

Food and Public Health Policies About BCM-7 in A1 Allele & B-Casein Protein in A2 Allele in Milk Variants

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Keywords

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Abstract

This article critically investigates the type of beta-casein protein traced in cow species' indigenous (A2) and exotic (A1) milk and its adverse effects on health. Medical practitioners and health care stakeholders are the respondents to address the gap between the type of milk intake and non-epidemic diseases. The sample size ranges from 40-50 respondents from the different disciplines of the medical fraternity through survey methods. Analyze Likert scale data using R Software. There is a strong association between the lack of awareness level of public health imbalances and the benefits of indigenous cow milk against non-epidemic diseases. However, it also establishes the significant effect of bovine milk as an antioxidant on human health. There is a need to explore further robust lacto-epidemiological studies to correlate the prevalence of A1 casein-related metabolic with non-epidemic disorders and the nature of milk allergies and antigens.

1. Introduction

Milk is always associated with preventing diseases. The relationship between different cow species variants specific to A1 and A2 type breeds is intriguing with non-epidemic diseases. The concept of indigenous and exotic milk is still an altercation in medical and dairy science. A1 milk beta-casein protein is associated with a chain of amino acids at the 67th position with a bioactive peptide bond of Beta-Casomorphin-7 entering the human body's digestive tract. (Kumar & et al. 2019). Indigenous cow milk of A2 allele possesses specific sweet taste characteristics, cooling in nature, highly nutritive, and tonic for vital human organs. A hump on the neck of desi cows with a particular vein absorbs the energy from the sun rays and produces Milk that has medicinal value.

Indian cattle breed descendants with a hump and adapted to indigenous climatic conditions are A1 allele. In contrast, cattle breed descendants with the non-hump physical structure easily survive in tropical environmental conditions. The lactating capacity of A1 type allele as compared to indigenous cow milk.

Previous evidence exists of a confident presence of beta-casein protein at position 67 with histidine (CAT) due to a

single amino-acid nucleotide in their mitochondrial DNA and proline (CCT) at 67th position is the significant difference between indigenous and exotic cow species the Taurine and zebu type of cow species have different mutations in the bovine beta-casein gene, leading to 12 genetic variants. The presence of beta-casein protein at position 67 with histidine (CAT) due to a single amino-acid nucleotide in their mitochondrial DNA and proline (CCT) at 67th position is the significant difference between indigenous and exotic cow species (Chan and et al., 2010).

It has been examined in various medical research that the presence of bioactive peptide beta casomorphin in the A1 variant has severe health impacts. Infants who consume exotic milk quickly absorb BCM-7 in their immature gastrointestinal tract compared to adults shown in figure (Fig.1),



Fig. 1: Diagrammatic Representation of Difference between A1 type Variant and A2 Type Variant in Cow Milk.

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Source : Digestion of A1 and A2 beta casein (<https://dietitianconnection.com/resources/clinical-dietetics/a2-milk/>).

Literature Review: A1 Milk and its correlation with non-communicable disorders (diabetes, autism). Hence it is difficult to reject Milk consumption of the A1 allele vehemently. Asian countries like India have the largest populated countries facing a disequilibrium in consumption and production pattern of Milk in the dairy sector fails to promote A2 type milk that possess health benefits compared to A1 type variant (Parashar and Saini, 2015).

A1 Milk and its correlation with non-communicable disorders like diabetes, autism, and cardiovascular diseases(CVD)are strong evidence in previous epidemiological studies. Hence it is difficult to reject Milk consumption of the A1 allele vehemently. Asian countries like India have the largest populated countries facing a disequilibrium in consumption and production pattern of Milk in the dairy sector that fails to promote A2 type milk that possess health benefits compared to A1 type variant (Parashar and Saini, 2015).

In milch animals, mainly the indigenous lactating species, after birth through mammary glands, produce colostrum in milk which is a rich source of nutrients for development and growth. Antibodies are also present in their milk, known as immunoglobulins (Igs) play a vital role in biological processes to boost immunity and longevity. Bovine colostrum Igs indirectly affect the immune system of newborn calves and infants. The major Ig classes in bovine and human Milk are IgA, IgG and IgM, traced in the lymph and blood fluid to fight against infectious and epidemic diseases.(Korhonen and Marnila, 2009).

Further scientific investigation to understand the effects of BCM-7 present in the A1 allele and beta-casein present in A2 milk variants concerning its human consumption and health issues. However, in many studies, there is a direct relation between non-epidemic diseases like Type1diabetes, cardiovascular diseases, gastrointestinal and neurological disorders and consumption of A1 type variant milk (Misra et al.,2009).

The distribution of conventional Milk in the unorganized Indian dairy sector poses a serious health threat. Traceability of free fatty acids, urea content, a high percentage of somatic cell count, coliform bacteria count,

lactose, and non-solid fat at low freezing point incidents the problem of adulteration and contamination

(Kourimska, Legarova et al. 2014).

Stringent policies and regulatory frameworks are required in the dairy sector to differentiate between the indigenous and exotic cow species based on their DNA configuration and level of β casein protein and colostrum to trace their health risks. (De, Paradkar and Vaidya,2015).

Motivation of Research : This research aims to address the gaps in understanding the awareness level about the presence of beta-casein milk protein in A2 allele milk variants for public health concerns.

Research Gaps: In India, in contrast to Europe and the U.S., the general public is reluctant to emphasize the quality and adverse effects of type of milk consumption as an essential part of daily diet due to the niche market of A2 type milk and its exorbitant price. There is a need to focus on A2 or indigenous cow milk health benefits among dairy farmers, producers and consumers. (Narayan,2019). This research aims to address the gaps in understanding the awareness level about the presence of beta-casein milk protein in A2 allele milk variants for public health concerns.

The study's hypothesis: For the research hypothesis, we fail or reject the null hypothesis.

$H_0 : H_1 = 0$

H₀1.1:There is no significant relationship between A2 milk benefits and public awareness.

H₀1.2: There is no strong relationship between indigenous cow milk as an antioxidant and health benefits.

H₀1.3: There is no significant relationship between A1 Vs. A2 deficiency imbalances and A2.milk against non-epidemic Diseases.

H₀1.4: There is no significant relationship between awareness level about FSSAI certification of milk and its usage.

2. Methodology

The preliminary study is conducted on medical practitioners, and stakeholders like healthcare providers, managers, policymakers and others in

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India are the respondents. Snowball sampling method and the online survey method record the responses due to the COVID-19 outbreak. Semi-structured questionnaire on a five-point Likert scale (5-Strongly Agree (SA),4-Agree (A),3-Neutral (N),2-Disagree (DA),1-Strongly Disagree (SDA), with close and open-ended questions for expert opinions for this study. Responses are gathered from seventeen statements in a close-ended form of questions to understand the gravity of the subject and the concern issue shown in the above (Table 1).

https://docs.google.com/forms/d/e/1FAIpQLSe5HHxLucjrBw48jA7W4aeydrj-IYOgMQzVaRfM_Ny557_oaw/viewform?usp=sf_link.

Data Analysis and Interpretation: The Regression Analysis technique predicts a relationship between one target variable and one or more explanatory variables selected randomly from a sample statistic. The X's are the independent variables (I.V.s). Y is the dependent variable.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_p X_p + \epsilon$$

A correlation matrix and a linear regression model are used as statistical tools through R software version 1.4.1717 to analyze and interpret the outcomes.

Table 1: Profiling of Explanatory Variables

Variable No.	Variable Name	Variable code
Var. 1	Organic Farming Beneficial	ORGFARM
Var.2	Importance of A2 Milk during COVID	IMILKCOVID
Var.3	A2 Milk Against Non-Epidemic Diseases	A2MNED
Var.4	A1 Vs. A2 Milk Benefits	A1A2MBF
Var.5	Composition of Casein in Milk	COSCESNIM

Var. 6	A1 Negative Effect on Health	A1NEEFOH
Var. 7	A1 Vs. A2 Deficiency Imbalances	A1A2DI
Var. 8	A1 Milk Immune Disruptive Effects	A1IDEFF
Var. 9	Aflatoxin in Milk Samples	ALFAMS
Var. 10	Importance of Awareness Program about Milk Benefits among Dairy Farmers	IMPPAPMS
Var. 11	Desi Cow Milk as Antioxidant	DESIOX
Var. 12	Steps to Avoid Contamination	CONM
Var. 13	Ayurvedic Importance	AI
Var. 14	Certification and FSSAI Guidelines	CFSSAI
Var. 15	Balanced diet	BD
Var. 16	Nutritive Value	NV

Linear Regression Equation:

$$lm = \text{LOAW (Y)} \sim \beta_0 + \beta_1 X_{A1.A2DI} + \beta_2 X_{A1X2..A2MBF} + \beta_3 X_{A1IDEFF} + \beta_4 X_{A1NEFFOH} + \beta_5 X_{A2MNED} + \beta_6 X_{IMPAPMF} + \beta_7 X_{IMLKCOVID} + \beta_8 X_{DESIOX} + \beta_9 X_{CONM} + \beta_{10} X_{10}$$

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$$\text{COCESINM} + \beta_{11}X_{11}.\text{CFSSAI} + \beta_{12}X_{12}\text{AI} + \beta_{13}X_{13}\text{ALFAMS} + c \dots \dots \dots (1).$$

$$\text{lm} = \text{LOAW}(Y) \sim \beta_0 + \beta_1 X_1.\text{DESIOX} + \beta_2 X_2.\text{CFSSAI} + \dots (2).$$

$$\text{lm} = \text{LOAW}(Y) + \beta_0 + \beta_1 X_1.\text{A2MNED} + c \dots \dots \dots (3).$$

The observations are normally distributed in the linear model. The Shapiro-Wilk test was used through R Software to test the normal distribution of explanatory variables in the dataset. The p-value of all the explained variables and target variable in the output table (Table 2) mentioned below is less than 0.05, at a 5% significance level and C.L. of 95%. Hence the H_0 hypothesis is rejected and concludes that data is normalized.

Table 2: Shapiro-Wilk normality test.

Variable Name	Type of Variable	W	P-value
A2MNED	IV	0.72138	0.0000001351
A1.A2MBF	IV	0.816	0.000009888
DESIOX	IV	0.816	0.00000006654
LOAW	DV	0.63366	0.00000005302
CFSSAI	IV	0.87021	0.0002072

Scale Reliability : Alpha reliability for Var.1. The Importance of A2 against non-epidemic diseases (A2NED) was more significant than 0.7. In a linear equation scale reliability of explanatory variables was similar to and more than significant value of 0.7.

The sample statistic drawn from the population ranges lies between a min. value of -2.40985 and a max. value of 1.46726. meaning there is a standardization between observations scattered towards the central. The sample's median is -0.07, negatively skewed and approximately a normal distribution.

From the above output table (Table 3), the residual SE for this model is 0.5854 on (n-1) 28 degrees of freedom, where the coefficient of determination (Multiple R²) is 0.4943 and Adj. R² is 0.2596, where the probability value of the F-statistic is **0.04822**, less than a threshold value. **t= Estimates/S.E.**

Where,
 't'= Inferential statistic to understand the significant difference between two variables.

'Estimates coefficients' = Actual population parameters. 'S.E' = Average using the units of response variable.

From the above linear equation (2) and output table (Table 3), LM 2, the p-value of t-statistic for the explanatory variables, variables: DESIOX, CFSSAI are less than the threshold value of 0.05 and strong significant relationship with a response variable. However, the magnitude of the t-statistic is more significant than one and has a high possibility of evidence against the null hypothesis. The model does not fully explain all the variables. The coefficient of determination explained only 19% of the variables in the regression model. The p-value of the F statistic is less than 0.05, degree of freedom (n-1) is more than thirty. Hence, null hypothesis is rejected, and the model is significant. But it is not considered a good model, so we further developed the linear equation and suggested that the model is robust.

Table 3: Summary table of Linear Regression Model 1.

	Coefficients			
	Estimates	Standard Error	t-value	Pr(> t)
Intercept	-1.77798	2.44104	-0.728	0.4724
A1.A2DI	-0.26989	0.17015	-1.586	0.1239
A1.A2MBF	-0.02379	0.24004	-0.099	0.9218

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A1IDEF F	0.23036	0.15757	1.462	0 0.1549
A1NEFF OH	-0.04068	0.19901	-0.204	0.8395
A2MNE D	0.33758	0.20923	1.613	0.1179
IMPAP MF	0.02261	0.20266	0.112	0.9120
IMLKC OVID	-0.03462	0.12968	-0.267	0.7915
DESIOX	0.44911	0.19544	2.298	0.0292 *
CONM	0.27513	0.23207	1.186	0.2458
COCESI NM	0.35611	0.29604	1.203	0.2391
CFSSAI	-0.34640	0.13573	-2.552	0.0164 *
AI	0.12589	0.13695	0.919	0.3658
ALFAM S	0.41752	0.18028	2.316	0.0281 *

The sample statistic drawn from the population ranges lies between a min. value of -2.53067 and a max. value is 1.59040, meaning there is a standardization between observations scattered towards the central. The sample's median is 0.04, positively skewed and approximately a normal distribution.

From the below mentioned output table (Table 4), the residual SE for this model is 0.6246 on (n-1) 39 degrees of freedom, where the coefficient of determination (Multiple R²) is 0.4943 and Adj. R² is 0.2596, where the

probability value of the F-statistic is **0.01349**, less than a threshold value.

From the below mentioned linear equation (2) and output table (Table 4), LM 2, the p-value of t-statistic for the explanatory variables, variables: DESIOX, CFSSAI are less than the threshold value of 0.05 and strong significant relationship with a response variable. However, the magnitude of the t-statistic is more significant than one and has a high possibility of evidence against the null hypothesis. The model does not fully explain all the variables. The coefficient of determination explained only 19% of the variables in the regression model. The p-value of the F statistic is less than 0.05, degree of freedom (n-1) is more than thirty. Hence, null hypothesis is rejected, and the model is significant. But it is not considered a good model, so we further developed the linear equation and suggested that the model is robust.

Table 4: Summary table of Linear Regression Model 2.

Variable Name	Type of Variable	W	P-value
A2MNED	IV	0.72138	0.0000001 351
A1.A2MB F	IV	0.816	0.0000098 88
DESIOX	IV	0.816	0.0000000 6654
LOAW	DV	0.63366	0.0000000 05302
CFSSAI	IV	0.87021	0.0002072

The sample statistic drawn from the population ranges lies between a min.-2.7308 value of -2.7308 1.26972 and a max. the value is 1.26972, meaning there is a standardization between observations scattered towards the central. The sample's median is -0.01, negatively skewed and approximately a normal distribution.

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From the below mentioned output table (Table 5), the residual SE for this model is 0.6512 on (n-1) 40 degrees of freedom, where the coefficient of determination (Multiple R²) is 0.1062 and Adj. R² is 0.08382 where the probability value of the F-statistic is **0.03523**, less than a threshold value.

Table 5: Summary table of Linear Regression Model 3.

Coefficients				
Response Var.: LOAW	Estimates	Standard Error	t-value	Pr(> t)
Intercept	2.5769	0.6714	3.8380	0.000431 ***
A2MNE	0.3846	0.1765	2.180	0.035225 *

From the above linear equation (3) and output table (Table 5), LM 3, the probability value of t- statistic for the explanatory variable A2 milk against non-epidemic diseases. (A2MNE) are less than the threshold value of 0.05 and have a highly significant relationship with a response variable. However, the magnitude of the t- statistic is more important than one and has a high possibility of evidence against the null hypothesis.

The model thoroughly explains the variable. The coefficient of determination Adjusted R² explained 83 % of the variables in the regression model. The probability value of the F statistic is 0.03, and the degree of freedom (n-1) is significantly higher than the threshold value. Hence, the H₀ is rejected, and the model is significant. The robust linear relationship between the response and predicted variable in model LM 3 suggests that the model is best fitted.

3. Result

Hypothesis Testing:

In the output table (Table 6), the regression S.S. is 2.0147, and The p-value of the sample estimate is 0.003523. Hence the overall significance value of the F-statistic is

significant, and a strong association exists between the predicted and explanatory variables.

Table 6.: ANOVA table for hypothesis testing.

Response Var.: LOAW	Sum of Squares	Degree of Freedom	F-Value	Pr(>F)
A2MNE	2.0147	1	4.7511	0.03523 *
Residuals	16.96	15	40	

Residual Diagnostics: In Linear Regression, there are three stages in developing a statistical model: formatting and fitting a model to data and evaluating the model. The summary table checks the condition of heteroscedasticity, normality and Influential observations used in the diagnostics plots. Here, standardized residuals are used to measure the gap between observed and expected values.

Residuals Vs. Fitted Values in (Fig. 2) graph indicates fitness of linear model 3. It also tests the linearity between the target variable and explanatory variables. In this model, the red line is not straight in the first graph of fitted values, meaning there is a polynomial relationship between Dependent and Independent Variables. Observations 20 and 30 are outliers that significantly affect the regression results. The residuals are spread across the plot around the line y=0 uniformly, which meets the condition of homoscedasticity.

Residuals v/s Leverage Plot indicates each data point's influence on the leverage regression model. Observations 5 and 10 are the outliers in linear model 3 that altered the results. In this plot, the horizontal gray line near the red smoothed line that no points indicate inside the graph plotted, i.e. less than the value of 0.5. Cook's distance in the graph is represented by a red dash line at the corner of both the sides on the graph plotted.

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Advanced Residual Diagnostics test the problem of overfitting in the linear model 3. Potential outliers drag the model towards its side in every estimated regression function. The standardized criterion cannot detect these influential outliers, and studentized residuals are an alternative criterion for evaluating the potential outliers. The Rcmdr package Bonferroni test the type of residuals in R software. Observations 5 and 30 in linear model 3 have extreme values and are considered an outlier. The p-value of linear model 3 is 0.000030735, which is less than the threshold value of 0.05. Hence null hypothesis is rejected.

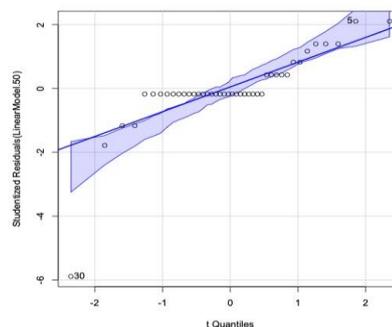


Fig. 3: Advanced Residuals diagnostics- Q-Q plot for Linear Model 3

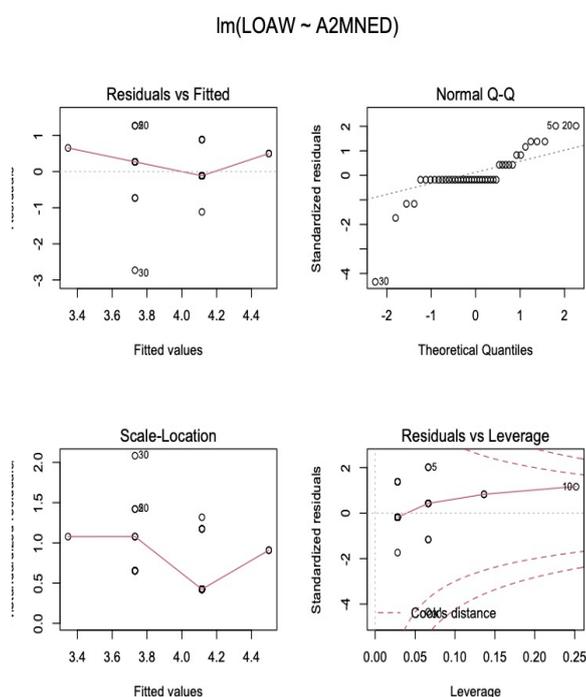


Fig.2 : Diagnostics plots for Linear Model 3.

The Studentized residuals are plotted (Fig 3) against a t-distribution with an $(n-p-1)$ degree of freedom, normality of the sample statistic is measured, where p is the number of regression parameters (including the intercept), and n is the sample size in the Q-Q plot () function plots of R software. In linear model 3, Q-Q plot for studentized residual, observations 5 is an outlier. Leverage is a measure used to detect outliers based on independent variables (I.V.s). In linear model 3 it. If empirical observations of an independent variable are distant from other statements, the leverage value is high.

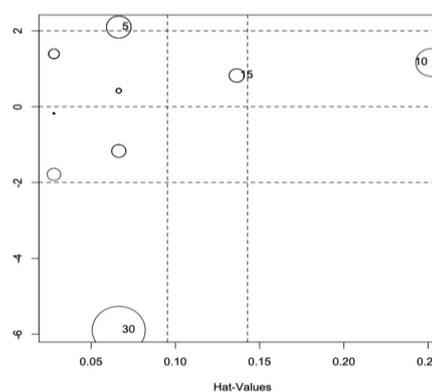


Fig.4.: Influence Plot for Linear Model 3.

The diagnostics plot of leverage v/s residual plot in (Fig.2) suggests that these observations 5 and 15 are not genuinely influential in their regression results. Deleting these points does not affect intercept and slope values in regression lines. Another way of representing Cook's observations is that distance depends on the circle's size. In the figure (Fig.4.) Observations 5 and 15 have more considerable Cook's distance, and 30 and 10 highly are influential.

Breusch-Pagan's test evaluates whether the residuals are distributed with equal variance over the predictor variable across the regression line in a linear model. Here in the case of linear model 3, the p-value is 0.00006394 less than the threshold value of 0.05. Hence, it violates the homoscedasticity assumption in this linear model LM 3. The Non-Constant Variance test is used for test whether the residuals of

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the model after fitted values have constant variance. If non-constant is not present, it is said to be the condition of Homoscedasticity.

Homoskedasticity in the given data indicated that the conflict around the regression line is the same for all values of the feature X. Here, the p-value of linear model 3 is 0.000063939 less than the level of significance. So, reject the null hypothesis, and residuals are not constant. In linear model 3, the variance of the residuals is not consistent, and in the case of linear model 3, it is 15.98225 and the suggested power transformation is 4.342733, which is more than the value of one. Hence the residuals are not homoskedastic.

In the case of linear model 3, the problem of multicollinearity has no existence of a non-linear relationship between independent variables in a given dataset. There is only one response variable, and the explanatory variable explains the model.

The linear regression algorithm assumes that the relationship between each feature and target is linear—the crPlots() function in R software tests the linear relationship and line of fit. In the plotted graph (Fig. 5) indicated by the blue line linear model 3, if the pink line represents the estimated model that overlaps the blue line, there is a linear relationship between predicted and explained variable.

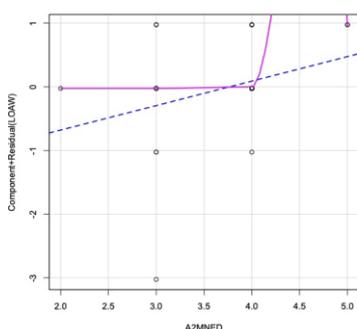


Fig. 5 : Component +Residual Plot for linear Model 1.

Durbin-Watson test measures an autocorrelation between a variable of present and past values in the given dataset. It comes into existence when residuals are not independent of each other. The p-value of linear model 3 is 0.9918, which is more than the significance level of 0.05. Hence H_0 fails to reject, indicating the absence of autocorrelation and independence of errors conversely.

In summary (Fig. 6), evaluate and determine the quality of the model based on the p-value of coefficients, Multiple R^2 ANOVA and residual analysis. Linear Model 3 is considered the best model, as its variance is explained 83.3% compared to other models using two variables and is considered moderate. In this case, For evaluating the model, Akaike Information Criterion (AIC) estimates the relative quality of the model and out-of-sample prediction error. AIC of linear model 3 87.10854, which has a minor AIC criterion among linear Model 3.1 considered the best model.

	Model 4	Model 8	Model 3	Model 1
Multiple R^2	0.4376	0.4312	0.3808	0.4328
AIC	79.7445 5	78.712 73	74.919 7	69.88914

Fig. 6: Model Comparison Table

4. Discussion

Research outcomes suggested strong evidence that indigenous cow milk is an antioxidant and has health benefits. The hypothesis for the A2 type milk variant is significant from a public health point of view as it proves a correlation in this study. It is observed that there is a shred of evidence to support that A2 milk posses nutritive value to fight against non-epidemic diseases and minimize the risks of type-I diabetes, Cardiovascular Diseases and obesity, which is evident from previous literature. There is a need to protect the interest of public health related to the use of additives, synthetic colors and hormones while selling and grading milk and dairy products. There is an urge to develop health policies to create awareness among the general public about the guidelines and certification parameters for FSSAI, underlying the importance of indigenous cow milk. It has been observed that A2 milk has its ayurvedic relevance, evident in Vedic literature. FSSAI and dairy research organizations should address the gaps in providing certification for the traceability of chemical composition of milk allergens and detection of the presence of β casein protein in indigenous and exotic milk.

5. Conclusion:

In the past five decades, there has been substantial and incremental growth in the field of dairy technologies which is widely accepted and cow's milk is considered an alternative source of nutritional food supplement for different gender groups and age segments. Epidemiological research to identify the A1 and A2 allele Discover innovative genome sequencing, breed improvement techniques, recombinant enzyme technologies, microbial fermentation, and advanced proteomics approaches is a breakthrough in this dairy research in India from public health and nutrition point of view. Fair policies in the Indian dairy sector and transparent regulatory guidelines are needed to develop awareness about health benefits, homogenization of milk and its harmful effects.

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