Changes in Immunological, Inflammatory, and Biochemical Markers Following Donkey Milk Consumption: A Pilot Prospective Single-Group Before–After Study

Maria Ivanova Kireva-Komitova[1] MD, Tanya Borisova Beleva[1] MD, Aleksandar Ivanov Aleksandrov[1] MD, Valentina Taneva Koleva-Hadzhieva MD[1], Anna Dimitrova Dimitrova[2*]

[1] Hadji Dimitar Multiprofile Hospital for Active Treatment Ltd., Sliven, Bulgaria
[2] Department of Material Science and Technology, Technical University of Sofia, 8 Kliment Ohridski Blvd., 1000 Sofia, Bulgaria
* *Corresponding author* a_dimitrova@tu-sofia.bg

Abstract **- This study aimed to characterize changes in serum immunoglobulins (IgA, IgM, IgE, and IgG), C-reactive protein (CRP), and magnesium (Mg) during two successive courses of donkey milk consumption. A pilot prospective, single-group before–after study enrolled 90 participants assessed at four time points. The first course consisted of five days of freeze-dried donkey milk; the second consisted of ten days of sequentially administered pasteurized and freeze-dried milk. Laboratory measurements were obtained before and 20 days after each course. Within-participant differences were analyzed using the Wilcoxon signed-rank test with Holm adjustment across six markers; exploratory subgroup comparisons used the Kruskal–Wallis and Mann–Whitney U tests. After the first course, no marker reached statistical significance following adjustment. After the second course, significant decreases were reported for all four immunoglobulins and CRP, alongside an increase in Mg. Changes did not differ significantly by age group or recorded chronic disease status. These findings describe temporal associations in laboratory measurements. In the absence of a control group, they neither establish a causal effect of donkey milk nor demonstrate clinical benefit.**

Keywords**—donkey milk, immunoglobulins, inflammation, magnesium, prospective study.**

1. INTRODUCTION

Donkey milk has adducted interest as a food supplement with a distinctive protein, lipid, and mineral composition and a range of biologically active constituents, notably lysozyme and lactoferrin [1]–[4]. Published studies have examined its nutritional value, antimicrobial properties, and processing and preservation technologies. Nevertheless, the presence of compounds with potential biological activity does not, in itself, establish beneficial changes in laboratory markers or clinical efficacy in humans.

Methodologically, assessing the hypothesized biological effects of any substance in humans necessitates a clear separation between its chemical profile, laboratory-proven mechanisms, and actual clinical results. While data regarding components like lactoferrin and lysozyme point to a certain biological potential, they are insufficient for forecasting how systemic immunoglobulin levels will fluctuate. This clear divergence between the properties of a product and the physiological response of the host highlights why continuous laboratory and clinical monitoring remains essential [1]–[4].

Human clinical trials have target highly diverse patient groups and therapeutic goals. These include evaluating dietary tolerance in pediatric cohorts with cow's milk allergies, developing donkey milk alternatives for targeted nutritional support, and conducting exploratory research within specific clinical environments. However, comparing these outcomes directly is heavily restricted by discrepancies in trial methodologies and product compositions. Specifically, there is a distinct lack of long-term data that simultaneously tracks blood immunoglobulins, inflammatory parameters, and metabolic markers over consecutive periods of dietary use [5]–[8].

Interpreting existing research requires a careful distinction between the varying hypotheses tested by different authors. Investigations into pediatric milk allergies assess whether a substitute food is well tolerated, whereas clinical trials on dietary supplements or adjunctive treatments feature distinct target groups, co-therapies, and outcomes. For this reason, their conclusions do not provide a direct basis for confirming alterations in circulating IgA, IgM, IgE, or IgG concentrations within the current cohort [5]–[8].

Immunoglobulins IgA, IgM, IgE, and IgG represent different aspects of humoral immunity, and their concentrations are influenced by numerous physiological and pathological factors. C-reactive protein (CRP) is a nonspecific laboratory marker of inflammation, whereas magnesium (Mg) participates in enzymatic reactions, energy metabolism, and neuromuscular function. Measuring these markers together provides a broader description of laboratory dynamics but cannot substitute for clinical assessment or independently establish the efficacy of the consumed product.

The aim of this pilot prospective, single-group before–after study was to describe changes in IgA, IgM, IgE, IgG, CRP, and Mg in 90 participants during two successive courses of donkey milk consumption. The principal analytical focus was the within-participant comparison of measurements before and after each course. Exploratory analyses also examined whether the magnitude of change differed by age category or the presence of a recorded chronic disease. The observational design is suitable for generating hypotheses but cannot establish causality.

2. MATERIALS AND METHODS

A. Study design

A prospective, single-group before–after study was conducted at Hadji Dimitar Multiprofile Hospital for Active Treatment Ltd., Sliven, Bulgaria, under project BG16RFPR001-1.001-0507-C01, 'Development of an Innovative Donkey Milk-Based Food Supplement'. The analysis included 90 participants identified in the anonymized analytical dataset by codes U001–U090. Two successive courses were undertaken, with laboratory assessments before and after each. No randomization, placebo, or parallel control group was employed. Accordingly, the comparisons describe changes between specified time points without isolating the influence of the product from other individual or temporal factors.

The unit of observation was the individual participant; the unit of the primary statistical analysis was the paired set of measurements obtained for that participant within a given course. For each analyte, the pre-course value was compared with its corresponding follow-up value. This approach describes the direction of change and tests the null hypothesis for paired differences, but it does not control for changes in diet, seasonality, infections, concurrent treatments, or other events between assessments.

The four assessment points define two distinct within-course contrasts rather than a controlled longitudinal estimate of treatment efficacy. Each contrast is anchored to its own pre-course measurement, thereby avoiding an assumption that T1 and T2 represent the same physiological state. In the absence of untreated observations over the same intervals, spontaneous fluctuation, regression toward the mean, measurement variability, and time-varying exposures remain plausible explanations for some or all of the changes.

B. Participant characteristics

Participants were classified into three age categories: 12–30 years (n = 15; 16.7%), 31–50 years (n = 34; 37.8%), and over 50 years (n = 41; 45.6%). Fifty-two participants (57.8%) had no recorded chronic disease, whereas 38 (42.2%) had at least one recorded chronic condition (Table 1). Age category and chronic disease status were used for exploratory subgroup comparisons.

The age and chronic-disease categories are descriptive strata, not matched or randomized groups. The broadest age category combines participants with potentially heterogeneous baseline physiology, while the binary chronic-disease indicator does not distinguish diagnosis, severity, duration, or therapy. Consequently, these characteristics can support exploratory comparisons of change but cannot provide adequate control of clinical confounding.

TABLE I
Characteristics of the study population

Characteristic	Number	Proportion (%)
Age 12–30 years	15	16.7
Age 31–50 years	34	37.8
Age >50 years	41	45.6
No recorded chronic disease	52	57.8
Recorded chronic disease	38	42.2
Total participants	90	100

Age category and chronic disease status are two separate classifications of the same 90 participants; percentages are calculated independently within each classification.

C. Consumption regimen

Two successive courses differing in duration and product processing were administered. The first was a five-day course of freeze-dried donkey milk, in an amount equivalent to 100 mL of milk twice daily. The second course lasted ten days: pasteurized donkey milk was consumed for the first five days, followed by freeze-dried donkey milk for the next five days, according to a regimen of 100 mL twice daily. No laboratory assessment was conducted between the two product forms during the second course. Therefore, post-course findings characterize the complete sequential regimen rather than the independent effect of either form.

Differences in duration (five versus ten days) and the order of product forms are important for interpretation. The equivalent volume of starting milk describes the consumption regimen but does not permit quantitative comparison of exposure to individual biologically active constituents.

The specified regimen corresponds to a nominal milk-equivalent intake of 200 mL per day in each course. This equivalence concerns starting milk volume; it should not be interpreted as demonstrated equivalence of protein, bioactive molecule, or mineral exposure across processing methods. Because the second course combines two formulations in a fixed sequence, formulation, cumulative duration, and order are inseparable in the available comparison.

D. Assessment time points

Laboratory markers were evaluated at four time points: T0, before the first course; T1, 20 days after completion of the first course; T2, before the second course; and T3, 20 days after completion of the second course. The primary within-participant comparisons were T0–T1 and T2–T3. T1 and T2 were treated as distinct time points, not interchangeable baseline measurements. The interval between courses was not analyzed as a separate intervention.

The 20-day post-course interval means that the reported endpoints represent laboratory measurements after cessation of consumption, not immediate responses during intake. Changes detected at follow-up may reflect processes occurring during consumption, during the subsequent interval, or both. Without additional intermediate samples, the temporal onset, peak, and persistence of the laboratory differences cannot be established.

E. Laboratory markers

The markers assessed were serum immunoglobulins IgA, IgM, IgE, and IgG, C-reactive protein (CRP), and magnesium (Mg). Results are expressed in g/L for IgA, IgM, and IgG; IU/mL for IgE; mg/L for CRP; and mmol/L for Mg. Each within-participant comparison included only individuals with measurements at both relevant time points. Missing observations were neither replaced with zeros nor statistically imputed. Analyses used the recorded laboratory values without further classification according to reference intervals.

The outcome panel includes markers with distinct biological interpretations: the measured immunoglobulins characterize circulating humoral components, CRP reflects a nonspecific acute-phase response, and serum Mg reflects extracellular mineral status rather than total-body stores. These six measurements should therefore be interpreted as separate laboratory endpoints, not combined into an unvalidated composite measure of immune or metabolic benefit. Differences in assay methodology, specimen handling, analytical precision, or reference intervals could influence between-time-point comparability; such laboratory-specific information is not available in the reported methods.

F. Statistical analysis

Paired observations were analyzed using the two-sided Wilcoxon signed-rank test. Holm's step-down procedure was applied to adjust for multiple testing across the six primary comparisons within each course. Statistical significance was assessed at $\alpha = 0.05$ using the adjusted p-values. Differences in the magnitude of change across the three age categories were evaluated using the Kruskal–Wallis test, whereas comparisons between participants with and without a recorded chronic condition used the Mann–Whitney U test. Subgroup analyses were exploratory: failure to detect a statistically significant difference does not demonstrate equivalence. Results are presented as means, mean changes, and p-values. Medians, interquartile ranges, and confidence intervals were not calculated for the analysis presented.

For each marker and course, the analysis is defined on the set of participants with both the corresponding pre-course and follow-up observation. Let d_i denote the follow-up value minus the pre-course value for participant i. The paired analysis concerns the distribution of these individual differences, not the independence of two cross-sectional samples. The signed-rank procedure ranks the absolute nonzero differences and assesses their signed pattern; its conventional location interpretation depends on the shape of the difference distribution, whereas a general null formulation concerns symmetry around zero. The summaries supplied here do not allow those distributional assumptions or the handling of ties and zero differences to be audited.

The Wilcoxon signed-rank test accounts for the paired structure of the observations and does not require normally distributed individual differences. Holm's procedure controls the family-wise Type I error rate across the six markers within each course. Adjusted statistical significance should not be equated with clinical importance. The magnitude and precision of the observed differences should be considered in light of the available paired measurements and potential confounding factors.

Multiplicity was addressed separately within each six-marker course-specific family. Under Holm's procedure, ordered unadjusted p-values are evaluated sequentially against progressively less stringent thresholds, or equivalently converted to monotone adjusted p-values. This reduces the risk of at least one false-positive result across the six specified marker tests in a course. It does not correct across both course-specific families jointly, and it does not extend to the exploratory age- and chronic-disease subgroup tests. Those subgroup p-values should therefore be treated as descriptive and hypothesis-generating rather than confirmatory evidence of effect modification.

The reported arithmetic means and mean changes are descriptive summaries. The Wilcoxon signed-rank test evaluates paired within-participant differences rather than a difference between the reported group means. Statistical significance was interpreted only for the observed paired measurements; it does not imply a clinically important change.

Arithmetic mean changes quantify the average recorded direction and magnitude in the analyzed pairs, but a statistically significant signed-rank result is not a test that the population mean change differs from zero. In particular, mean values alone cannot reveal skewness, extreme observations, or the proportion of individuals whose measurements increased versus decreased. Reporting the median paired difference, its distribution and a compatible effect-size estimate would improve interpretability, but these statistics cannot be reconstructed reliably from aggregate means and p-values. No additional dispersion measures or confidence intervals have therefore been inferred for the present article.

3. RESULTS

A. Completeness of laboratory data

The number of complete paired observations differed across markers (Table 2). During the first course, complete pairs were available for all four immunoglobulins in 90 participants, but for CRP and Mg in only 20 participants each. During the second course, there were 90 pairs for each immunoglobulin, 87 for CRP, and 86 for Mg. These differences in data availability affect the precision and comparability of estimates, particularly for CRP and Mg.

Pairwise completeness was 100% for all immunoglobulin comparisons, but only 22.2% for CRP and Mg in the first course (20/90); corresponding completeness in the second course was 96.7% for CRP (87/90) and 95.6% for Mg (86/90). Thus, first-course estimates for CRP and Mg describe a substantially smaller observed subset. In the absence of a documented reason for missing measurements, missingness cannot be assumed to be random; the complete-pair results may differ from those that would have been obtained for all 90 participants.

TABLE II
Number of complete paired laboratory measurements

Marker	First course T0–T1	Second course T2–T3
IgA	90	90
IgM	90	90
IgE	90	90
IgG	90	90
CRP	20	87
Mg	20	86

B. Changes following the first course

Following the initial five-day course, mean IgA, IgM, IgG, CRP, and Mg concentrations decreased, whereas IgE increased slightly (Table 3). None of the six changes remained statistically significant after Holm adjustment. The lowest unadjusted p-value was observed for IgG ($p=0.022$), increasing to 0.131 after adjustment. Thus, the available evidence was insufficient to reject the

corresponding null hypotheses for the first-course comparisons. Interpretation of CRP and Mg was further constrained by the small number of complete pairs (n = 20).

TABLE III
Laboratory markers before and after the first course

Marker	n	T0	T1	Δ	p	p (Holm)
IgA (g/L)	90	3.905	3.570	−0.335	0.148	0.620
IgM (g/L)	90	1.891	1.887	−0.004	0.649	1.000
IgE (IU/mL)	90	77.807	80.112	+2.305	0.124	0.620
IgG (g/L)	90	17.941	16.714	−1.227	0.022	0.131
CRP (mg/L)	20	4.365	4.325	−0.040	0.850	1.000
Mg (mmol/L)	20	0.839	0.836	−0.003	0.126	0.620

Note: T0 and T1 are arithmetic means; Δ denotes the mean within-participant change (T1 − T0). p denotes the reported unadjusted p-value; p (Holm) denotes the adjusted p-value reported in the original analysis.

C. Changes following the second course

Following the second, ten-day course, mean values decreased for IgA (2.800 to 2.468), IgM (1.341 to 1.200), IgE (76.603 to 67.703), IgG (12.406 to 11.392), and CRP (5.014 to 4.114), whereas Mg increased (0.815 to 0.868). Holm-adjusted p-values were below 0.05 for all six markers (Table 4), indicating statistically significant changes between T2 and T3 among participants with complete paired observations. This statistical significance does not, by itself, establish the clinical magnitude, desirability, or cause of the observed differences.

TABLE IV
Laboratory markers before and after the second course

Marker	n	T2	T3	Δ	p	p (Holm)
IgA (g/L)	90	2.800	2.468	−0.332	0.000094	0.000471
IgM (g/L)	90	1.341	1.200	−0.141	0.000056	0.000338
IgE (IU/mL)	90	76.603	67.703	−8.901	0.002	0.005
IgG (g/L)	90	12.406	11.392	−1.014	0.000190	0.000760
CRP (mg/L)	87	5.014	4.114	−0.900	0.017	0.017
Mg (mmol/L)	86	0.815	0.868	+0.053	0.005	0.010

Note: T2 and T3 are arithmetic means; Δ denotes the mean within-participant change (T3 − T2). p denotes the reported unadjusted p-value; p (Holm) denotes the adjusted p-value reported in the original analysis. Small discrepancies may arise when differences are calculated from the rounded values displayed.

D. Analysis by age group

Potential differences in the magnitude of change were examined across the three age categories. The Kruskal–Wallis test did not identify statistically significant between-group differences for any of the six markers.

E. Analysis by chronic disease status

To explore the possible influence of concurrent chronic disease, changes were compared between participants with and without a recorded chronic condition. The Mann–Whitney U test did not identify statistically significant between-group differences in the magnitude of change.

TABLE V
Between-group comparisons following the second course

Marker	Kruskal–Wallis p	Mann–Whitney p
IgA	0.547	0.521
IgM	0.292	0.928
IgE	0.868	0.971
IgG	0.275	0.653
CRP	0.156	0.711
Mg	0.772	0.934

Note: Values are unadjusted p-values from the exploratory between-group comparisons.

Exploratory subgroup tests did not identify statistically significant differences in the magnitude of change according to age category or chronic disease status. However, the absence of statistical significance does not demonstrate identical or equivalent biological responses between groups.

4. DISCUSSION

The principal finding was the contrasting pattern of laboratory changes observed after the two courses. None of the six markers remained statistically significant after Holm adjustment following the five-day course, whereas all six showed statistically significant changes following the ten-day course. This contrast does not establish greater efficacy of the second course, because the courses differed in duration, sequence of processed product forms, and completeness of measurements. Values immediately preceding the second course also differed from those recorded after the first: for example, IgA was 3.570 at T1 and 2.800 at T2, and IgG was 16.714 at T1 and 12.406 at T2. Changes during the intervening period cannot be attributed to either course.

The observed contrast between course-specific significance patterns must also be distinguished from a formal comparison of the courses. A significant result in one period and a nonsignificant result in another does not, on its own, demonstrate that the changes differ significantly between periods. Such an inference would require a prespecified direct analysis of course-related differences on comparable paired observations, with consideration of the distinct baselines and inter-course interval. The current findings do not establish a dose-response relationship or comparative superiority of either preparation.

A. Immunoglobulin dynamics

The statistically significant reductions in IgA, IgM, IgE, and IgG following the second course indicate changes in circulating components of humoral immunity. These immunoglobulin classes have distinct physiological roles in host defense. IgA is particularly important in mucosal immune protection; IgM contributes to early humoral immune responses; IgG is a major component of systemic humoral immunity; and IgE participates in certain allergic and antiparasitic immune responses.

The direction of change in these four immunoglobulin classes is insufficient to establish an unequivocal ‘improvement’ in immune status. In an individual, a reduction in a particular immunoglobulin may have different implications depending on laboratory reference intervals, previous infections, immunological diagnoses, and clinical presentation. “Immunostimulation” and

"immunomodulation" cannot be claimed as demonstrated clinical outcomes of this investigation, accordingly. Interpretation should remain confined to the statistically documented laboratory dynamics.

Immunoglobulin concentrations may be influenced by infection, age, chronic inflammatory disease, pharmacological treatment, and individual physiological variation. Published evidence concerning donkey milk proteins, including lysozyme and lactoferrin [1]–[4], supports interest in their biological properties but does not establish a mechanism for the serum changes observed here. In the absence of individual clinical assessments incorporating laboratory reference intervals, the reductions in immunoglobulin concentrations cannot be characterized as beneficial immunomodulation.

Interpretation of serum immunoglobulin concentrations requires consideration of individual reference ranges, clinical status, and concomitant therapy. Therefore, a statistically significant laboratory difference is not, in itself, evidence of improved immune function.

B. C-reactive protein dynamics

CRP is a nonspecific marker whose concentration may vary in association with numerous inflammatory conditions. A statistically significant reduction in mean CRP was observed following the second course. This direction of change is of interest in light of publications discussing the potential anti-inflammatory properties of selected donkey milk constituents [1], [3], [4]. However, the present study cannot determine whether the CRP reduction resulted from donkey milk consumption, the natural course of coexisting conditions, or other factors.

The biological nonspecificity of CRP is central to its interpretation as an outcome measure. Isolated inflammatory or infectious episodes between assessments may alter CRP independently of dietary intake. Without information on clinical symptoms, intercurrent infections, and concomitant treatments, these explanations cannot be distinguished. Therefore, the observed mean reduction of 0.900 mg/L following the second course is a quantitative laboratory finding rather than direct evidence of a therapeutic anti-inflammatory effect.

Only 20 complete CRP pairs were available for the first course, compared with 87 for the second. This substantial difference in sample size complicates direct comparison between periods. Clinical pilot studies have investigated donkey milk as an adjunct to conventional treatment in specific disease contexts [8]; however, their findings cannot be directly extrapolated to the present cohort because of differences in study design and participant characteristics.

C. Magnesium dynamics

Magnesium is an essential mineral involved in numerous physiological and biochemical processes, including cellular energy metabolism, enzymatic reactions, and normal nervous and muscular function. Mean serum Mg increased significantly following the second course. Although noteworthy, this finding cannot be attributed to a specific constituent of the donkey milk product. Serum Mg concentrations may be affected by dietary intake, comorbidities, renal function, medication use, and other factors.

The increase in mean Mg from 0.815 to 0.868 mmol/L following the second course was relatively small in absolute terms. Statistical significance alone does not establish its clinical relevance. Serum Mg concentration is not a comprehensive measure of total-body magnesium stores; thus, the finding does not demonstrate correction of magnesium deficiency.

D. Relevance of product processing

The second course involved sequential consumption of pasteurized and freeze-dried donkey milk. These processing methods serve different purposes in food preparation and preservation. Pasteurization reduces microbiological risk through controlled heat treatment, whereas freeze-drying removes water under reduced pressure and low-temperature conditions, yielding a product with lower moisture content. Existing literature addresses the technological characteristics and potential applications of different donkey milk preparations [2], [3].

E. Differences by age and chronic disease status

Exploratory subgroup analyses did not identify significant differences in the magnitude of changes by age group or recorded chronic disease status. However, the age categories were broad, and chronic disease was represented by a binary variable. These classifications limit assessment of the influence of specific diagnoses, disease severity, and concurrent treatment.

Between-group tests assess differences in individual changes rather than changes occurring within each subgroup. Accordingly, nonsignificant findings do not establish equivalent responses. In particular, the relatively small number of participants aged 12–30 years (n = 15) may limit power to detect moderate subgroup differences.

F. Scientific significance of the findings

The main contribution of this study is its concurrent description of four classes of circulating immunoglobulins, CRP, and Mg across four time points in a single cohort. Separate analyses of the two courses and explicit reporting of incomplete measurement pairs help distinguish observed laboratory dynamics from unsupported interpretations of clinical benefit. These findings provide a basis for hypothesis generation and the design of controlled follow-up studies using standardized interventions and prespecified clinical endpoints.

5. STUDY LIMITATIONS

The primary limitation is the single-group design without a parallel control group, precluding causal attribution of the observed changes. The courses differed in duration, and the second combined pasteurized and freeze-dried forms sequentially without an intervening laboratory assessment. Quantitative exposure to individual bioactive constituents was not established, and individual-level information on concurrent infections, treatments, and dietary intake was insufficient. These limitations prevent reliable separation of product-related effects from temporal and individual influences.

The reporting of the study also has limitations relevant to reproducibility. Participant recruitment and eligibility criteria, the laboratory assay methods and analytical platforms, and the precise interval between the two courses are not specified in the present report. Accordingly, the

representativeness of the cohort, analytical comparability of the measurements, and possible influences during the interval between courses cannot be fully evaluated from this article alone.

The same considerations limit the assessment of measurement bias. Without assay-level information, it is not possible to establish whether samples across visits were analyzed using identical platforms, calibration conditions, or quality-control procedures. Nor can the article independently verify adherence to the stated intake regimen. These are uncertainties in the evidentiary record rather than proof that the underlying procedures were inadequate; they constrain reproducibility and the strength of causal interpretation.

Only 20 complete paired observations for each of CRP and Mg were available during the first course, compared with 87 and 86, respectively, during the second. Reporting means and p-values without medians, interquartile ranges, confidence intervals, or effect sizes limits assessment of the distribution and precision of within-participant changes. Subgroup analyses were exploratory. Neither clinical endpoints nor a systematic safety analysis were reported.

A further inferential limitation follows from repeated observation of the same cohort across the two courses. Although the paired tests account for dependence within each course, the two sets of tests are not independent experiments. Differences in baseline status, intervening events, completeness and cumulative exposure preclude attributing changes specifically to product duration or processing. The lack of individually contextualized clinical reference intervals additionally prevents classification of the numerical laboratory shifts as beneficial, harmful, or clinically neutral.

6. CONCLUSION

Among 90 participants, statistically significant changes in IgA, IgM, IgE, IgG, CRP, and Mg were observed between T2 and T3 after the second course, following Holm adjustment. No changes remained statistically significant after correction for multiple comparisons following the initial five-day course. Exploratory subgroup analyses did not reveal significant differences in the magnitude of change by age group or chronic disease status.

The scientific value of this study lies in its structured description of six laboratory markers and their temporal dynamics across two courses. The observations may inform the planning of subsequent controlled investigations. In the absence of a control group, the observed changes cannot be interpreted as a demonstrated causal effect or clinical benefit of donkey milk consumption. Future studies should incorporate a control group, standardized characterization of the product, prespecified clinical endpoints, more complete laboratory follow-up, and a documented safety assessment.

ACKNOWLEDGMENTS AND FUNDING

This research was conducted under project BG16RFPR001-1.001-0507-C01, “Development of an Innovative Donkey Milk-Based Food Supplement”, implemented by Hadji Dimitar Multiprofile Hospital for Active Treatment Ltd. under the Competitiveness and Innovation in Enterprises Programme 2021–2027, co-financed by the European Regional Development Fund.

DATA AVAILABILITY

The anonymized participant-level analytical dataset, comprising records coded U001–U090, is retained in the project documentation. Access may be granted subject to appropriate institutional authorization and applicable personal data protection requirements.

ETHICAL CONSIDERATIONS AND INFORMED CONSENT

Informed consent declarations were completed for all 90 participants. Individual consent documents are stored separately from the anonymized analytical dataset in accordance with applicable personal data protection requirements.

The youngest age category spans 12–30 years; the aggregate age data do not establish whether any participants were minors. Consequently, the available summary does not permit separate reporting of age-specific consent procedures, where applicable. No ethics committee approval identifier is reported in this article.

Consent to participate and review by an independent ethics body address related but distinct dimensions of research governance. The documented presence of individual consent declarations should not be presented as evidence of a particular ethics committee decision. Since the summarized age categories cannot determine whether participants younger than 18 years were enrolled, the applicability of guardian permission and age-appropriate participant assent cannot be evaluated from the aggregate age distribution. The available reporting does not identify an ethics committee decision or an approval number; no such authorization is assumed here.

The analytical dataset is described using participant codes rather than direct identifiers, with consent documents retained separately. Pseudonymized or coded health information nevertheless remains subject to applicable confidentiality and data-protection safeguards. The stated conditional availability of participant-level data does not constitute open access and does not override institutional authorization or participant privacy restrictions. These measures delimit the reproducibility of the analysis while protecting participant information.

REFERENCES

[1] M. Z. Khan et al., “Is there sufficient evidence to support the health benefits of including donkey milk in the diet?” Frontiers in Nutrition, vol. 11, art. 1404998, 2024. doi: 10.3389/fnut.2024.1404998.

[2] M. Živkov Baloš, D. Ljubojević Pelić, S. Jakšić, and S. Lazić, “Donkey milk: An overview of its chemical composition and main nutritional properties or human health benefit properties,” Journal of Equine Veterinary Science, vol. 121, art. 104225, 2023. doi: 10.1016/j.jevs.2023.104225.

[3] M. Martini, I. Altomonte, D. Tricò, R. Lapenta, and F. Salari, “Current knowledge on functionality and potential therapeutic uses of donkey milk,” Animals, vol. 11, no. 5, art. 1382, 2021. doi: 10.3390/ani11051382.

[4] M. P. Singh et al., “Donkey milk as a non-bovine alternative: A review of its nutri-functional properties, applications, and challenges,” Journal of Food Science and Technology, vol. 61, no. 9, pp. 1652–1661, 2024. doi: 10.1007/s13197-024-05939-9.

[5] G. Monti et al., “Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: An in vivo and in vitro

study," Pediatric Allergy and Immunology, vol. 18, no. 3, pp. 258–264, 2007. doi: 10.1111/j.1399-3038.2007.00521.x.

[6] S. Vincenzetti et al., "Hypoallergenic properties of donkey's milk: A preliminary study," Veterinaria Italiana, vol. 50, no. 2, pp. 99–107, 2014. doi: 10.12834/VetIt.219.125.5.

[7] C. Peila et al., "The use of a novel donkey milk-derived human milk fortifier in the neonatal period had no effect on the frequency of allergic manifestations during the first years of life: The FORTILAT Trial follow-up," Breastfeeding Medicine, vol. 19, no. 3, pp. 223–227, 2024. doi: 10.1089/bfm.2023.0320.

[8] V. Kolarov et al., "Exploring the potential of donkey milk in enhancing pneumonia treatment outcomes - Pilot study," Antibiotics, vol. 15, no. 2, art. 121, 2026. doi: 10.3390/antibiotics15020121.