Early versus delayed fortification of human milk in preterm infants: a systematic review

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Short Title: timing of human milk fortification in preterm infants: a systematic review

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1. Abstract

Expressed breast milk (EBM) is commonly supplemented with commercially prepared human milk fortifier to meet the additional nutritional needs of preterm infants. The optimal milk intake at which to introduce fortification is unknown. The objective of this systematic review was to compare the effect of early fortification (EF) versus that of delayed introduction of human milk fortifier (DF) on short-term outcomes including growth, feeding intolerance, length of hospital stay and maturity at discharge in very low birth weight infants. The search was carried out until March 2019 using five electronic databases (PubMed, Ovid Medline, web of science, Ovid Embase and the Cochrane Library). The search was supplemented with a search of the clinical trial registry and reference lists. Eligible studies involved randomized controlled trials that had been designed to compare EF against DF using multi-nutrient fortifier for infants of birth weight <1500 g who were fed exclusively or predominantly EBM. Four authors independently screened the studies for eligibility. A total of 1972 articles were screened; two studies met the inclusion criteria and were included with a total number of participants of 171. The definition of EF and DF was not consistent between the two studies. There was no significant impact of EF versus DF on all outcomes. In conclusion, current data are limited and do not provide evidence on the optimal time to start fortification. The definition of EF and DF needs to be agreed and further larger randomized controlled trials are required.

2. Introduction

The third trimester of pregnancy is a period of nutrient accretion and growth. Preterm infants have missed some or all of that period, and therefore their nutritional requirement is much higher than that of term infants. Preterm infants accumulate significant nutrient deficiency in early postnatal life which may result in growth restriction (weight below the 10th centile for postmenstrual age). Poor growth is a very common problem in the preterm infant population, with poor postnatal growth found in 99% of extremely low birth weight infants (1) and can be associated with a higher risk for short term morbidities (2-4). Postnatal growth restriction has been associated with poorer neurodevelopmental outcomes and may continue to affect ex-preterm infants during later childhood and adulthood (5-7). Expressed breast milk (EBM) confers numerous advantages for preterm infants, especially those born with a very low birth weight (VLBW) (8). However, when fed as the only source of nutrition, EBM does not meet the preterm infant's increased nutritional requirements (9, 10). The protein requirement of growing preterm infants is between 3.5 and 4.5 g/kg/day (11). Feeding with 150 ml/kg/day of unfortified EBM (often considered full enteral feeding) provides only about 1.8 g/kg/day of protein. Providing optimal early nutrition to preterm infants is challenging, but it can be facilitated by the use of multi-nutrient human milk fortifier (HMF). The addition of a HMF to EBM is recommended but there is no consensus on the timing at which HMF can safely be introduced and practice vary greatly. Fortification alters both the nutrient density and osmolality of EBM, which may affect intestinal peristalsis and feed tolerance, and increase the risk of necrotizing enterocolitis (NEC) (12). Historically, this has been used as a justification for delaying the introduction of HMF. There is some evidence that these adverse outcomes may be more common with the use of bovine-based HMF, but the data are limited (13-15). However, any risk needs to be balanced against the early introduction of HMF to avoid postnatal growth failure, which itself may have adverse neurodevelopmental consequences. The aim of this systematic review was to compare in-hospital outcomes of early versus delayed introduction of multi-nutrient human milk fortifier. Outcomes of interest were short-term growth, feeding intolerance, length of hospital stay and post-menstrual age (PMA) at discharge.

3. Methods

Search

Published literature was searched up to 28th of March 2019, using five databases. The reference lists of the most relevant papers and reviews were searched manually. The clinical trial registration websites (clinicaltrial.gov) and Current Controlled Trials (isrctn.com) were searched for ongoing or completed trials. Abstracts were considered for inclusion only if they contained the necessary information. The search strategy was based on MeSH terms/subject headings and separate keywords. The MeSH terms were the following: fortified food, human milk, premature infant, growth & development, mortality, low birth weight, breast milk and diet supplementation. The keywords were human milk, breast milk and fortification. Boolean terms (AND, OR) were used to connect the search words. The study designs included were randomized or quasi-randomised controlled trials in the English language and there was no date restriction. Details of the systematic review protocol and search strategy are available in supplementary material (Tables a and b).

Inclusion criteria

A study was eligible if it was a randomized or quasi-randomized controlled trial and participants were both of very low birth weight (<1500 grams) and fed exclusively or predominantly with EBM (either mother`s own milk or pasteurized donor human milk). We included only studies designed to evaluate early fortification (i.e. added at small enteral volume) versus delayed fortification (i.e. added at larger enteral volume). At least one of our primary outcomes had to be measured. A study was excluded if it did not meet these criteria or included babies with congenital anomalies.

Outcomes

The primary outcomes of this systematic review were short-term growth parameters (length, head growth and weight gain), Feeding intolerance (defined as clinical signs only and/or cessation of feeding), length of hospital stay (number of days that the baby remained in the neonatal unit) and PMA (i.e. gestational age plus chronological age) at discharge. Secondary outcomes were NEC and sepsis.

Study selection and data extraction

To avoid bias in the study selection, the first reviewer, WA, screened all titles. Three other reviewers, CE, ALG and JS, screened all the titles again independently to make the final decision and ensure no study was missed. After that, WA reviewed the abstracts of all potentially relevant studies and obtained their full text. WA assessed full texts against the predetermined inclusion and exclusion criteria with the help of an inclusion checklist form (Appendix, form 1). Excluded studies were double-checked by two reviewers, CE and ALG. WA extracted the data from the studies meeting the inclusion criteria using data extraction sheet (Appendix, form 2). The following data items were extracted: publication year, authors, study settings, country where study carried out, design, participants, nutrition intervention and outcomes of interest. Data were entered onto Microsoft Excel.

Individual study risk of bias and quality assessment

Three reviewers (WA, CE and AG) independently assessed the risk of bias and the overall quality of each study. Criteria suggested by the Cochrane Collaboration tool were applied. Judgment for potential bias in each study methodology was classified as being of low, high or unclear risk according to the following domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data and selective reporting (16). The quality of each individual study was assessed using a scoring tool that we developed. The criteria for quality assessment considered the following aspects: the number of centres involved in the study, sample size, description of the intervention, reporting outcomes definition and method of measurement, study groups' homogeneity and consideration of confounding factors. Details of our systematic review protocol are available in the supplementary material.

4. Results

Searches

Database searches returned 5442 articles, and an additional 25 articles were found through a manual search. After duplicates were removed, the titles of 1972 articles were screened for relevance, resulting in the exclusion of 1805 articles. The remaining 167 articles were then assessed by reference to their full

texts. After applying our eligibility criteria, 165 records were excluded, and two studies (17, 18) were included in this review (Figure 1).

Description of the included studies (n=2)

The studies' characteristics are summarized in Table 1. The initial sample size was 100 in the trial by Shah et al. (17) and 80 in the trial by Alizadeh et al. (18). The studies occurred in two distinct geographical regions. One study was carried out in the USA between 2013 and 2015 (17), while the other was done in Iran between 2012 and 2013 (18). Both studies were published in 2016. One study was conducted in two NICUs (17) and the other in one NICU (18). Both studies included only preterm infants. In one study, the eligibility criteria included infants below 1500 g at birth (17), while the other study allowed low birth weights (<2000 g) to be included; this study was included in the review because the average birth weight of the participants was 1295 g. Both studies excluded infants with congenital anomalies. Infants in the study by Alizadeh who had to be fed formula because of insufficient MOM supply were excluded. The average gestational age in the two studies was 29.1 weeks.

There was inconsistency in the definitions of early fortification (EF) and later or delayed fortification (DF) between the two studies. In the study by Shah, EF started at 20 ml/kg/d, and DF started at 100 ml/kg/d. This study did not report the postnatal age at which fortification was initiated. In the study by Alizadeh, EF was reported as 'first feeding' and DF was commenced at 75 ml/kg/d. First feeding was described as starting on the 'first day', as trophic feeding at a rate specific to birth weight and maturity level. They reported that the mean postnatal age at introduction of fortification in the EF group was 3.7 days.

Infants were fed exclusively with human milk in both studies. In the study by Shah, infants were fed both MOM and DHM. Infants in the DF group received a higher proportion of DHM than those in the EF group (67% vs. 54%) but it was not reported if this was statistically significant. In the study by Alizadeh, infants were fed only MOM. In both studies, the fortifier used was of bovine origin and the method used for fortification was the addition of a standard amount for all babies rather than an individualized approach. Both trials aimed to increase EBM caloric density to 24 kcal/oz. Fortification was continued until the weight reached 3000 g in the study by Alizadeh, but the study by Shah did not report a criterion for discontinuing fortification.

The definition of NEC, feeding intolerance and sepsis outcomes varied between the two studies. In Shah's study, feeding intolerance was defined as symptoms or signs leading to feeding cessation, whereas in the study by Alizadeh it was defined as signs of feeding intolerance only. NEC was defined in Shah's study using Bell's criteria (Stage II or more), but the other study used a list of clinical, laboratory and radiological diagnostic criteria. In the study by Shah, sepsis was defined by clinical signs of sepsis proven by blood culture, whereas the other study defined it by clinical signs only. Definitions for all reported outcomes are listed in the supplementary file (Table E).

Individual studies' risks of bias and quality assessments

There was a low risk of selection bias in the study by Shah. In Alizadeh's study, the risk of selection bias was unclear, as no details were provided on the tool used to perform block randomization, and the allocation was concealed. There was a high risk of performance bias in the trial by Shah, as the blinding of infants' caregivers and the research investigator was not possible due to the fortification of EBM occurring at the bedside. In the trial by Alizadeh, the risk of performance bias was unclear, as no details were provided. The risk of detection bias was unclear in both studies. There was a low risk of attrition

bias in both studies, as most of the participants remained in the study until the intervention ended (90 % in Alizadeh's study and 99 % in Shah's study). No selective reporting was observed in either trial (Table 3). Shah's study was evaluated to be of high quality, while the other study was evaluated to be of moderate quality (Table 4).

Effect of EF versus DF on outcomes

Most of the primary and secondary outcomes were measured in both studies with the exception of length of hospital stay, which was reported by only one study (Shah's study). Neither study reported PMA at discharge. The primary outcome in the study by Shah was days to reach full feeding, while in-hospital growth was the primary outcome in Alizadeh's study. Due to the small number of included studies, meta-analysis was not performed. Data on all primary outcomes except length of stay were available for 171 infants in the two trials. Length-of-stay data were available for 99 infants in the trial by Shah. Neither study reported differences between groups including NEC or sepsis (Table 2).

5. Discussion/Conclusion

The main finding of this systematic review is that there is insufficient evidence to evaluate the effects of EF against DF of human milk in VLBW infants. Despite screening a large number of international studies using a robust search strategy, we found little evidence on the benefits of EF versus DF. The number of studies was small, and the definitions of EF and DF varied. In one of the included trails, by Shah et al., the primary outcome was days to achieve full feeding volume. They showed that the median days to achieve full feeding and the number of events of feeding intolerance were similar in the EF and DF groups. Some published studies that did not meet our inclusion criteria showed some benefits of EF. One observational study compared EF (80 ml/kg/day) with DF (160 ml/kg/d) in late preterm infants and found that infants in the EF group regained their birth weight faster and had fewer events of feeding intolerance (21). A clinical trial by Sullivan et al. (which was not designed to compare EF with DF) showed that fortification with human-milk-based fortifier was tolerated at 40 ml/kg/day (22). An observational study by Tillman et al. showed that EF (from the first feeding) improved bone mineralisation (lower alkaline phosphate) and did not have higher events of feeding intolerance (23). Nevertheless, proper randomised clinical trials designed to evaluate the effectiveness and safety of EF in high-risk infants are needed to inform feeding practice.

Although optimising nutrition for preterm infants is advantageous in terms of improved short-term growth, there remains a lack of evidence on the longer-term benefits of fortification of EBM. A recently published Cochrane meta-analysis comparing the growth and developmental outcomes of using fortified EBM against unfortified EBM found insufficient data from which to draw any meaningful conclusions (19). Despite a rigorous search methodology, the authors concluded that there is limited evidence for evaluating the benefits of multi-nutrient fortification of EBM against unfortified EBM.

In summary, current evidence does not provide guidance on the optimal time to start fortification. Further and larger randomised clinical trials comparing the effect of EF versus DF are needed. Future studies should measure core outcomes that are important for both parents and health professionals to allow results to be compared and combined(24).

6. Appendix

Form 1. Eligibility checklist

Form 2. Data extraction sheet

7. Supplementary Material

Table a. Systematic review protocol
Table b. Search strategy
Table c. Criteria for assessing risk of bias
Table d. Quality assessment tool
Table e. Definition of outcomes in the included studies

8. Statements

8.1. Acknowledgement

This work was supported by the Faculty of Applied Medical Sciences, the Clinical Nutrition Department at Imam Abdurrahman Bin Faisal University, Dammam, the Kingdom of Saudi Arabia.

8.2. Statement of Ethics

The authors have no ethical conflicts to disclose.

8.3. Disclosure Statement

CAE has been involved in workshops for ILSI Europe. Other authors have indicated they have no potential conflicts of interest to disclose.

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8.5. Author Contributions

Mrs Alyahya conceptualized and designed the systematic review, carried out the searches, screened the studies, assessed the full text, extracted the data, carried out the analysis, and drafted and revised the manuscript.

Professor Edwards and Dr. Garcia conceptualized and designed the systematic review, screened the studies, supervised the data extraction and analysis and critically reviewed the manuscript.

Dr. Simpson screened the studies and reviewed the analysis and critically reviewed the manuscript.

Dr. Mactier conceptualized and designed the systematic review and critically reviewed the manuscript.

All the authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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10. Figure Legends

Figure 1 PRISMA flowchart

11. Table legends

Table 1 Characteristics of the included studies

Table 2 Effect of EF versus DF on outcomes

Table 3 Risk of bias assessment

Table 4 Quality assessment of the included studies

Figure 1 Figure 1 PRISMA flowchart

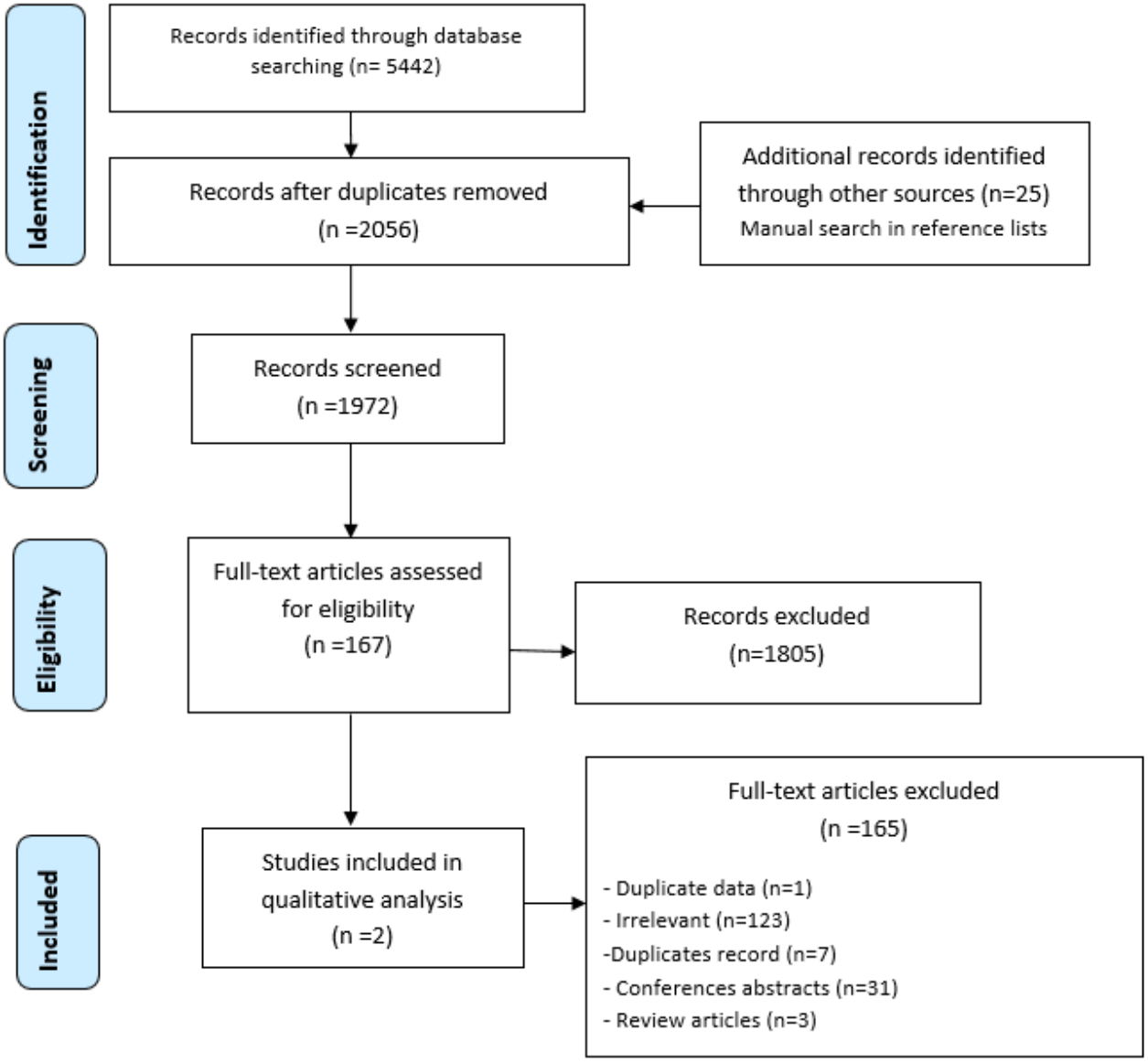

Table 1. Characteristics of the included studies

	Shah et al	Alizadeh et al
Methods		
Settings /Study period	Two NICUs in USA/ /2013-2015	One NICU in Iran/ 2012-2013
Objective	To compare human milk fortification at two different feeding volumes on time to reach full feeding and feeding intolerance	Compare early versus delayed fortification on growth, feeding intolerance, sepsis and NEC.
Participants		
Inclusion criteria	Preterm, Birth weight < 1500 g	Preterm, Birth weight <2000 g
Exclusion criteria	Died or expected to die before 72 hours of life, major congenital or chromosomal abnormality, no MOM or refused DHM	Major congenital anomalies, transferred to another hospital, did not attend the neonatal clinic follow-up, had to be fed with formula due to insufficient MOM.
N randomized /analyzed	100/99	80/72
Gestational age	Median (25th ,75th centile)=27.8 (26, 30)[1]	Mean ± standard deviation =30.4 ± 1.9[1]
Birth weight	Median (25th ,75th centile)= 990 (810,1225)[1]	Mean ± standard deviation =1295 ± 379[1]
Nutrition management		
Day EN started	3 (2.5 ,4)	One
EN advancement rate	10-20 ml/kg/d	10-20 ml/kg/d
Milk of feeding	MOM or DHM	MOM
Intervention description		
Definition of EF	20 ml/kg/d	First feeding
Definition of DF	100 ml/kg/d	75 ml/kg/d
Age at fortification	No data	EF: 3.7 ± 2.76 DF: 9.48 ± 5.31
Fortifier name	Enfamil HMF liquid	Aptamil FMS
Fortifier type	Bovine origin Acidified liquid HMF	Bovine origin Powder
Fortifier dose	Five ml added to 25 ml of EBM	4.4 g added to 100 ml of EBM
Duration of fortification	No data on criteria to stop fortification	Until weight reaches 3 kg.
Fortified EBM energy density	24 kcal/oz.	24 kcal/oz.
Fortification method	Standard	Standard

[1] Average value is calculated for the two study groups

Table 2. Effect of EF versus DF on outcomes

Outcome	Study	Unit	Early fortification	Delayed fortification	*P* value
Weight gain					
	Shah, g/kg/d ,4 wk of life	median (IQR)	8.7 (6.4,12.2)	7.7 (5.1,10.6)	0.08
	Shah, g/kg/d ,36 wk PMA	median (IQR)	18.3 (14.9,20.7)	16.7 (13.9,22)	0.3
	Alizadeh	z score	-1.24 (-3.74 to 0.84)	-1.23 (-3.92 to 1.16)	0.864
Head growth					
	Shah ,4 wk of life	mean (SD)	-0.97 (0.91)	-0.95 (1.0)	0.90
	Shah, 36 wk PMA	mean (SD)	-0.71 (1.08)	-0.65 (1.07)	0.80
	Alizadeh	z score	-5.15 (-0.95 to 1.66)	-0.43 (-2.77 to 1.10)	0.787
Length gain					
	Shah, 4 wk of life	mean (SD)	-1.0 (0.57)	0.97 (0.69)	0.81
	Shah, 36 wk PMA	mean (SD)	-1.58(0.93)	1.59 (0.89)	0.93
	Alizadeh	z score	-0.14 (-3.74 to 2.26)	-0.44 (-4.11 to 1.82)	0.348
Feeding intolerance					
	Shah	N (%)	15 (31)	15 (30)	1
	Alizadeh	N (%)	5 (14)	3 (9)	0.771
Length of hospital stay (d)					
	Shah	median (IQR)	68 (41,101)	63 (50,83)	0.49
Necrotizing enterocolitis					
	Shah	N (%)	2 (4)	2 (4)	1
	Alizadeh	N (%)	2 (6)	0	0.223
Sepsis					
	Shah	N (%)	3 (6)	6(12)	0.49
	Alizadeh	N (%)	2 (6)	1(3)	0.572

IQR: interquartile range, SD: standard deviation. wk : weeks, The number of participants analysed for all outcomes in study by shah (EF=49, DF=50) and in study by Alizadeh (EF= 36, DF=36)

Table 3. Risk of bias assessment

	Shah et al		Alizadeh et al	
Domain	**Review authors' judgement**	**support for judgement**	**Review authors' judgement and**	**support for judgement**
Selection bias				
Random sequence generation	Low risk	Use of blocked stratified randomisation approach by computerized software.	Unclear risk	Block randomisation method, no details how.
Allocation concealment	Low risk	The research coordinator and principal investigator performed the enrolment and assignment of infants after randomization	Unclear risk	Double blinded, no details how
Performance bias				
Blinding of participants and personnel (all outcomes)	High risk	Infants' caregiver and research investigators were not blinded. Fortification (intervention) occurred at bedside, blinding was not possible.	Unclear risk	It was not reported if the caregiver and researcher were blinded.
Detection bias				
Blinding of outcome assessment	Unclear risk	Was not clearly reported if the outcomes measurers were blinded	Unclear risk	It is unclear if the outcome measurer was blinded at the time of outcome measurement
Attrition bias				
Incomplete outcome data	Low risk	99 % of the participant were analysed	Low risk	90 % of the participant were analysed
Reporting bias				
Selective reporting	Low risk	Outcomes were reported	Low risk	Outcomes were reported

Table 4. Quality assessment of the included studies

	Score Shah et al	Rational	Score Alizadeh et al	Rational
Multicentre	1	Two centers	0	One centre
Sample Size	1	99 infants included	1	72 infants included
Description of Intervention	2	EF and DF was clearly defined as ml/kg/d	1	EF was defined as 'first feeding', specific volume was not reported.
Baseline characteristics comparable	1	EF and DF groups had similar demographic	1	EF and DF groups had similar demographic
Outcomes defined	1	Yes	1	Yes
Outcomes measurement described	1	Yes	0	Yes
Confounding factors	1	RCT but 13 % difference in DHM intake between groups. Not reported if statistically significant.	1	RCT
Score (Quality level)	8 (High)		5 (moderate)	