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Effect of A1 / A2 milk protein on Biochemical and haematological parameters of Mice

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Abstract
In present study one group of mice was treated with A1 milk whereas another group was treated with A2 milk. Different parameters of Haematological and Biochemical test on above two groups of mice revealed non-Significantly higher Haemoglobin Hb (mg%) was noticed in A2 treated group of mice compare to A1 milk treated mice. A1 milk treated group of mice showed significantly higher TLC mean value compared to control group and A2 treated group. Non-significantly but higher mean value of PCV (%) and RBC was noticed in A2 milk treated group of mice. The mean value of Neutrophils (%) of A1/A2 milk treated group of mice showed non-significant difference with each other. Non-significantly but higher mean value of Lymphocytes (%) was noticed in A2 milk treated group of mice compared to A1 milk treated group. A1 milk treated group of mice showed significantly higher Eosinophils (%) mean value 02.00^{b}±0.60 compared to A2 milk treated group 01.00^{a}±0.85. So A1 milk may cause allergic condition. The mean value of Monocytes (%) of A1/A2 milk treated group of mice showed non-significant difference with each other. A2 milk treated group of mice showed significantly higher Total protein (g/dl) mean value 6.64 ^{b}±2.10 compared to A1 milk treated group 5.75^{a}±2.75. A2 milk treated group of mice showed significantly lower Cholesterol (mg/dl) mean value 105.91^{a}±6.55 compared to A1 milk treated group 138.37^{b}±7.94. A2 milk treated group of mice showed significantly lower Triglyceride (mg/dl) mean value 72.71^{a}±5.35 compared to A1 milk treated group 105.41^{b}±9.20.

Keywords: A1 milk, A2 milk, caseins, whey proteins

Introduction
Milk is regarded as one of the staples of whole world diets due to its high nutritional value. More than 95% of the cow milk proteins are constituted by caseins and whey proteins. Among the caseins, beta casein is the second most abundant protein and has excellent nutritional balance of amino acids. In this context, a type of milk called A2 has recently received attention from the industry. This type of milk, characterized by a difference in an amino acid at position 67 of the β-casein polypeptide chain, releases much smaller amounts of bioactive opioid peptide β-casomorphin 7 upon digestion, which has been linked to harmful effects on human health. A1 /A2 polymorphism leads to a key conformational change in the secondary structure of expressed β-casein protein. Gastrointestinal proteolytic digestion of A1 variant of β-casein (raw/processed milk) leads to generation of bioactive peptide, beta casomorphin 7 (BCM7) Elliott *et al.* (1999) [2]. By the uptake of A1 milk oxidant of low dietary lipoproteins (LDL) and oxidation of LDL form arterial plaque. Epidemiological evidences claim that consumption of beta-casein A1 milk is associated as a risk factor for type-1 diabetes, coronary heart disease, arteriosclerosis, sudden infant death syndrome, autism, schizophrenia etc. Laugesen *et al.* (2003) [3] & Tailford *et al*. (2003) [5]. A broad range of studies from American and European investigations has shown reduction in autistic and schizophrenic symptoms with decrease in A1 milk intake Cade *et al*. (2000) [6] Further, animal trials have also supported the linking of type-1 diabetes to milk exposure in general and A1 beta-casein in particular.

Material and Methods
Haematological Test
The following hematological parameters were carried out as per the procedure described by using Automatic Blood Cell Counter Make Diatron, Model Abacus 380.
1. Haemoglobin (Hb) Concentration (gm%)
2. Packed Cell Volume (PCV) (%)
3. Total Erythrocyte Count (TEC) (Million/ cu.mm)
4. Total Leucocytes Count (TLC) (Thousand/cu.mm)

Differential leucocytes count
The blood smears were prepared on grease free clean slides. Smears were stained by Wright's stain and examined microscopically for differential leukocyte count under oil immersion (100X) magnification.

Biochemical parameters
Total protein
Total protein concentration was estimated in serum using biuret method as described by Tietz (1986) [9] and expressed as g/dl.

Principle
The peptide bonds of protein react with copper II ions in alkaline solution to form blue-violet complex (biuret reaction). Each copper ion complexing with 5 or 6 peptide bonds. Tartarate is added as a stabilizer whilst lodide is used to prevent auto- reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 546nm.

Method
Reaction mixture consists of copper II sulphate (19 mmol/L), potassium sodium tartarate (43 mmol/L), potassium iodide (30 mmol/L) and sodium hydroxide (600 mmol/ L). One ml of reaction mixture was taken and reaction was initiated by addition of 20 µl serum and allowed to incubate for 10 minutes at 37°C. The absorbance of serum was read at 546 nm against reagent blank and compared with the standard protein 6.0 g/dl.

Albumin
Albumin concentration was estimated in serum using modified Daumas method as described by Daumas *et al.* (1972) and expressed as g/dl.

Principle
Albumin binds with Bromocresol green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye converting it to green colour. The colour produced to the concentration of albumin which is read at 630nm.

Method:
Reaction mixture consists of Bromocresol green (0.08 mmol/L), succinate buffer (pH 4.2, 50.0 mmol /L), sodium azide 1.0 g /L. 1.0 ml of reaction mixture was taken and 10 µl of serum was added to initiate reaction 12 and incubated for 1.0 minute at 37°C. Absorbance of standard (3.6 g/dl) ane each sample read at 630nm against reagent blank.

Globulin
The globulin fraction in serum samples was determined by subtracting serum albumin from serum total protein.
Globulin (g/dl) = Total protein – Albumin

Albumin:Globulin Ratio (A/G ratio)
Albumin:Globulin ratio was obtained by dividing serum albumin concentration by serum globulin concentration.

Total cholesterol:
Total cholesterol was determined by CHOD - PAP method as described by Allain *et al.*, (1974) [1] in serum.

Principle

$$\text{Cholesterol ester} \xrightarrow{CE} \text{Cholesterol + Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + 4AAP + \text{Phenol} \xrightarrow{POD} 4H_2O + \text{Quinoneimine}$$

CE: Cholesterol esterase
CHOD: Cholesterol Oxidase
4AAP: 4-Aminoantipyrine

Method
Reaction mixture consists of cholesterol esterase (200 IU /L). cholesterol oxidase (150 IU/L), peroxidase (2000 IU/L), sodium phenolate (20 mmol /L), 4-aminoantipyrine (0.5 mmol /L), and phosphate buffer (pH 6.5, 68 mmol /L). One ml of reaction mixture was taken and reaction was started by adding 20 µl of serum sample and incubated for 10 minutes at 37 °C. Absorbance of each serum and standard was read at 505 nm against blank and compared with the standard cholesterol 200 mg/dl.

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X Concentration of standard (mg/dl)}$$

Triglycerides
Triglyceride was estimated in serum by GPO-TRINDER method described by Mc Gowan *et al.*, (1983) [4].

Principle

$$\text{Triglycerides} + H_2O \xrightarrow{LPL} \text{Glycerol + free fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{GK/Mg^2} \text{Glycerol-3-phosphate} + ADP$$

$$\text{Glycerol -3-Phosphate} + O_2 \xrightarrow{GPO} DAP + H_2O_2$$

LPL: Lipoprotein lipase
GK: Glycerol Kinase
GPO: Glycerol Phosphate Oxidase
DAP: Dihydroxyacetone Phosphate
ATP: Adenosine triphosphate
4-AAP: 4 Aminoaantipyrine
DHBS: 3, 5-Dichloro-2-hydroxybenzene sulfonate

Method
Reaction mixture consists of ATP (2.5 mmol /L), Mg^{2+} (2.5 mmol /L), 4-aminoantipyrine (0.8 mmol /L), 3-5 DHBS (1 mmol /L), peroxidase (2000U/ L), glycerol kinase (550U / L), GPO (8000 U/L), lipoprotein lipase (3500 U/L) and buffer (pH 7.0, 53 mmol/ L). One ml of reaction mixture was taken and reaction was initiated by adding 10 µl of serum sample and incubated for 10 minutes at 37°C. The absorbance standard and each serum was read at 505 nm against blank and compared with the triglycerides standard 200 mg/dl.

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X Concentration of standard (mg/dl)}$$

Result and Discussion

1. Hematological test: CBC

CBC was performed to access the parameters like hemoglobin, TLC, DLC, PCV and RBC. The results are shown in Table no. 01. No relevant reference was found for above findings as it is a novel work.

Table 1: Means of CBC in the blood of mice of A1/A2 group

Parameters	Control group	A1 group	A2 group
Hb (mg%)	14.20±2.05	14.00±1.71	14.50±2.63
TLC (10^3/ cu.mm)	7810^{a}±87.58	14750^{b}±101.95	9050^{a}±75.55
PCV (%)	46.00±7.03	42.00±8.93	43.50±6.34
RBC (10^6)	7.00±2.09	7.00±3.63	7.25±2.78
DLC			
Neutrophils (%)	27.00±4.50	28.00±5.91	28.00±3.43
Lymphocytes (%)	70.00±3.50	69.00±3.20	70.00±2.08
Eosinophils (%)	02.00^{b}±0.75	02.00^{b}±0.60	01.00^{a}±0.85
Monocytes (%)	01.00±0.34	01.00±0.20	01.00±0.28

Means bearing the different superscript differ significantly ($p<0.05$)

1. **Haemoglobin Hb (mg%):** In Comparative study of A2 milk showed non-Significantly higher Haemoglobin Hb (mg%)14.50±2.63 compare to A1 milk 14.00±1.71.
2. **Total leukocyte count (TLC (10^3/ cu.mm):** A1 milk treated group of mice showed significantly higher TLC mean value14750^{b}±101.95 compared to control group7810^{a}±87.58 and A2 treated group 9050^{a}±75.55.
3. **Pack cell Volume PCV (%):** Non-significantly but higher mean value of PCV (%) 43.50±6.34 was noticed in A2 milk treated group of mice.
4. **Red Blood Cell, RBC (10^6):** Non-significantly but higher mean value of RBC 7.25±2.78 was noticed in A2 milk treated group of mice.
5. **Neutrophils (%):** The mean value of Neutrophils (%) of A1/A2 milk treated group of mice showed non-significant difference with each other.
6. **Lymphocytes (%):** Non-significantly but higher mean value of Lymphocytes (%) 70.00±2.08 was noticed in A2 milk treated group of mice compared to A1 milk treated group.
7. **Eosinophils (%):** A1 milk treated group of mice showed significantly higher Eosinophils (%) mean value 02.00^{b}±0.60 compared to A2 milk treated group 01.00^{a}±0.85. So A1 milk may cause allergic condition.
8. **Monocytes (%):** The mean value of Monocytes (%) of A1/A2 milk treated group of mice showed non-significant difference with each other.

2. Biochemical test

Various Biochemical parameters like Total protein, Albumin, Cholesterol and Triglyceride were evaluated and the results are shown in table no. 02. No relevant reference was found for above findings as it is a novel work.

Table 2: Means of Biochemical parameters in the blood of mice of A1/A2 group

Parameters	Control group	A1 group	A2 group
Albumin (g/dl)	2.35±0.11	2.13±0.85	2.62±0.34
Globulin (g/dl)	3.90^{a}±0.15	3.62^{a}±0.67	4.02^{b}±0.97
Total protein (g/dl)	6.25^{b}±3.01	5.75^{a}±2.75	6.64^{b}±2.10
Albumin: Globulin ratio	0.60±0.07	0.58±0.04	0.65±0.06
Cholesterol (mg/dl)	$131.69^{b\pm}$10.12	138.37^{b}±7.94	105.91^{a}±6.55
Triglyceride (mg/dl)	112.22^{b}±7.75	105.41^{b}±9.20	72.71^{a}±5.35

Means bearing the different superscript differ significantly ($p<0.05$)

1. **Albumin (g/dl):** Non-significantly but higher mean value of Albumin (g/dl) 2.62±0.34 was noticed in A2 milk treated group of mice.
2. **Globulin (g/dl:** A2 milk treated group of mice showed significantly higher Globulin (g/dl) mean value 4.02^{b}±0.97compared to A1 milk treated group 3.62^{a}±0.67.
3. **Total protein (g/dl):** A2 milk treated group of mice showed significantly higher Total protein (g/dl) mean value 6.64 ^{b}±2.10 compared to A1 milk treated group 5.75^{a}±2.75.
4. **Albumin Globulin ratio:** Above all three groups showed non-significant difference with each other (Tab. No.02)
5. **Cholesterol (mg/dl):** A2 milk treated group of mice showed significantly lower Cholesterol (mg/dl) mean value 105.91^{a}±6.55 compared to A1 milk treated group 138.37^{b}±7.94.
6. **Triglyceride (mg/dl):** A2 milk treated group of mice showed significantly lower Triglyceride (mg/dl) mean value 72.71^{a}±5.35 compared to A1 milk treated group 105.41^{b}±9.20.

Conclusion

Recently, a relationship between disease risk and consumption of a specific bovine β-casein fraction with either A1 or A2 genetic variants has been identified and Various parameters of Hematological and Biochemical test showed that the better effect of A2 milk on the health whereas the parameters like Triglyceride (mg/dl), Cholesterol (mg/dl), Total protein (g/dl), Eosinophils (%),TLC (10^3/ cu.mm) and PCV (%) evidences claim that consumption of beta-casein A1 milk is associated as a risk factor for health.

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