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Comparative evaluation of A1A2 and A2A2 cow milk-containing diets on biochemical and histological parameters of Wistar rats

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Research Article

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Abstract

This Research Communication aims to compare the effect of A1A2 and A2A2 cow milk diets on the biochemical and histological parameters of rats. The rats were divided into four groups and fed with a normal diet, A2 milk powder, A1A2 or A2A2 cow milk diets for 90 d. Blood glucose, kidney function, liver function and lipid profile were examined during the experimental period. The study showed an increase in the body weight of the A1A2 group whereas a slight decrease in the A2A2 group, and blood glucose levels increased from d 0 to day 90 in all experimental groups. However, none of these changes were found to be statistically insignificant ($P > 0.05$). Moreover, no significant changes were recorded in other parameters (serum glutamic pyruvic transferase and serum glutamic-oxaloacetic transaminase for liver function, bilirubin direct, cholesterol, triglycerides, creatinine and uric acid). The histology of the liver, kidney and pancreas also showed no changes in all groups. Overall, this study revealed no significant difference in the nutritional values of A1A2 and A2A2 milk types and hence equally beneficial for health. Although the present study showed no significant difference in the effect of both milk types in 90 d, further studies might be conducted to evaluate their longer term effects.

Cow milk is an important source of food and nutrition for people around the world. It contains a wide range of nutrients, including proteins, vitamins, and minerals, that are essential for body health. A1 milk is produced from animals with the β-casein A1A1 genotype and A2 milk is produced from the A2A2 genotype whereas A1A2 heterozygous genotype animals produce milk containing both A1 and A2 β-casein (Şahin and Boztepe, 2023). The A1 and A2 variants of β-casein differ for a point mutation in the amino acid sequence at position 67 where histidine is present in the A1 variant whereas proline is in the A2 variant (Guantario *et al.*, 2020). It has been reported that the gastrointestinal digestion of A1 and A2 alleles of β-casein showed different impacts on health due to the difference in amino acid sequence in the 67th position. The presence of histidine in the A1 allele of β-casein was found to reduce the protein susceptible to proteolytic cleavage by digestive enzymes and hence produces β-casomorphin-7 (BCM-7) peptide (Brooke-Taylor *et al.*, 2017; Guantario *et al.*, 2020). BCM-7 is a μ-opioid receptor ligand and is widely studied clinically because of its possible role in various human health issues including gastrointestinal problems, cardiovascular disorders/arteriosclerosis, type 1 diabetes mellitus and autism (Elliott *et al.*, 1999; Cade *et al.*, 2000; McLachlan, 2001; Zoghbi *et al.*, 2006; Semwal *et al.*, 2022). On the other hand, the A2 allele of β-casein does not produce BCM-7 due to the presence of proline in the 67th position of its amino acids sequence and hence considered safe as compared to the A1 variant (Sodhi *et al.*, 2012).

A1A1, A1A2 and A2A2 are the three important genotypes of milk-producing cow breeds worldwide with an average frequency of 0.112, 0.434 and 0.454, respectively for $n = 7667$ (Nuomin *et al.*, 2022). A recent study by Şahin and Boztepe (2023) conducted in Italy also found an average frequency of 0.145, 0.46 and 0.395 of A1A1, A1A2 and A2A2 genotypes, respectively for $n = 400$. The average frequency of A1 and A2 alleles for the above reports ($n = 8067$) was recorded to be 0.352 and 0.648, respectively. These data clearly indicate that

the share of A1A1 genotype breeds is too low as compared to A1A2 and A2A2 genotypes. It showed that the A1 allele of β-casein found in regular milk mainly comes from the A1A2 genotype and rarely from A1A1 due to the difference in their frequencies. Particularly in the Indian scenario, due to cross breeds of cattle, regular market milk with pure A1 allele is rarely available (Mukesh *et al.*, 2022). Hence, A1A2 and A2A2 cow milk were compared in the present study in which milk containing both A1 and A2 β-casein was obtained from the A1A2 genotype whereas A2 β-casein from the A2A2 genotype cow.

Nowadays, the increasing aged population is one of the big challenges globally, particularly their diet and healthcare. There are various factors responsible for ageing and dietary deficiency is one of them (Soenen *et al.*, 2016). Within this context, milk consumption is particularly required for the elders, as it contributes to the intake of necessary macro and micronutrients. However, the selection of suitable milk in a diet with ideal protein and fat content is equally important. Hence, the present study is an attempt to compare the dietary effects of A1A2 and A2A2 cow milk on rats using different parameters that are known to involve in cardiovascular, metabolic and gastrointestinal issues.

Material and methods

A complete description of Materials and methods is provided in the online Supplementary File. All procedures were performed according to Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the study was approved by the Institution's Animal Ethics Committee as No. 585/05/A/CPCSEA. Milk samples collected from the Badri and Jersey cows from Dehradun, Uttarakhand were analysed at the ICAR-National Bureau of Animal Genetic Resources (NBAGR), Karnal Haryana for identification of A1/A2 genotypes. After the authentication of A1/A2 genotypes, the milk of Jersey and Badri cows was collected before the onset of the experiment to prepare rat feed. Moreover, a standard A2 milk powder, obtained from the ICAR-National Bureau of Animal Genetic Resources, Karnal (India) as a gift sample, was also used in the study. The collected milk was dried separately using a vacuum rotary evaporator at the temperature of 40°C. The dried milk powder was mixed with the pelleted feed for rodents purchased from the local market that contained maize starch (467.5 g/kg), amino acid mix (140 g/kg), maltodextrin (155 g/kg), sucrose (100 g/kg), cellulose (50 g/kg), soya oil (40 g/kg), mineral mix (35 g/kg), vitamin mix (10 g/kg), choline chloride (2.5 g/kg) and *t*-butylhydroquinone (0.008 g/kg). A total of 120 g/kg each of A1A2, A2A2 and standard A2 milk powder was added to the market diet to prepare other diets. The diets were prepared by modifying the AIN-93M diet for rodents as described by Reeves (1997).

The rats were divided into four groups ($n = 6$ per group): normal diet control, standard A2 supplement, A1A2 supplement and A2A2 supplement. For 90 d the normal control group received the basal pelleted market diet, the standard group received the basal diet plus 120 g/kg of standard A2 milk powder, the A1A2 group received the basal diet 1 plus 120 g/kg A1A2 milk powder and the A2A2 group received the basal diet plus 120 g/kg A2A2 milk powder. Blood samples were collected at day 0, 15, 30, 45, 60, 75 and 90 *via* retro-orbital plexus under ether anaesthesia. The blood samples were collected in clot activator tubes and fluoride tubes (for glucose test) and left at 4°C for 3 h. Thereafter, the blood samples were centrifuged using Mini Spin Eppendorf, Germany, at 3000 rpm for 10 min to separate the serum (Kumar *et al.*, 2022). The isolated serum was used to evaluate blood glucose, liver function (SGPT, SGOT and bilirubin direct), lipid profile (cholesterol and triglycerides) and kidney function (creatinine and uric acid) with the help of Erba Semi Auto Biochemistry Analyzer using Erba diagnostic kits at the Pathology Laboratory, Uttarakhand Ayurved University Hospital, Dehradun, India. The body weight of experimental animals was also measured regularly. Data were entered into Microsoft Excel 2019 and entered into SPSS V21.0 for statistical analysis. The quantitative data of each group were expressed as mean and standard deviation. The variances between-group and within-group were compared by one-way analysis of variance (ANOVA) using *F*-test. *P* values were calculated using *F* distribution calculator and values of $P \leq 0.05$ were considered statistically significant.

Results and discussion

Body weight results are in Fig. 1a. Overall, there was relatively little change in body weight during the 90 d of the experiment. This lack of growth may in part be related to the experimental blood sampling protocol and needs to be taken into account when interpreting the data. The standard A2 group increased slightly in body weight (from 80 ± 4.0 to 86 ± 4.8 g as did the A1A2 group (89 ± 4.3 to 106 ± 5.4 g), whereas the body weight of the A2A2 group decreased from 96 ± 5.1 to 88 ± 4.0 g. These changes were not statistically significant ($P > 0.05$) when compared with the control group. Studies have shown that cow milk consumption can affect the body weight of humans and other animals. The impact of cow milk on body weight can depend on several factors, including the type and amount of milk consumed, as well as the individual's overall diet and lifestyle (Visioli and Strata, 2014). In some studies, rats fed a high-fat diet supplemented with cow milk or dairy products gained more weight than rats fed a similar diet without dairy. This may be due to the high calorie and fat content of cow milk, as well as its potential effect on appetite and metabolism (Eller and Reimer, 2010). However, other studies have found that cow milk or dairy products can have a neutral or even reductional effect on body weight. For example, some studies have suggested that consuming low-fat or fat-free milk or dairy products can help to promote or maintain body weight, possibly due to the high protein and calcium content of these foods (Thorning *et al.*, 2016). It is important to consider the overall quality and nutrient density of the diet, as well as other lifestyle factors such as physical activity and stress management.

The blood glucose levels are shown in Fig. 1b. They increased from 95.46 ± 6.45 to 104.4 ± 6.09, 87.25 ± 5.87 to 102.1 ± 7.09 and 91.74 ± 6.90 to 98.66 ± 6.04 mg/dl in the standard, A1A2 and A2A2 groups, respectively. The highest changes in the blood glucose levels were noticed in the A1A2 group. However, once again the statistical analysis showed that the changes recorded in blood glucose levels were not significant ($P > 0.05$) when compared with the initial day as well as with the control group (Fig. 1b). There is a reported statement which supports that the consumption of A1 milk induces diabetes mellitus whereas A2 milk does not affect the level of blood glucose (Elliott *et al.*, 1999; Joshi *et al.*, 2021). Earlier studies also suggested that A1 β-casein forms β-casomorphin-7 (BCM-7) peptide on digestion and is responsible for some adverse health effects such as inflammation, indigestion and an increased risk of certain chronic diseases (Semwal *et al.*, 2022). Cow milk contains lactose, a type of sugar that can increase blood glucose levels when consumed. However, it also contains protein and fat, which can slow down the absorption of sugar into the

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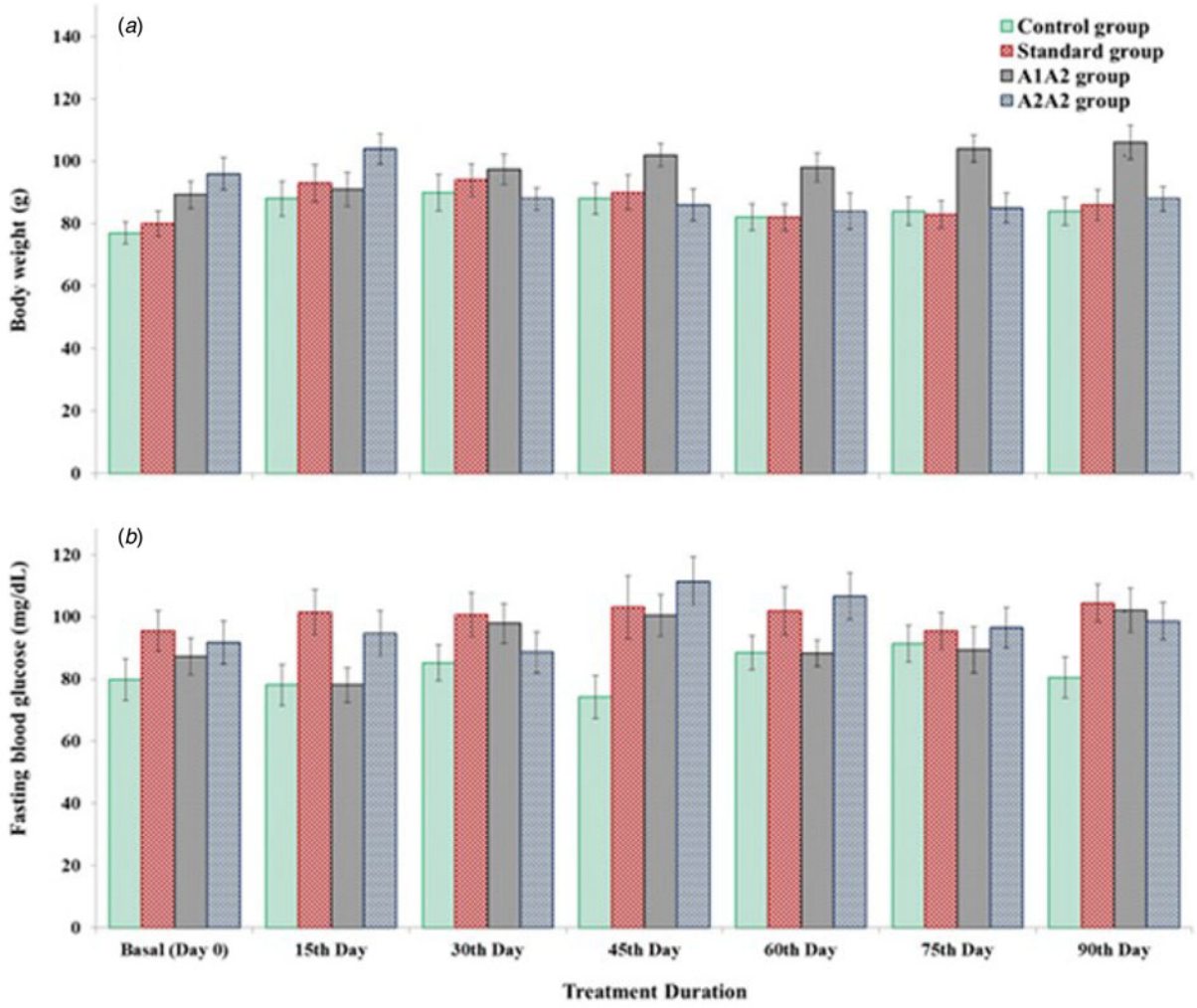

Figure 1. Effect of diets supplemented with A2, A1A2 or A2A2 milk powder on body weight (a) and fasting blood glucose levels (b) of rats.

bloodstream and may help to prevent spikes in blood glucose levels. Some studies have suggested that consuming cow milk or dairy products may be associated with a lower risk of developing type 2 diabetes, possibly due to the presence of bioactive compounds such as calcium, magnesium, and vitamin D in milk. However, other studies have suggested that excessive consumption of dairy products may be associated with a higher risk of developing diabetes, especially in individuals who are overweight or obese (Kalergis *et al.*, 2013). It is important to note that the impact of cow milk on fasting blood glucose levels can vary depending on the individual and the specific circumstances. Consumption of moderate amounts of milk is generally not expected to significantly affect fasting blood glucose levels in healthy individuals, but larger amounts may lead to a more significant increase, especially in individuals with lactose intolerance or insulin resistance. In the present study, the average changes in blood glucose levels in both A1A2 and A2A2 groups were found similar and non-significant at the end of the study when compared with the initial day as well as with the control group.

The liver function test data are in Table 1. SGPT levels remained relatively constant throughout, but did increase slightly in the A1A2 and A2A2 groups (from 73.74 ± 3.86 to 83.41 ± 5.32 U/l and from 82.41 ± 4.67 to 90.14 ± 4.75 U/l, respectively). However, the values remained the same in the case of the normal diet and standard A2 diet after 90 d of study. On the other hand, the levels of SGOT in rats were increased by similar amounts in all three supplemented groups with a change ranging from 41 to 47 U/l, a change that was not seen in the basal control diet. Nevrtheless, the results were found to be statistically nonsignificant ($P > 0.05$) for both SGPT and SGOT. In the case of bilirubin direct levels, a slight increase was noticed in the standard and A2A2 groups whereas a slight decrease was recorded in the A1A2 group. However, this change was found statistically nonsignificant ($P > 0.05$) when compared with the control group as well as with the initial day (Table 1). Consuming cow milk is not known to have a significant effect on liver function in healthy individuals. However, certain populations, such as those with pre-existing liver disease, may need to be cautious about their cow milk consumption. The present study showed insignificant changes in the levels of SGPT and SGOT as well as in the levels of bilirubin direct in rats of all groups.

Cholesterol data are in Table 1. Cholesterol decreased in the basal diet, standard A2 and A2A2 groups whereas a slight increase was noticed in the case of the A1A2 group. In comparison to the control group, the changes in all three supplemented groups were not significant ($P > 0.05$). Similarly, the changes in triglycerides levels were found statistically nonsignificant in all three groups when compared with values of the initial day as well as with the

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Table 1. Effect of different diets on liver function (SGPT/ALT, SGOT/AST and bilirubin direct) and lipid profile (cholesterol and triglycerides) of rats

Day	Control group	Standard group	A1A2 group	A2A2 group
SGPT/ALT (U/l)				
Basal (0) Day	74.56 ± 3.87	75.68 ± 4.57	73.74 ± 3.86	82.41 ± 4.67
15th Day	78.54 ± 4.43	76.5 ± 5.12	80.11 ± 4.23	85.5 ± 4.87
30th Day	81.24 ± 4.56	78.5 ± 3.45	84.4 ± 4.47	77.8 ± 3.87
45th Day	76.53 ± 3.87	81.33 ± 5.34	70.2 ± 3.56	86.5 ± 3.56
60 Day	83.45 ± 5.87	85.24 ± 5.67	73.18 ± 3.12	102.5 ± 5.78
75th Day	81.75 ± 4.98	75.81 ± 4.76	75.24 ± 4.65	93.48 ± 4.96
90th Day	79.28 ± 3.76	74.15 ± 4.65	83.41 ± 5.32	90.14 ± 4.75
SGOT/AST (U/l)				
Basal (0) Day	55.85 ± 2.98	55.65 ± 3.65	49.58 ± 3.29	58.54 ± 2.76
15th Day	58.45 ± 3.87	52 ± 4.87	47.13 ± 2.51	54.81 ± 4.76
30th Day	54.57 ± 3.76	113.9 ± 7.87	108.6 ± 7.43	104.5 ± 7.59
45th Day	48.45 ± 3.12	99.3 ± 6.59	109.1 ± 5.84	113.2 ± 5.81
60th Day	61.47 ± 3.91	112.91 ± 4.76	97.5 ± 4.98	102.6 ± 6.28
75th Day	63.14 ± 2.56	98.74 ± 3.98	95.21 ± 5.10	94.58 ± 5.38
90th Day	58.25 ± 2.25	102.4 ± 3.54	96.56 ± 5.43	99.41 ± 5.73
Bilirubin direct (mg/dl)				
Basal (0) Day	0.47 ± 0.23	0.65 ± 0.21	0.74 ± 0.15	0.78 ± 0.17
15th Day	0.56 ± 0.24	0.68 ± 0.06	0.73 ± 0.29	0.8 ± 0.02
30th Day	0.48 ± 0.20	0.8 ± 0.28	0.68 ± 0.54	0.94 ± 0.23
45th Day	0.52 ± 0.21	0.74 ± 0.15	0.65 ± 0.16	0.79 ± 0.04
60th Day	0.61 ± 0.19	0.72 ± 0.17	0.62 ± 0.09	0.81 ± 0.05
75th Day	0.63 ± 0.18	0.74 ± 0.23	0.66 ± 0.12	0.85 ± 0.01
90th Day	0.61 ± 0.22	0.74 ± 0.32	0.62 ± 0.27	0.88 ± 0.12
Cholesterol (mg/dl)				
Basal (0) Day	85.14 ± 3.76	88.42 ± 4.71	75.2 ± 4.67	75.14 ± 3.46
15th Day	79.54 ± 3.54	90.28 ± 4.94	77.31 ± 4.87	71.44 ± 4.23
30th Day	71.45 ± 4.01	84.5 ± 5.65	77.3 ± 4.94	77.64 ± 3.86
45th Day	71.24 ± 3.86	73.5 ± 4.56	75.68 ± 3.65	65.41 ± 4.76
60th Day	75.85 ± 2.98	74.57 ± 4.15	76.46 ± 3.76	67.6 ± 3.41
75th Day	74.54 ± 3.77	71.52 ± 3.87	73.51 ± 4.45	62.62 ± 3.74
90th Day	69.51 ± 4.23	69.57 ± 3.62	79.85 ± 4.83	66.84 ± 4.34
Triglycerides (mg/dl)				
Basal (0) Day	36.45 ± 2.45	38.24 ± 3.56	42.14 ± 4.17	46.45 ± 3.61
15th Day	38.14 ± 2.67	41.2 ± 3.78	43.5 ± 4.26	41.03 ± 3.72
30th Day	35.74 ± 3.12	37.5 ± 3.36	46.83 ± 2.94	46.84 ± 2.27
45th Day	28.47 ± 2.85	39.5 ± 3.87	43.4 ± 3.25	41.72 ± 2.86
60th Day	31.95 ± 2.94	31.26 ± 2.87	43.98 ± 3.29	43.92 ± 2.65
75th Day	34.4 ± 3.56	28.65 ± 2.76	38.46 ± 3.58	46.41 ± 3.45
90th Day	33.21 ± 3.32	31.41 ± 2.93	43.49 ± 3.60	42.14 ± 3.90

control group (Table 1). Cow milk consumption can have an effect on lipid profile, which refers to the levels of cholesterol and other fats in the blood. Cow milk is a source of saturated fat, which can increase levels of low-density lipoprotein (LDL) cholesterol (so-called 'bad' cholesterol. High levels of LDL cholesterol are associated with an increased risk of heart disease and stroke. However,

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cow milk also contains unsaturated fats, which can have a positive effect on lipid profile by increasing levels of high-density lipoprotein (HDL) cholesterol ('good'). HDL cholesterol helps to remove LDL cholesterol from the bloodstream, which can reduce the risk of heart disease and other related conditions. The effect of cow milk on lipid profile can vary depending on the amount consumed, as well as individual factors such as genetics, age, and overall diet. It's also important to note that other factors, such as physical activity, weight management and smoking, can also affect lipid profiles. In general, it's recommended that individuals with high cholesterol or a history of heart disease limit their intake of saturated fats, including those found in cow milk. This can be achieved by choosing low-fat or skim milk instead of whole milk, and by limiting consumption of other high-fat dairy products such as cheese and butter. Cow milk consumption can affect the lipid profile, but the impact can vary depending on individual factors and overall diet (Lordan *et al.*, 2018). It is also reported that A1 milk consumption can negatively alter the blood lipid profile in comparison to the positive effects of A2 milk, but our data do not provide any support for that view.

The renal function test data are in online Supplementary Table S1. Creatinine levels increased from 0.64 ± 0.12 to 0.88 ± 0.32 mg/dl in the A1A2 group but no changes were recorded in other groups. Uric acid levels increased slightly in the A2A2 group (1.42 ± 0.84 to 1.82 ± 0.27 mg/dl) but decreased from 1.62 ± 1.13 to 1.08 ± 0.45 mg/dl in the A1A2 group. These changes were found statistically nonsignificant when compared with the control group). Cow milk consumption is generally safe for individuals with healthy kidneys and is not known to harm renal function. however, individuals with pre-existing kidney disease or a high risk of developing kidney disease may need to be cautious about their cow milk consumption. This is because cow milk contains protein, which can increase the workload on the kidneys and may be difficult to metabolize in individuals with impaired kidney function (Gopinath *et al.*, 2016). In this study, the creatinine and uric acid levels of rats were insignificantly changed during the entire experiment. Furthermore, the kidney, liver and pancreas histology of rats did not show any signs of adverse effects (online Supplementary Fig. S1).

In conclusion, all of the changes that we observed in rats in response to different β-casein genotype dietary supplementations were small and non-significant. The data need to be interpreted with caution due to the frequent blood sampling and its possible adverse effects on growth. Nevertheless, our data provide no support for the belief that β-casein A1 and A2 have negative and positive effects, respectively, on health.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000663

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