozzarella
Milk	kg	100	100
Water	m^3	0.17	0.50
Sodium chloride	g	139	400
Cleaners	g	36.6	396
Lubricating oil	g	0.58	0.17
Transport	tkm	8.68	8.58
Rennet[a]	g	3.00	30.6
Citric acid	g	0.00	160
Natural gas	kWh	10.63	18.8
Electricity	kWh	7.20	9.60
Refrigerant gas	kg	–	0.002
Packaging film	kg	0.18	3.61

Lysozyme was not considered.

[a] Primary data from one of the world's leading producers of rennet were used.

In addition, normalization was carried out: Recipe Midpoint (H) has European normalization references (the average European inhabitant environmental load, for each impact category). The normalization step allows obtaining a dimensional scores that are useful for understanding the relative importance of different impact categories (Guinée et al., 2002).

Weighting was also performed using the endpoint method (ReCiPe Endpoint (H) V1.10/Europe ReCiPe H/H).

2.2. Statistical analysis

The complete data set was analyzed using SAS 9.4 (2012; SAS Institute Inc., Cary, NC), computing descriptive statistic procedures including least square means (Proc LSMEANS).

Data were analyzed by Proc GLM to test the influence of β-casein genetic variants, stage of lactation, number of lactation and season on animal performances, milk cheese making characteristics and cheese yield. Also, the interaction between β-casein genetic variants and stage of lactation, number of lactation, and season were tested by Proc GLM.

The model was:

$$Y_{ijklm} = m + A_i + B_j + C_k + D_l + A_iB_j + A_iC_k + A_iD_l + e_{ijklm}$$

where:

Y_{ijklm} = dependent variable (animal performances, milk cheese making characteristics and cheese yield);

m = general mean;

A_i = effect of β-casein genetic variants, with i A1A1, A1A2, A2A2;

B_j = effect of stage of lactation, with j from 1 to 3;

C_k = effect of number of lactation with k from 1 to 3;

D_l = effect of season with winter or summer;

A_iB_j = effect of interaction between β-casein genetic variants and stage of lactation;

A_iC_k = effect of interaction between β-casein genetic variants and number of lactation;

A_iD_l = effect of interaction between β-casein genetic variants and season;

e_{ijklm} = residual error.

Table 2
Inventory data concerning cheese and by products characteristics.

	Unit	Grana Padano	Mozzarella
DM[a] content			
Fresh cheese	%	61	37.5
Whey[b]	%	6	6
Cream	%	29	–
Economic value[c]			
Grana Padano PDO[d] first quality[e]	euro/kg	7.45	–
Grana Padano PDO[d] second quality[e]	euro/kg	7.35	–
Non PDO[d] grated chees[e]	euro/kg	6.35	–
Cheese	euro/kg	–	4.50

[a] Dry matter content.

[b] Whey and stretching water for mozzarella cheese (Gernigon et al., 2009).

[c] Clal, 2020.

[d] Protected designation of origin.

[e] 10 months ripening.

3. Results and discussion

3.1. Milk and cheese yield for the β-casein variants, and allocations at cheese factory level

Results concerning milk, cheese and by-products are summarised in Table 3.

The number of animals belonging to the A1A1 group was slightly lower compared to the other groups, due to the herd composition; however, other studies conducted on a greater number of animals, detected a low frequency of A1A1 genetic variants, compared to the other two (Heins et al., 2021; Potočnik et al., 2016).

As reported in Table 3, low statistical differences occurred among the genetic variants, concerning daily milk quantity, but the highest differences were due to lactation number and stage of lactation (probability not shown in Table 3). However, a tendency ($P = 0.09$) was detected for individual daily milk production: cows belonging to A1A1 group seemed to have lower milk production, compared to the others (Table 3). Heins et al. (2021) highlighted that milk production was not statistically different with regard to A1 or A2 genotype in organic dairy herds. Considering the estimation of whole production at the end of lactation for each cows throughout genetic merit the difference among genetic variants were not significant for FPCM and parameters of quality (data not shown). This result is consistent with the international bibliography, indicating that selection on A2A2 β-casein genotype would not have implications for traits of dairy production (Potočnik et al., 2016).

No statistical differences for milk quality parameters were observed, for genetic variants. Also Nguyen et al. (2018) reported no significant differences in the concentration of fat, protein, lactose and total solids content between the milk with the two different β-casein phenotypes (A1A1 and A2A2). However, Ristanić et al. (2020) found that milk yield and milk fat concentration were significantly higher in A2A2 compared to both A1A1 and A1A2 genotypes, while milk protein concentrations were significantly higher in A2A2 compared to A1A2 genotype. Heins et al. (2021) highlighted that milk production was not statistically different with regard to A1 or A2 genotype in organic dairy herds.

No differences statistically significant were detected also for cheese and by-products yields, both for Grana Padano PDO and mozzarella cheese (Table 3). Allocation method can strongly affect the impacts per kg of cheese product and, consequently, of co-products (Bava et al., 2018). For cheese production, in particular, considering DM allocation as the reference, most impact categories increased using economic allocation. The economic allocation is influenced by price fluctuations and, due to the low economic value of the co-products, assigns to cheese a higher share of the total impact (Bava et al., 2018).

In Table 4, are summarised the different allocation methods used for the impact assessment: 1) physical method (IDF International Dairy Federation, 2015), for milk at farm; 2) dry matter content (IDF International Dairy Federation, 2015) and economic value (selling prices of different products) for fresh cheese and by-products; 3) for Grana Padano PDO, economic value among the different cheese products (PDO cheese first quality, PDO cheese second quality, non PDO grated cheese).

3.2. Environmental impact of packaged cheese

A comparison among the different β-casein genetic variants was made, considering packaged cheese: the results are reported in Fig. 2.

Table 3
Milk quality and cheese yield for the β-casein variants (Least Square means).

	Unit	A1A1	A1A2	A2A2	SE[a]	Model	GENETIC	GENETIC * STAGE	GENETIC * LACTATION	GENETIC * SEASON
						P	P	P	P	P
Milk										
FPCM[b]	kg day cow^{-1}	29.6	37.1	34.6	2.47	0.03	0.09	0.47	0.95	0.86
Milk fat	%	3.91	3.49	3.96	0.306	0.92	0.48	0.99	0.65	0.37
Milk protein	%	3.24	3.28	3.29	0.084	<0.0001	0.90	0.87	0.35	0.79
Milk casein	%	2.71	2.74	2.83	0.071	<0.0001	0.47	0.30	0.96	0.16
Grana Padano cheese										
RCT[c]		18.6	19.1	19.6	1.41	0.30	0.87	0.28	0.67	0.92
a30[d]		17.8	16.7	19.2	2.37	0.61	0.75	0.20	0.89	0.97
Cheese yield[e]	%	7.49	7.30	7.59	0.204	0.07	0.60	0.92	0.60	0.71
Cream yield[f]	%	5.04	2.58	5.38	1.33	0.14	0.19	0.86	0.14	0.12
Whey yield	%	86.5	89.1	86.0	1.33	0.31	0.20	0.86	0.16	0.16
Mozzarella cheese										
Cheese yield[g]	%	18.0	17.0	18.3	0.843	0.57	0.48	0.99	0.52	0.37
Whey yield	%	82.0	83.0	81.7	0.843	0.57	0.48	0.99	0.52	0.37

[a] Standard error.
[b] Fat and protein corrected milk.
[c] Milk coagulation time.
[d] Curd firmness.
[e] At 6 months ripening (Cassandro et al., 2016).
[f] Considering that all the cream produced was sold as is.
[g] Barbano (1984).

Results on the environmental impact of 1 kg of packaged cheese showed that, among the β-casein genetic variants, A1A1 seemed to be the most impactful, for all the impact categories considered, and for both the cheeses. A1A2 and A2A2 β-casein genetic variants, overall, showed similar results for environmental impact of both cheeses considered (Fig. 2). That outcome was only due to the lower individual daily milk production of cows belonging to A1A1 group, compared to the cows belonging to A1A2 and A2A2 groups (29.6, 37.1 and 34.6 kg FPCM day cow^{-1}, respectively). This outcome, indeed, was obtained despite both the cheeses made with A1A1 milk showed to have a similar yield compared to A2A2 cheeses and even a slightly higher yield than A1A2 cheeses (Table 3). Milk production at farm represents the highest contribution to the environmental impact of the cheese production chain (Palmieri et al., 2017), making the animal productivity a stronger driver rather than cheese yield.

Table 4
Different allocation factors among the different products.

	Unit	beta-Casein polymorphs		
		A1A1	A1A2	A2A2
Milk				
Physical method				
FPCM[a]	%	91.1	92.9	92.4
Meat	%	8.89	7.10	7.62
Cheese factory mozzarella				
DM allocation				
Cheese	%	57.8	56.1	58.3
Whey[b]	%	42.2	43.9	41.7
Economic allocation				
Cheese	%	98.5	98.4	98.5
Whey[b]	%	1.50	1.61	1.47
Cheese factory Grana Padano				
DM allocation				
Fresh cheese	%	43.8	45.4	43.8
Whey	%	43.9	47.9	43.2
Cream	%	12.4	6.70	13.1
Economic allocation				
Fresh cheese	%	83.4	89.5	82.8
Whey	%	2.16	2.44	2.11
Cream	%	14.4	8.1	15.1
Ripening Grana Padano				
Economic allocation				
PDO[c] cheese 1st quality	%	85.0	85.0	85.0
PDO[c] cheese 2nd quality	%	12.4	12.4	12.4
Non PDO[c] grated cheese	%	2.57	2.57	2.57

[a] Fat and protein corrected milk.
[b] Whey plus stretching water.
[c] Protected designation of origin.

These results are consistent with the international bibliography (Potočnik et al., 2016) confirming that A2A2 milk seems not to have advantages concerning technological properties: in the present study, sustainability concerning the β-casein genetic variants seemed, indeed, to be more related to milk production at farm, rather than cheese yield. Considering results obtained in studies relying on much more high number of animals, indeed, it is inadvisable to use milk containing the A2 alleles for cheese production, as these are associated with a slightly worsening of milk coagulation properties, and as a consequence, a less efficient cheese-making process (Bisutti et al., 2022).

A slightly higher cheese yield, however, was detected for A2A2, compared to A1A1, even though not statistically different (Table 3), but this was mainly due to a depression of cheese yield of A1A1 milk, due to the high SCC content of that milk (4.65 vs. 4.42 and 4.56 $\log_{10}$ SCC/mL for A1A1, A1A2 and A2A2, respectively, no statistically different), confirmed also by lower individual daily production of cows belonging to A1A1 group (Table 3). As reported by Summer et al. (2015), indeed, the increase of milk SCC content is associated with a reduction in fat, protein and casein content of the milk, and with lower cheese yield. The quantity of cheese produced per unit of milk, therefore, mainly depends on the total amount of solid contents, in particular fat and casein (Auldist et al., 2004; Verdier-Metz et al., 2001). However, also Ristanić et al. (2020) highlighted a lower milk production of cows in the first lactation with A1A1 genotype, compared to A1A2 and A2A2 genotypes. The lower milk production of cows belonging to the A1A1 group, indeed, may be related not only to the health status of those cows. In an environmental impact perspective, therefore, considering the whole production process, this aspect needs to be taken into account, since raw milk production represents a very high contribution, together with cheese yield.

Impacts of packaged Grana Padano PDO (first quality) and mozzarella cheeses are reported in Table 5 (economic allocation).

Considering the economic allocation, the environmental impact of Grana Padano PDO cheese resulted to be, overall, higher than the environmental impact of mozzarella cheese. Particularly, for climate change, the impact of Grana Padano PDO production was almost the double of mozzarella cheese (Table 5). This is mainly due to the different moisture

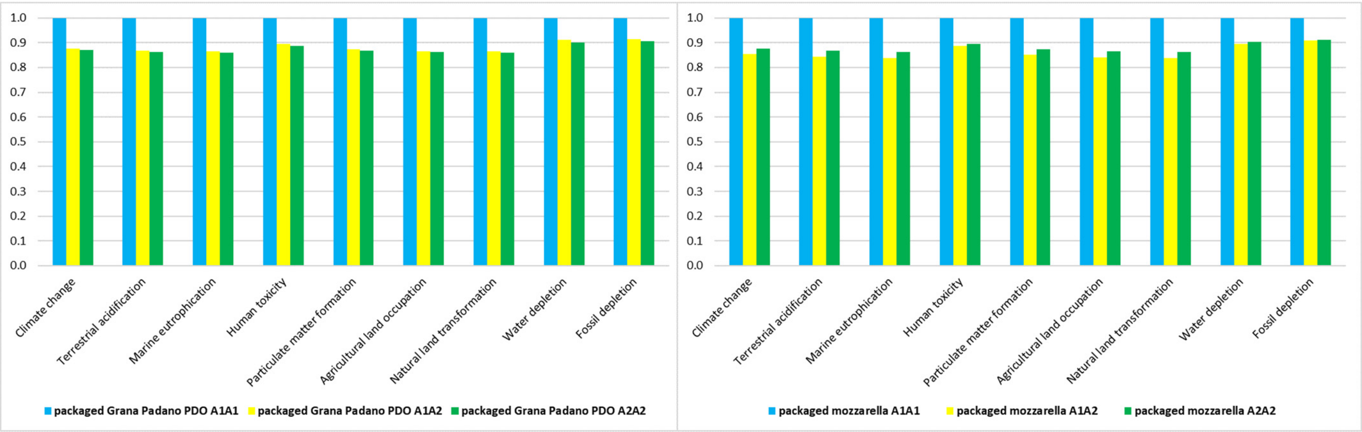

Fig. 2. Comparison of 1 kg of packaged cheese obtained with milk with different β-casein genetic variants, data normalized as compared with the maximum value.

content of the two cheeses (61 vs. 37.5 % fresh cheese) and, as a consequence, to the different yield of Grana Padano PDO and mozzarella (Table 3). Grana Padano PDO showed an average climate change of 20.7 kg CO2eq/kg packaged cheese, while mozzarella cheese showed, on average, a climate change of 10.7 kg CO2eq/kg packaged cheese. The highest values were related to A1A1 variant, for both the cheeses: 22.5 and 11.7 kg CO2eq/kg packaged cheese, for Grana Padano PDO and mozzarella, respectively.

Regarding climate change, the impact of Grana Padano PDO resulted to be slightly higher, compared to values reported in the international bibliography, on cheese with similar yield and economic allocation method (Bava et al., 2018; González-García et al., 2013; van Middelaar et al., 2011). Higher values of environmental impact were detected also for terrestrial acidification (Dalla Riva et al., 2018; González-García et al., 2013), human toxicity (Dalla Riva et al., 2018) and water depletion (Dalla Riva et al., 2018). This lower sustainability of Grana Padano PDO, compared with the studies, is probably due to the high environmental impact of milk in the present study (e.g. climate change 1.71 kg CO_2 eq/kg FPCM, on the average for the three β-casein genetic variants).

Mozzarella with economic allocation showed similar value for climate change to Kristensen et al. (2015) and for climate change, marine eutrophication and human toxicity to Dalla Riva et al. (2017). However, values related to terrestrial acidification were slightly lower while values related to water depletion were higher than Dalla Riva et al. (2017). Water depletion depended on raw milk production at farm, energy consumption and packaging, probably determining the differences between our results and values reported in the international bibliography. In particular, the higher values obtained in the present study for water depletion were probably related to the method of irrigation adopted in Carpaneta farm, involving high water consumption, as well as energy mix used for the impact assessment, considering hydropower.

Impacts of packaged Grana Padano PDO and mozzarella cheeses are reported in Table 6 (DM allocation).

If allocation factors based on DM content is considered, results concerning the environmental impact of Grana Padano PDO seemed to be more comparable with those of mozzarella (Table 6), even though the latter was still more sustainable. For climate change, indeed, Grana Padano PDO showed, on the average, an impact of 11.2 CO_2eq/kg packaged cheese, compared to the one of mozzarella that was 6.2 kg CO_2eq/kg packaged cheese. As for economic allocation, A1A1 variant reported the highest value for every impact category considered, both for Grana Padano PDO and mozzarella (Table 6). However, biggest differences were detected for the two cheeses, rather than for the β-casein genetic variants, as occurred also for economic allocation.

As confirmed also by Finnegan et al. (2018), therefore, based on the literature, fresh cheese has lower environmental impact than semi-hard cheese, particularly concerning direct energy consumption.

Considering DM allocation, water depletion resulted to be, on average, 9.2 m^3/kg of the cheese for Grana Padano PDO and 5.1 m^3/kg of the cheese for mozzarella cheese.

Considering DM allocation, results obtained for climate change of Grana Padano PDO was almost the double than those reported by Borghesi et al. (2022) on Parmigiano Reggiano PDO cheese (6.74 kg

Table 5
Environmental impact of packaged cheese, economic allocation.

Impact categories	Unit	Packaged Grana padano PDO[a]			Packaged mozzarella cheese		
		beta-casein polymorphs					
		A1A1	A1A2	A2A2	A1A1	A1A2	A2A2
Climate change	kg CO_2 eq	22.5	20.4	19.4	11.7	10.3	10.1
Terrestrial acidification	kg SO_2 eq	0.20	0.18	0.17	0.10	0.09	0.09
Marine eutrophication	kg N eq	0.11	0.10	0.09	0.05	0.05	0.05
Human toxicity	kg 1,4-DB eq	1.17	1.08	1.02	0.67	0.61	0.60
Particulate matter formation	kg PM_{10} eq	0.03	0.03	0.03	0.02	0.02	0.02
Agricultural land occupation	m^2a	24.1	21.5	20.5	12.0	10.4	10.3
Natural land transformation	m^2	0.03	0.03	0.03	0.02	0.01	0.01
Water depletion	m^3	17.0	15.9	15.1	9.38	8.65	8.39
Fossil depletion	kg oil eq	1.94	1.81	1.72	1.19	1.11	1.08

[a] Protected designation of origin.

Table 6
Environmental impact of packaged cheese, DM allocation.

Impact categories	Unit	Packaged Grana padano PDO[a]			Packaged mozzarella cheese		
		beta-casein polymorphs					
		A1A1	A1A2	A2A2	A1A1	A1A2	A2A2
Climate change	kg CO_2 eq	12.2	10.7	10.6	6.84	5.85	5.98
Terrestrial acidification	kg SO_2 eq	0.11	0.09	0.09	0.06	0.05	0.05
Marine eutrophication	kg N eq	0.06	0.05	0.05	0.03	0.03	0.03
Human toxicity	kg 1,4-DB eq	0.66	0.59	0.59	0.39	0.35	0.35
Particulate matter formation	kg PM_{10} eq	0.02	0.02	0.02	0.01	0.01	0.01
Agricultural land occupation	m^2a	12.8	11.1	11.0	7.03	5.91	6.08
Natural land transformation	m^2	0.02	0.01	0.01	0.01	0.01	0.01
Water depletion	m^3	9.80	8.95	8.83	5.50	4.93	4.97
Fossil depletion	kg oil eq	1.12	1.02	1.01	0.70	0.64	0.64

[a] Protected designation of origin.

CO2 eq/kg packaged cheese). However, despite the production system considered by Borghesi et al. (2022) was organic, the impact of raw milk involved in the cheese production was equal to 0.98 kg CO_2eq/kg FPCM, that is much lower than the impact of the milk considered in the present study (1.71 kg CO_2 eq/kg FPCM, on average for the three β-casein genetic variants). Probably, that was the main driver of this difference detected among the studies, since cheese yield is almost equal between Grana Padano PDO and Parmigiano Reggiano PDO. The water footprint of 1 kg of cheese, indeed, was also much higher than those reported by Borghesi et al. (2022), that is equal to 3.39 m^3.

Environmental impact of mozzarella cheese was comparable to the values reported by Dalla Riva et al. (2017), for climate change, terrestrial acidification, marine eutrophication and human toxicity. On the contrary, water depletion related to the mozzarella cheese of the present study is much higher than those reported by Dalla Riva et al. (2017). As for economic allocation, the higher values obtained in the present study for water depletion were probably related to the method of irrigation adopted in Carpaneta farm, involving high water consumption, as well as energy mix used for the impact assessment, considering hydropower.

Climate change of mozzarella was lower than the one reported by Kim et al. (2013), while comparable with marine eutrophication. In comparison to the results obtained by Kim et al. (2013), water depletion resulted to be much higher. Both, Dalla Riva et al. (2017) and Kim et al. (2013) reported results based on dry solids basis allocation method.

3.3. Contribution to climate change

Fresh cheeses were analyzed for the different contribution to the climate change (Fig. 3). Ripening and packaging were not considered in order to put in evidence the cheesemaking phase, since these phases should be considered negligible.

Raw milk production at farm was the most impactful phase along the considered supply chain, irrespective of the cheeses and of the β-casein genetic variants (Fig. 3). This result is consistent with the conclusions of other studies on Grana Padano PDO cheese (Bava et al., 2018), mozzarella cheese (Palmieri et al., 2017) and on other cheeses (Borghesi et al., 2022; Dalla Riva et al., 2018; van Middelaar et al., 2011).

A remarkable contribution of the dairy farm in the climate change was given by methane emission from the enteric fermentation process, followed by feed production, as confirmed by the international bibliography (Gislon et al., 2020a). The impact of milk was 1.91, 1.56, 1.66 kg CO_2eq/kg FPCM, for milk A1A1, A1A2 and A2A2, respectively.

Transportation of milk from dairy farm to cheese factory seemed not very important and this is consistent with the short distance between the locations of the farms and the factory. This result is consistent with other findings of the international literature (Palmieri et al., 2017).

Among the different β-casein genetic variants, other small contribution was given by rennet utilization, cleaning products, sodium chloride and lubricating oil, for both Grana Padano and mozzarella cheese (Fig. 3). For rennet, primary data from one of the world's leading producers were used.

Electricity and natural gas consumed had a slightly higher impact on mozzarella, rather than on Grana Padano. This is due to the different production chain of the cheeses, requiring more energy inputs for mozzarella cheese production. This aspect is true for all the β-casein genetic variants considered. In accordance with our findings, Flysjö et al. (2014) reported that, excluding milk production, the use of energy had the highest contribution on environmental impact of cheese production and other dairy products.

Overall, the differences among the three β-casein genetic variants were not so strong, but slightly differences were detected more between the different cheese production chains (Fig. 3). Therefore, considering only the cheesemaking phase, the selection of milk with A2A2 β-casein may be not so convenient.

3.4. Environmental impact of packaged cheese: normalization

Although it is not a required step in life cycle impact assessment, normalization of results eases their interpretation, even though, only few studies of the international bibliography take into account a normalization step (McClelland et al., 2018). Particularly, normalization was useful to identify the impact categories, which are important for mozzarella and Grana Padano PDO production sectors, by scaling the impact categories up or down. Normalization, therefore, represents a tool, allowing contextualizing, on a regional basis, the emissions impacts. The total emissions of a specific impact category for the European region were estimated and, subsequently, normalized to a per head basis, for the Europe. Results of normalization are reported in Fig. 4.

According to Kim et al. (2013), that conducted a normalization analysis on cheddar cheese, with US normalization factors, aquatic eutrophication and terrestrial acidification are important categories where improvement activities can be focused, concerning cheese production chain. Also Palmieri et al. (2017) found, through a normalization analysis conducted on mozzarella cheese, acidification and eutrophication as the impact categories emerged for their magnitude, in accordance with the present findings. Marine eutrophication and terrestrial acidification were found also by Dalla Riva et al. (2017) as the impact categories, which are important for mozzarella cheese production sector. However, these authors did not consider natural land transformation that, in the present study, emerged as the impact category that is particularly relevant for the cheese industry.

Natural land transformation was the highest impact category, due to the fact that, as above commented, raw milk subsystem was responsible for almost all the harmful impact in this category, particularly due to cows feed production. As reported by Rota Graziosi et al. (2022), the use of soybean meal is positively correlated to the environmental impact of the ration and, as a consequence, of the milk production, mainly

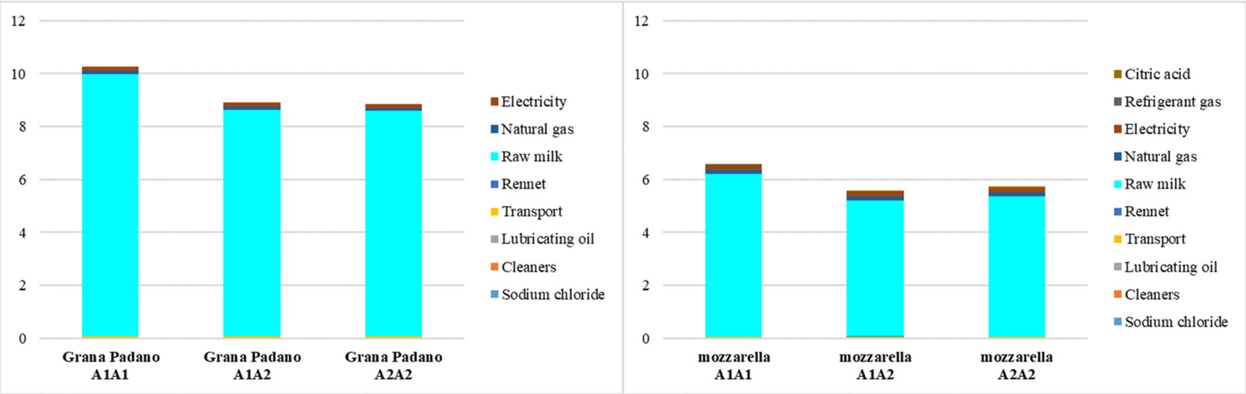

Fig. 3. Contribution to climate change on fresh cheese (without considering ripening and packaging) regarding beta-casein polymorphism.

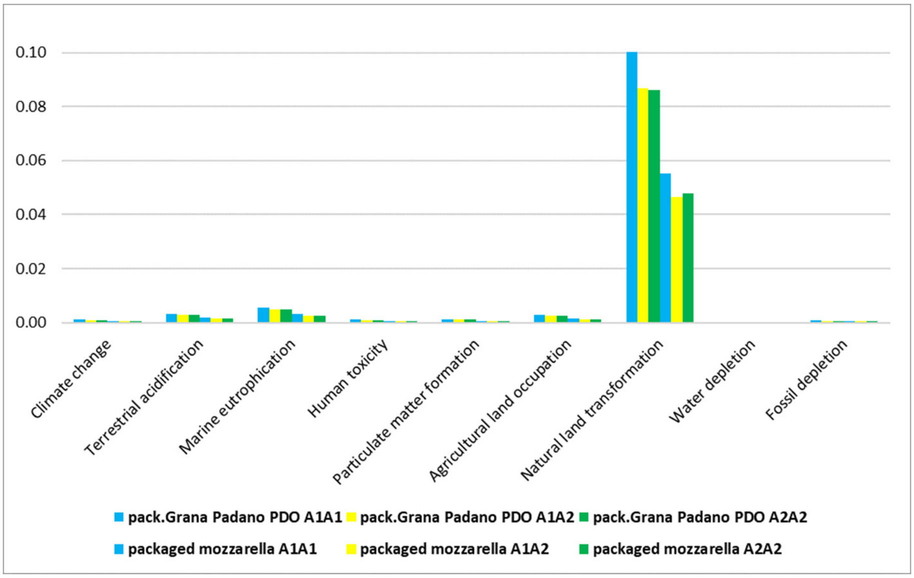

Fig. 4. Results of normalization on packaged cheese.

for its geographical origin. Also Canellada et al. (2018) found that, concerning cheese production, the most significant category was natural land transformation. Since soybean meal constitutes the most popular protein source included in the ration for lactating cows in northern Italy (Gislon et al., 2020b), the high impact on natural land transformation might be valid for the actual cheese varieties and, in particular, for Grana Padano PDO, where the production regulations do not allow using several feed protein sources such as, for example, rapeseed meal.

Overall, the two cheeses showed similar results concerning normalization, highlighting the same impact categories as those on which to focus improvement activities (i.e. natural land transformation, marine eutrophication and terrestrial acidification).

Among the β-casein genetic variants, no differences were detected concerning normalization, with natural land transformation, marine eutrophication and terrestrial acidification as the impact categories emerged for their magnitude, for either A1A1, A1A2 or A2A2.

3.5. Environmental impact of packaged cheese: weighting (endpoints method)

Weighting is the final part of the life cycle impact assessment and it can help decision-makers determine where to prioritize and focus efforts aimed at reducing a product's environmental impact, even though only in few studies of the international bibliography is carried out (McClelland et al., 2018). Going from the midpoint to the endpoint level, through damage pathways, may be useful for better understanding the environmental problem related to the cheese production. Results obtained for weighting are presented in Fig. 5.

Endpoint impact categories explain damage at the three areas of protection: human health, ecosystem quality and resource scarcity (Huijbregts et al., 2017). Endpoint approach is complementary to the midpoint one: midpoint characterization typically has a strong relation to the environmental flows, the endpoint characterization makes easier to interpret the relevance of the environmental flows (Hauschild and Huijbregts, 2015).

Results of the productive processes analyzed highlighted that, greater damage was given to the ecosystem quality, followed by human health and, finally, resource scarcity, both for Grana Padano PDO and mozzarella cheese. β-casein genetic variants didn't show important differences concerning weighting phase, showing the same behaviour among the endpoints indicators (Fig. 5).

For both the cheeses and the different β-casein genetic variants, climate change, photochemical ozone formation, freshwater eutrophication, toxicity, terrestrial acidification, water consumption and land use are the impact categories insisting on the ecosystem quality (Huijbregts et al., 2017).

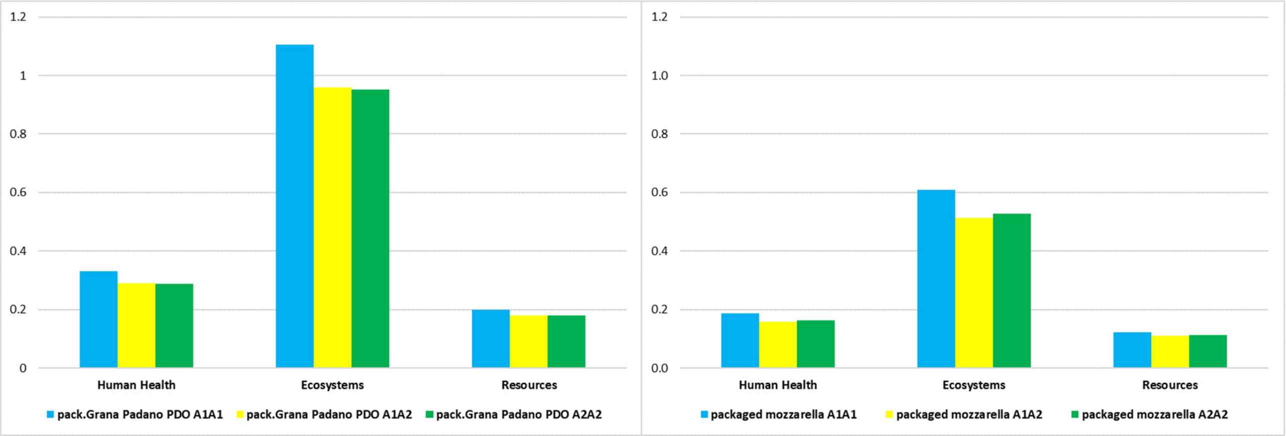

Fig. 5. Results of weighting on packaged cheese at endpoint level.

Human health is damaged by climate change, stratospheric ozone depletion, ionising radiation, particulate matter formation, photochemical ozone formation, toxicity and water consumption.

Mineral and fossil scarcity represent the overall resource scarcity (Huijbregts et al., 2017).

4. Conclusion

Recently, the interest in beta-casein polymorphism has been gaining popularity, among researchers, milk producers and consumers, mainly because of their influence on human health, principally gastrointestinal physiology and digestive discomfort. Concerning environmental sustainability, however, differences were detected mainly between Grana Padano PDO and mozzarella cheese, rather than among A1A1, A1A2 and A2A2 β-casein genetic variants. LCA results, indeed, showed that A1A1 seemed to be the most impactful, only due to the lower individual daily milk production of cows belonging to A1A1 group, compared to the cows belonging to A1A2 and A2A2 groups.

Normalization and weighting can help decision-makers determine where to prioritize and focus efforts aimed at reducing Grana Padano PDO and mozzarella's environmental impact. Particularly, natural land transformation was the most notably affected of the studied categories and, in an endpoint view, greater damage was given to the ecosystem quality, for both Grana Padano PDO and mozzarella cheese and for all the three β-casein genetic variants.

However, since the slightly differences among β-casein genetic variants seem to be due to the lower individual daily milk production of A1A1 cows, more than anything, to clarify the effects of A1 and A2 genetic variants on environmental sustainability of cheese production, further investigations were required. More extensive investigation on higher numbers of animals, indeed, is needed.

In conclusion for the environmental impact of cheese production the selection of milk with A2A2 β-casein may be not so convenient if only the technological properties useful for cheesemaking phase was considered.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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