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Integrated immune and protein profiles predict growth in preweaning lambs independent of feeding regimen and maternal oxytocin administration

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Abstract

Early-life nutrition and maternal management are important determinants of neonatal physiological development in ruminants. This study evaluated the effects of alternative feeding strategies and maternal oxytocin administration on growth, hematological traits, and immune-related biomarkers in preweaning lambs. A total of 96 newborn lambs were followed for 60 days under four management regimens: natural suckling (NS), colostrum followed by milk replacer feeding (MC+MR), early separation after colostrum intake with exclusive milk replacer feeding (MR), and natural suckling with maternal oxytocin administration (NS+OT). Body weight, serum protein fractions, immunoglobulin G (IgG), and hematological parameters were measured longitudinally. Data were analyzed using linear mixed-effects models. Principal component analysis was used to summarize the biomarker dataset, and the first principal component (PC1) was subsequently evaluated as an integrated physiological predictor of body weight. Body weight and serum IgG changed significantly with age across all groups ($P < 0.001$), whereas no significant main effects of feeding regimen or oxytocin treatment were detected for the primary growth outcome. PC1, representing a coordinated immune-protein and inflammatory profile, was positively associated with body weight. These findings suggest that preweaning lamb growth is more strongly associated with intrinsic physiological status than with the specific management interventions examined under the conditions of this study.

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Introduction

The preweaning period is a critical window for the physiological and immunological programming of lambs. Hematological and biochemical profiles serve as essential diagnostic tools, providing objective data on immune status (1) and systemic health (2). Because physiological abnormalities are often preceded by deviations from established hemato-biochemical baselines (3), these parameters are invaluable for evaluating the efficacy of nutritional interventions and dietary supplements (4,5). Furthermore, it is well-established that age-dependent maturation significantly influences blood metabolite concentrations in neonates (6).

A primary challenge in lamb management is the inherent vulnerability of the neonatal immune system. Due to the synepitheliochorial placental architecture in ruminants, maternal antibodies cannot be transferred to the fetus during gestation, rendering lambs immunologically naive at birth (7,8).

Consequently, lambs rely entirely on the intestinal absorption of colostral immunoglobulins during a transient period of high intestinal permeability. During this phase, the neonatal gut is non-selective, allowing for the potential absorption of both protective IgG and environmental pathogens. High mortality rates typically peak during the first month of life; if these underlying immunological deficits are not addressed, they can result in permanent impairments to lifetime productivity (9).

In regions like Bangladesh, local sheep breeds often exhibit limited lactational performance (10,11), leading to stunted growth trajectories and high mortality rates—often exacerbated by *Haemonchosis*, diarrhea, and pneumonia (12,13). In cases of maternal rejection, abandonment, or agalactia, lambs must transition to milk replacers (MR) for survival (14). While these strategies are widely practiced, their combined effects on the internal physiological and immunological status of lambs remain insufficiently understood.

Alternatively, maternal management may involve the use of exogenous oxytocin to enhance milk let-down. Oxytocin stimulates the contraction of myoepithelial cells within the mammary gland, facilitating the release of alveolar milk (15). Previous studies have suggested that oxytocin treatment can improve lactation length, persistence, and the nutritional density of milk (16-19). However, the downstream systemic effects of such maternal hormonal interventions on the lamb's internal biochemical environment remain poorly understood.

Traditional univariate approaches may not fully capture the complex interactions among metabolic, immune, and hematological parameters. Multivariate techniques such as Principal Component Analysis (PCA) provide a robust framework to integrate these variables and identify composite physiological patterns associated with growth (20–23).

We hypothesized that preweaning lamb growth would be more strongly associated with integrated immune-protein and hematological profiles than with external management interventions such as feeding regimen or maternal oxytocin administration. Therefore, the objectives of this study were to evaluate the effects of different feeding strategies and maternal oxytocin administration on growth and physiological status in preweaning lambs, and to determine whether multivariate physiological profiles derived from principal component analysis could explain variation in body weight beyond treatment effects. Unlike previous studies that emphasized individual biomarkers or single management interventions, the present study used an integrated multivariate approach to identify physiological correlates of early growth.

Materials and Methods

Ethical approval

This research was reviewed and approved by the Animal Welfare and Experimentation Ethics Committee (AWEEC), Bangladesh Agricultural University, Mymensingh, Bangladesh, under Ethical Approval Recommendations Number: AWEEC/BAU/2025(2)/42(a), Date: 20-12-2024.

Study area and experimental period

The study was conducted at the Sheep Research Farm (SRF) and Reproduction Laboratory, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh (24.73°N, 90.44°E), from mid-June to early September 2025. During the study period, ambient temperatures ranged from 16.46 to 29.13°C, with cumulative rainfall between 340 and 530 mm.

Animals and experimental design

A total of 96 newborn crossbred lambs (1/2 Dorper × 1/2 Jamuna River Basin) were enrolled immediately after birth. To ensure adequate passive transfer of immunity, all lambs received maternal colostrum within the first 6 hours of life under supervised suckling or assisted feeding.

Following colostrum intake, lambs were randomly allocated to four experimental groups (n = 24 per group): Group A, natural suckling with the dam throughout the preweaning period; Group B, maternal colostrum followed by separation and rearing on milk replacer; Group C, early separation after colostrum intake (within 12 h of birth) followed by exclusive milk replacer feeding; and Group D, natural suckling with maternal oxytocin administration to facilitate milk let-down. Randomization was performed at birth to minimize selection bias.

Milk replacer feeding protocol

The milk replacer used was MaxCare Lamb & Kid Milk Replacer (Maxu Animal Health, Australia), containing approximately 25% crude protein, 27% fat, 0.08 mg/kg vitamin B12, 11,000 IU/kg vitamin A, 3,200 IU/kg vitamin D3, 200 mg/kg vitamin E, 1 mg/kg folic acid, 0.3 mg/kg selenium, and 65 mg/kg zinc. The replacer was prepared by dissolving 200 g of powder per liter of warm water (38–40 °C). Each lamb received approximately 67.5 g of powder per day in three equal feedings via nipple bottles. Feeding volumes were adjusted according to body weight and appetite, and intake was monitored daily.

For lambs under natural suckling (Groups A and D), milk intake was not directly measured but was indirectly assessed through body weight gain and general health status.

Oxytocin injection

Oxytocin (2 IU; Otocin® Vet Injection, 10 IU/mL; Shinil Pharma) was administered intramuscularly to dams in Group D immediately prior to suckling (twice daily) to facilitate milk let-down, following established physiological protocols (18).

Given the short half-life of oxytocin (minutes), administration timing was synchronized with suckling events to ensure effective alveolar milk ejection. This approach was adopted to mimic practical field conditions where oxytocin is used to assist milk release without inducing prolonged systemic hormonal effects.

Blood sampling and laboratory analyses

Blood samples were collected via jugular venipuncture from each lamb at 3, 15, 30, 45, and 60 days of age. Samples were collected into K2EDTA vacuum tubes (CURE Medical Tech Co., Ltd., USA) for hematological analysis and plain tubes were used for serum separation.

Serum was obtained by centrifugation at 3000 rpm for 15 minutes at room temperature and stored at -20°C until analysis.

Serum immunoglobulin G (IgG) concentrations were determined using a commercial 96-well ELISA kit (Thermo Fisher Scientific, Australia), following the manufacturer's instructions. Total protein (TP) and albumin (ALB) concentrations were measured using biuret and bromocresol green colorimetric methods, respectively, employing Cromatest reagents (Linear Chemicals S.L.U., Spain).

Hematological parameters, including packed cell volume (PCV), hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), neutrophil-to-lymphocyte ratio (NLR), platelet count (PLT), and platelet-to-lymphocyte ratio (PLR), were analyzed using a Zybio Z4 VET automated hematology analyzer (Zybio Inc., China).

Growth performance measurements

Body weight (BW) of lambs was measured on the same days as blood sampling using a calibrated digital scale. Growth rate was calculated at 15-day intervals based on changes in BW throughout. Body weight (BW) was recorded at birth and subsequently at 3, 15, 30, 45, and 60 days using a calibrated digital scale. Key biological covariates were recorded, including birth weight, sex, litter size (single vs. twin) and dam parity (1–4). These variables were considered during statistical modeling to control for potential confounding effects.

Statistical analysis

Data were analyzed using linear mixed-effects models (LMM) implemented in the lme4 package in R (version 4.5.1) (24). The model included treatment group, sampling day, the interaction between treatment and day, birth weight, litter size, and parity as fixed effects, while lamb ID was included as a random effect to account for repeated measures. Residual normality and homoscedasticity were assessed using Q-Q plots and residual-versus-fitted plots, and post-hoc comparisons were performed using Tukey-adjusted estimated marginal means.

Multivariate analysis and growth prediction

To address multicollinearity among serum protein and hematological variables and to reduce data dimensionality, Principal Component Analysis (PCA) was performed using the prcomp function. Prior to PCA, all biomarkers were standardized (z-scores; mean = 0, SD = 1) to account for differences in measurement scale.

The number of retained components was determined using the Kaiser criterion (eigenvalues > 1) and visual inspection of scree plots. The first principal component (PC1), which explained the largest proportion of total variance, was interpreted as a composite immune–protein and hematological profile and was retained for downstream modeling.

PC1 scores were subsequently incorporated as a continuous covariate in a secondary linear mixed-effects model of body weight, alongside treatment, sampling day, and their interaction. This approach allowed growth trajectories to be estimated at a standardized physiological state, thereby isolating treatment-related effects while adjusting for underlying biological variation. Principal component loadings with absolute values ≥ 0.50 were used to aid biological interpretation of PC1. Multivariate visualizations, including scree plots, loading plots, and contribution plots, were generated using the "factoextra" and "ggplot2" packages (25,26).

Results

Growth trajectories in preweaning lambs

Lamb body weight (BW) increased progressively over the 60-day preweaning period in all treatment groups (Table 1). Mixed-effects modeling confirmed a significant effect of age on BW ($P < 0.001$), but neither the type of feeding intervention ($P = 0.573$) nor the interaction between treatment and time ($P = 0.418$) significantly influenced growth.

Birth weight, litter size, and dam parity were included as covariates in the model. Among these, birth weight showed a significant positive association with subsequent growth ($P < 0.05$), while lambs born as singles tended to have numerically higher BW compared to twins, although this effect was not statistically significant ($P > 0.05$). Parity did not significantly influence growth trajectories.

Integrated immune–protein and hematological profiles

The first principal component (PC1; eigenvalue = 2.13) explained 21.3% of the total variance and captured

a coordinated immune–protein and inflammatory profile (Figure 1). PC1 was characterized by positive loadings for total protein, albumin, and IgG, and negative loadings for platelet count and platelet-to-lymphocyte ratio, indicating that higher protein and immunoglobulin status together with lower inflammatory burden contributed to higher PC1 scores (Figure 2A). This pattern indicates that higher circulating protein and immunoglobulin levels, coupled with lower inflammatory indicators, define a coordinated physiological state associated with improved systemic balance.

protein status and lower inflammatory load—consistently exhibited greater growth.

Importantly, the distribution of PC1 scores overlapped substantially across treatment groups (Figure 3), indicating that this physiological advantage was independent of feeding regimen or oxytocin intervention**,** and likely reflects inherent biological variation among lambs.

Table 1: Body weight (BW, kg) of lambs under different treatments over time (mean ± SD). Row-wise P-values: one-way ANOVA; pooled P-values: mixed-effects model with lamb ID as random effect

Time (days)	Control	Colostrum & Milk Replacer	Milk Replacer Only	Oxytocin Dam	*P*-value
3	2.77 ± 0.97	2.60 ± 0.76	2.67 ± 0.77	2.61 ± 0.68	0.909
15	3.62 ± 1.02	3.41 ± 0.81	3.50 ± 0.86	3.41 ± 0.94	0.866
30	4.14 ± 1.04	3.85 ± 0.64	4.53 ± 0.88	3.88 ± 0.87	0.059
45	5.04 ± 0.94	4.63 ± 0.74	4.91 ± 1.10	4.72 ± 1.21	0.569
60	5.41 ± 1.00	5.24 ± 0.88	4.96 ± 1.24	5.23 ± 1.15	0.609

Row-wise P-values are from one-way ANOVA at each time point; pooled p-values reflect within-group time effects from a mixed-effects model with lamb ID as a random effect.

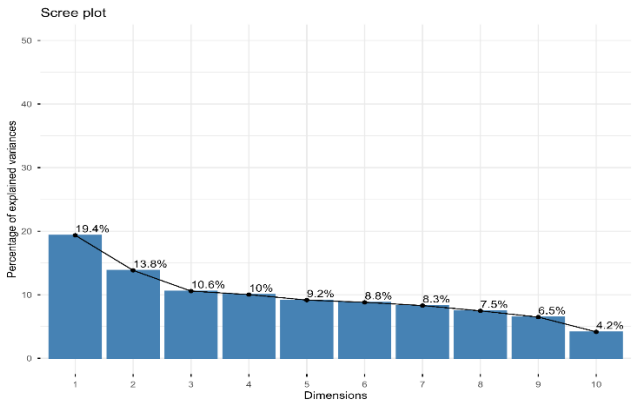

Figure 1: Proportion of total variance explained by each principal component. The labels indicate the percentage of variance captured, highlighting the relative contribution of each component to the overall data structure.

Physiological profile predicts growth

The composite PC1 score was positively associated with body weight ($F_{1,345.82} = 5.96$, $P = 0.015$; Figure 2B). Lambs with higher PC1 scores—reflecting stronger immune–

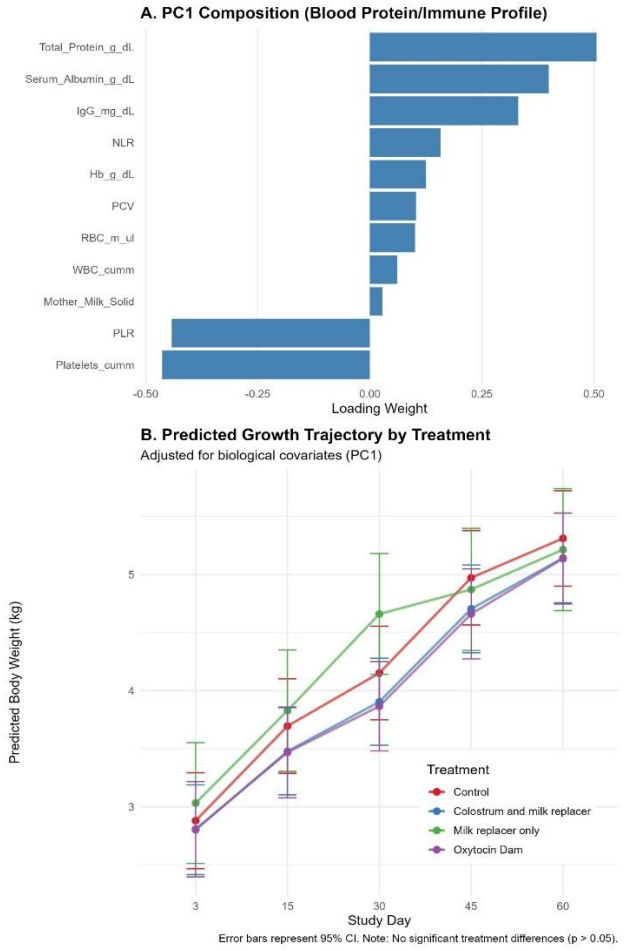

Figure 2: Principal component analysis and body weight trajectories where panel A shows PCA loadings for 11 biological variables and PC1 has strong positive loadings for total protein, serum albumin, and immunoglobulin G and panel B shows body weight across study days by treatment group with points indicating observed values, lines indicating fitted means and error bars indicating standard error.

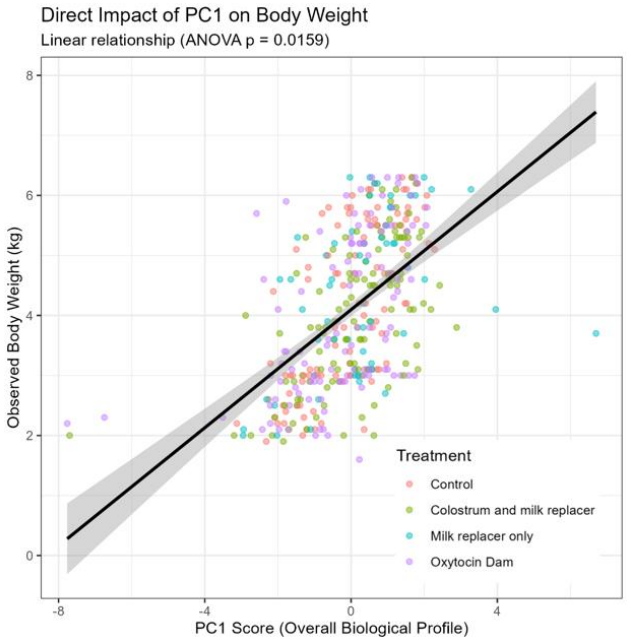

Figure 3: Relationship between the composite physiological score (PC1) and growth performance in preweaning lambs.

Hematological, biochemical, and inflammatory trends

Hematological and biochemical parameters remained within physiological reference ranges throughout the study period (Supplementary File 3). Age had a significant effect on several parameters, including increases in PCV ($P < 0.001$) and hemoglobin ($P = 0.026$), reflecting normal hematopoietic maturation.

Serum IgG concentrations showed a characteristic temporal pattern, with an initial decline followed by a gradual increase, consistent with the transition from passive to active immunity. No significant treatment effects were observed for most hematological or biochemical variables ($P > 0.05$), indicating that feeding strategy and oxytocin administration did not disrupt systemic physiological homeostasis **(**Figure 2B).

Discussion

This study demonstrates that preweaning growth in lambs is primarily driven by intrinsic physiological and immunological status rather than external management interventions such as feeding regimen or maternal oxytocin administration. Although all lambs exhibited normal growth and age-related increases in serum proteins and IgG, no significant differences were observed among treatment groups, indicating that alternative feeding strategies and oxytocin-assisted milk let-down did not confer measurable growth advantages under the conditions of this study.

A key finding was the significant association between the composite physiological index (PC1) and body weight. PC1 integrated markers of protein metabolism (total protein and albumin), immune competence (IgG), and inflammatory status (platelets and PLR), suggesting that growth is closely linked to a coordinated balance between nutritional status, immune function, and systemic inflammation. This aligns with previous studies in sheep and other livestock species, where multivariate approaches have demonstrated that composite physiological indices better reflect growth potential than individual biomarkers (20–23). Lambs with higher PC1 scores likely benefited from more efficient nutrient utilization, improved immune readiness, and reduced inflammatory burden, collectively supporting enhanced growth performance.

The absence of significant treatment effects on growth highlights the importance of baseline biological variation. Birth weight was positively associated with subsequent body weight, supporting previous evidence that prenatal development and intrauterine conditions shape postnatal performance (27). Although litter size and dam parity were not statistically significant in the present dataset, these factors remain biologically relevant and may contribute to the physiological variation captured by the principal component analysis (3). Because delivery type and maternal productivity were not recorded, their possible influence should also be considered when interpreting the results.

The temporal pattern of IgG observed in this study, characterized by an early decline followed by a gradual increase, is broadly consistent with the transition from maternally derived passive immunity to endogenous immunoglobulin production in neonatal ruminants (7,28-30). This pattern suggests that colostrum intake was achieved at the group level, although formal classification of passive transfer status was not possible because colostrum quality and threshold-based diagnostic criteria were not directly assessed. The relatively stable total protein and albumin concentrations further suggest maintenance of general nutritional and physiological status across groups (31).

The lack of significant effects of milk replacer feeding on growth performance suggests that, when properly formulated and managed, milk replacers can adequately support early-life development without compromising physiological health. Similar findings have been reported in studies where milk replacers did not significantly enhance growth when maternal milk supply was sufficient (32,33). Likewise, oxytocin administration, when applied in a physiologically appropriate manner, appears to facilitate milk ejection without exerting systemic effects on lamb growth or hematological parameters. This observation is consistent with the known short-term action of oxytocin, which primarily influences milk let-down rather than long-term metabolic processes (34–36).

From a practical perspective, these findings suggest that management interventions such as milk replacer feeding or oxytocin use may be less critical than ensuring optimal baseline physiological status. This shifts the focus toward improving prenatal care, ewe nutrition, colostrum management, and genetic selection to enhance neonatal resilience and growth potential. Previous studies have also emphasized the importance of maternal factors and early-life management in shaping hematological and growth outcomes (3,37,38).

Several limitations should be acknowledged. First, the study was conducted within a single seasonal window and under one management setting, which may limit the generalizability of the findings. Second, milk intake was not directly measured in the naturally suckling groups, reducing precision in the comparison of nutritional exposure among treatments. Third, colostrum quality, oxidative stress markers, endocrine indicators, delivery type, and maternal productivity were not measured directly. Future studies that include these variables, together with direct milk-intake measurements and validation across multiple environments, would provide a more complete understanding of the physiological determinants of preweaning growth.

Overall, this study highlights the importance of integrated physiological profiling in understanding growth dynamics in preweaning lambs and demonstrates that intrinsic biological factors play a more decisive role than external feeding interventions under well-managed conditions.

Conclusion

Preweaning lamb growth was more strongly associated with integrated physiological status than with feeding regimen or maternal oxytocin administration under the conditions of this study. Body weight changed significantly with age, whereas the management interventions evaluated here did not produce significant main effects on the primary growth outcome. Multivariate analysis indicated that lambs with higher integrated immune-protein profiles tended to have greater body weight. From a practical standpoint, these findings suggest that improving prenatal care, ewe nutrition, and colostrum management may be more beneficial for early lamb performance than relying on postnatal intervention alone. However, the findings should be interpreted considering the study limitations, particularly the lack of direct milk-intake and colostrum-quality measurements and the restricted environmental scope of the study.

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المناعة المتكاملة و ملامح البروتين المتوقعة في نمو الحملان قبل الفطام المستقلة عن نظام التغذية وإعطاء الأوكسيتوسين للأمهات

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الخلاصة

تعتبر التغذية في وقت مبكر من الحياة وإدارة الأمهات من المحددات المهمة للتطور الفسيولوجي لحديثي الولادة في المجترات. قيمت هذه الدراسة آثار استراتيجيات التغذية البديلة وإعطاء الأوكسيتوسين للأم على النمو، الصفات الدموية، والمؤشرات الحيوية المرتبطة بالمناعة في الحملان قبل الفطام. تمت متابعة ما مجموعه ٩٦ من الحملان حديثي الولادة لمدة ٦٠ يوما تحت أربعة أنظمة إدارة: الرضاعة الطبيعية (نس)، اللبأ تليها تغذية بديل الحليب (ماك+مر)، والفصل المبكر بعد تناول اللبأ مع تغذية بديل الحليب الحصري (مر)، والرضاعة الطبيعية مع إعطاء الأوكسيتوسين للأمهات (نس+أوت). تم قياس المعايير افقيا" التي شملت: وزن الجسم، بروتين المصل، الغلوبولين المناعي (IgG)، والمعايير الدموية. تم تحليل البيانات باستخدام نماذج التأثيرات المختلطة الخطية. تم استخدام تحليل المكون الرئيسي لتلخيص مجموعة بيانات العلامات الحيوية، وتم تقييم المكون الرئيسي الأول لاحقا كمؤشر فسيولوجي متكامل لوزن الجسم. تغير وزن الجسم ومستوى IgG في المصل بشكل معنوي (إ < ٠,٠٠١) مع التقدم في السن في جميع الفئات، في حين لم يتم الكشف عن آثار رئيسية كبيرة من نظام التغذية أو العلاج بالأوكسيتوسين لنتائج النمو الأولية. المكون الرئيسي الأول، الذي يمثل بروتينا مناعيا منسقا وملف تعريف التهابي، كان مرتبطا بشكل إيجابي بوزن الجسم. تشير هذه النتائج إلى أن نمو الحملان قبل الفطام يرتبط ارتباطا وثيقا بالحالة الفسيولوجية الجوهرية أكثر من التدخلات الإدارية المحددة التي تم فحصها في ظل ظروف هذه الدراسة.