# Evaluation of Whey Protein Wafer Supplementation on The Performance and Blood Profile of Weaning Sheep

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**Abstract.** The provision of feeder sheep is a problem for farmers due to a high mortality rate among pre- and post-weaned lambs. Whey protein contains amino acids, glucose, and immunoglobulin which facilitate immunity enhancement and body weight gain. This study aimed to evaluate the effectiveness of supplementing whey protein wafer on the performance and profile of weaning sheep. A total of 12 ewe were randomly allotted to 3 treatments: P0 (non-supplemented feed); P1 (1.5% whey protein wafer supplement; and P2 (3% whey protein wafer supplement). Each treatment was replicated four times. The results showed that the whey protein wafer supplement treatment significantly (P<0.05) affected erythrocytes, leukocytes, monocytes, glucose, and cholesterol. Wafer with 3% whey protein supplement showed the best average daily gain, a normal range of blood profile, and a good performance compared to other treatments. The normal blood profiles reflect the sound health of the sheep.

Keywords: Blood Profile, Industry By-product, Performance, Wafer, Whey protein.

**Abstrak.** Penyediaan domba bakalan menjadi masalah bagi para peternak karena tingginya angka kematian pada domba sebelum dan sesudah sapih. Protein whey mengandung asam amino, glukosa, dan imunoglobulin yang memfasilitasi peningkatan imunitas dan pertambahan berat badan. Penelitian ini bertujuan untuk mengevaluasi efektivitas suplementasi wafer protein whey terhadap performa dan profil domba sapih. Sebanyak 12 ekor induk domba dibagi secara acak ke dalam 3 perlakuan: P0 (pakan tanpa suplementasi); P1 (suplemen wafer protein whey 1,5%; dan P2 (suplemen wafer protein whey 3%). Setiap perlakuan diulang empat kali. Hasil penelitian menunjukkan bahwa perlakuan suplementasi wafer protein whey secara nyata (P<0,05) mempengaruhi eritrosit, leukosit, monosit, glukosa, dan kolesterol. Wafer dengan suplementasi protein whey 3% menunjukkan pertambahan berat badan harian rata-rata terbaik, rentang profil darah normal, dan performa yang baik dibandingkan dengan perlakuan lainnya. Profil darah yang normal mencerminkan kesehatan domba yang baik.

Kata Kunci: Profil Darah, Hasil Samping Industri, Performa, Wafer, Protein Whey.

## Introduction

Sheep have prolific traits (multiple bearing) and seasonal polyestrous, which enable them to mate throughout the year (Najmuddin and Narsich, 2019). Sheep birth rate is high but lamb mortality rate from pre-weaning to after-weaning can reach 20-50%, causing lack of lamb availability to breeders. After being weaned, sheep enter a transition period which requires balanced nutritional intake to boost growth (Maulana et al., 2020) and improve performance, health, and immunity of weaning sheep (Sholikhah, 2021).

Whey protein, a by-product of cheese processing, is the milk part that remains in curd (cheese) separation after coagulation. The composition of whey protein depends on the type of milk, the produced cheese, the lactation phase, and the processing. Whey protein generally contains 87.4% crude protein, 2.90% ash, 0.24% crude fiber, 0.44% Ca, and 0.2% P (Sang-Bum et al., 2019). The main types of whey protein are β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA), and immunoglobulin (IG). Immunoglobulins are antibodies which protect the immunity of young mammals (Ryan and Walsh, 2016). As a by-product, whey is loaded with nutrients from soluble protein and lactose to minerals, essential amino acids, and complex B vitamins. Whey protein has more branched-chain amino acids (leucine, isoleucine, and valine) and sulfur-containing amino acids (cysteine and methionine) (Eseceli et al., 2021).

Whey has been used in its natural form or powder, incorporated into drinking water or feed for pigs, sheep, and cattle. Dry whey is particularly common for non-ruminant and ruminant feeds. Whey positively affects weight gain, feed efficiency, protein, and fat digestibility, mineral absorption and retention, and nitrogen retention. The high branched-chain essential amino acids in whey facilitates muscle development in sheep (Eseceli et al., 2021).

Whey is highly abundant; every 10 L milk production can produce 1 kg of cheese with 8 to 9 L whey yields. Whey protein can bind water and create emulsions and froth. Since whey is easily damaged, it is important to increase its shelf life using preservation technology, such as feed wafer (Retnani et al 2020). Compared to other forms of feed, feed wafer can better increase nutrient intake, rumen digestibility, acetate, feeding activity, body weight gain, and feed conversion ratio (Karimidzeh et al., 2017). Feed supplement wafers generally contain high protein and energy, but the composition depends on the type of water to make.

Supplementing sheep with whey protein wafer expects to maintain lamb body immunity, as reflected from health status of the sheep (performance and blood profile), leading to a lower rate of lamb mortality. Giving whey protein to sheep as a milk substitute at 1.5% shows an increase in body weight gain and feed efficiency (Kareem et al., 2019). As of today, there has been limited research on whey wafer supplement to weaning sheep. The purpose of this study was to evaluate the effectiveness of whey protein wafer supplementation on the performance and blood profile of weaning sheep.

## Materials and Methods

A total of 12 local Indonesian female sheep with an average 8.25 ± 3.45 kg BW were reared semi-intensively and subjected to a randomized block design study. The sheep were allotted to 3 different treatments: P0 (non-supplemented feed); P1 (1.5% whey protein wafer supplement; and P2 (3% whey protein wafer supplement). The feed was made of tofu dregs, concentrate (palm oil cake, coconut cake, rice bran and others), and Dwarf Elephant Grass (*Pennisetum purpureum CV Mott*). The sheep were fed three times daily in the morning (one whey wafer for each sheep in P1 and P2), afternoon, and evening. Drinking water was provided *ad libitum*. The composition and nutritional content of the feed is presented in Table 1. The sheep were reared for seven weeks then administered orally with albenol-100 to minimize diseases caused by helminth infections.

**Feed processing**

The whey protein wafers were adjusted to meet the daily dry matter requirements for sheep (3.5% x 8 kg = 280 gr). The ingredients were whey, pollard, and whey protein. Wafer manufacturing starts with grinding, mixing, and forming wafers. The three ingredients are mixed and printed using a wafer machine. Whey protein wafer in P1 and P2 weighed 20g, containing 1.5% and 3% dry matter, respectively (see Table 1). The percentage of use of feed ingredients and nutrient content can be seen in Table 1.

**Sampling and measurements**

The sheep performance was measured from daily weight gain, recorded every two weeks. Blood sample (3ml) was drawn from the jugular vein, put into a EDTA tube, and brought to the laboratory in a cold box for blood hematology test on erythrocyte, leukocyte, hematocrit, and leukocyte differentiation (neutrophils, lymphocytes, basophils, and monocytes). Blood metabolite was analyzed to observe the levels of cholesterol, blood glucose, and blood urea nitrogen.

Table 1. Feed ingredients and chemical composition of whey protein wafer supplement

| Component | Treatments | |
|---|---|---|
| | P1 (1.5% whey protein) | P2 (3% whey protein) |
| Composition (%) | | |
| Pollard | 69 | 48 |
| Whey protein | 21 | 42 |
| Molasses | 10 | 10 |
| Total | 100 | 100 |
| Chemical Composition (%): | | |
| Ash | 5.66 | 5.17 |
| Crude protein | 11.87 | 11.57 |
| Ether extract | 2.73 | 2.20 |
| Crude fiber | 10.85 | 7.55 |
| Total digestible nutrient (TDN) | 53.33 | 37.97 |

**Statistical analysis**

The obtained data were subjected to analysis of variance (ANOVA) using SPSS v 25.0. Any significant differences ($p<0.05$) in the treatment groups were analyzed using Duncan's Multiple Range Test (DMRT).

## Results and Discussion

**Blood Hematology**

Blood hematology describes the effect of feeding on the health of livestock. In Table 2, the statistical results of blood hematology of weaning sheep across treatments were not significantly different ($P>0.05$). Hemoglobin levels in the study ranged from 9.3 to 9.4 % (Table 2), falling within the normal range of according to Nurani et al. (2019), that is 9-15%. It indicates that whey wafer supplement has provided sufficient nutrients and promoted nutrient metabolism for the formation of hemoglobin at normal levels (Maukaling et al., 2024). Hemoglobin levels bind oxygen in the blood; the higher the hemoglobin level, the more exchange of oxygen and carbon dioxide occurs. In contrast, hemoglobin deficiency can inhibit body metabolism (Rabbani et al., 2022).

The hematocrit percentage of weaning sheep in this study ranged from 24.7% to 27% (Table 2). The supplementation of whey protein wafers did not significantly affect the hematocrit of sheep ($P>0.05$) because the hemoglobin levels were not significantly different. P1 (no whey wafer) and P2 (3% whey wafers) showed a normal range of hematocrit by 26-45%, indicating a good health. While low hematocrit reflects unhealthy conditions, higher than normal hematocrit poses harm to the animal's body. The contributing factors to hematocrit value are age, sex, livestock type, and livestock physiological status (Astuti et al., 2022).

The mean erythrocyte level of post-weaning sheep in the study was 8.4 to 11.2 x $10^6$ $mm^{-3}$ (Table 2), falling under the normal range of erythrocytes according to Rahayu et al. (2017), namely 8 to 12 x $10^6$ $mm^{-3}$. It demonstrates that whey protein wafers have significantly affected the sheep's erythrocytes ($P<0.05$), fulfilled the sheep's nutritional needs, and promoted the formation of blood erythrocytes. The formation of erythrocytes in the spinal cord requires iron, vitamin C, folic acid, vitamin B12, and proteins. In the P1 and P2 treatments, erythrocyte level increased due to the added amino acid and protein content. It has been reported that the higher the nutritional content in feed, the higher the erythrocyte levels in the blood (Nahak et al., 2021).

The mean leukocyte count of post-weaning sheep in this study ranged from 8.1 to 9.5 $10^3$ $mm^{-3}$ (Table 2), which falls within the normal range according to Setiawati and Sarjono (2023), namely 4 to 12 x$10^3$ $mm^{-3}$. It demonstrates the significant effect of whey wafer supplement on leukocyte count of sheep ($P<0.05$). The state of a normal leukocyte count indicates that the sheep do not experience specific disorders.

Table 2. Hematological parameters of blood from weaning sheep in various treatments

| Parameters | Treatments | | |
|---|---|---|---|
| | P0 (Control) | P1 (1.5% whey protein) | P2 (3% whey protein) |
| Hemoglobin (%) | 9.4 ± 0.7 [a] | 9.7 ± 0.8 [a] | 9.3 ± 0.5 [a] |
| Hematocrit (%) | 27 ± 1.41[a] | 24.7 ± 0.47 [a] | 26.6 ± 1.06 [a] |
| Erythrocyte (x$10^6$ $mm^{-3}$) | 8.4 ± 0.4[a] | 11.2 ± 0.6[c] | 8.9 ± 0.8[b] |
| Leukocyte (x$10^3$ $mm^{-3}$) | 8.1 ± 0.2[a] | 9.5 ± 0.4[b] | 8.2 ± 0.2[a] |

The formation of leukocytes requires protein intake in the form of amino acids. Whey protein contains amino acids, including leucine, isoleucine, valine, cysteine, and methionine. The treatment in the study showed that whey wafer supplement increased leukocytes, supporting the immune system to fight infections or foreign bodies (Hariyani et al., 2020).

**Leukocyte Differentiation**

Lymphocytes are an important element in the immune system that can respond to antigens in the formation of antibodies. In this study, leukocyte differentiation is related to the sheep's response to whey protein wafer supplementation. Table 3 shows that the average lymphocytes in this study was 59.4–62.1%, which aligned with the range of 52-75% reported by Rahayu et al., 2017. It indicated that the whey wafer did not significantly affect sheep lymphocytes (P > 0.05). Lymphocytosis, or high lymphocyte count in the blood, is due to foreign parasites such as bacteria and viruses that enter the body, or a natural response of lymphocytes as it produces antibodies (Saputro et al., 2016). Lymphocytosis can be caused by virus, bacterial, and parasitic infections. In contrast, lymphopenia is when lower than normal lymphocytes are present in the blood circulation due to stress, physiological activity, age, and nutritional intake (Widhyari et al., 2020).

Neutrophils play the role of phagocytosis: killing foreign organisms, limiting the spread of microorganisms, and fighting infection. The feed treatment showed that whey wafer supplement did not significantly affect sheep neutrophils (P>0.05). The mean level of neutrophils in the study ranged from 29.8 to 31.3% (Tabel 3), which falls under the normal percentage of 30-48%. The percentage of neutrophils decreases and increases as a result of the body's normal response to acute inflammation (Rahmiati et al., 2018). The increase in neutrophils is attributed to inflammation, acute stress, tissue damage, or necrosis. In contrast, decreasing neutrophils is due to malnutrition, such as a deficiency of vitamin B12, folic acid, and copper and viral infections. It can cause a disruptive infection or general white blood cell damage (Setiawan et al., 2022).

Eosinophils play a role in detecting parasitosis, allergies, and other conditions. Eosinophils are abundant in skin tissue, respiratory tract, and digestive tract. This study showed that supplementing whey protein wafer to sheep feed did not significantly affect sheep eosinophils (P > 0.05). The average eosinophils ranged from 5.4 to 6.4% (Table 3), which falls under the normal range of 1 – 8%. Different percentages of eosinophils are attributed to the formation time of eosinophil, which affects the strength of sheep in responding to the entry of pathogenic bacteria (Setiawati and Sarjono, 2023). The contributing factors to eosinophil count are feed, environment, and total leukocytes. Stressful conditions can increase eosinophils as a body defense mechanism against disease agents (Purnomo et al., 2015).

Table 3. Parameters of leukocyte differentiation in released sheep in various treatments

| Parameters | Treatments | | |
|---|---|---|---|
| | P0 (Control) | P1 (Whey protein 1.5%) | P2 (Whey protein 3 %) |
| Lymphocyte (%) | 62.1 ± 3,01 | 59.4 ± 3,81 | 60.7 ± 5.15 |
| Neutrophil (%) | 29.8 ± 1.35 | 31.1 ± 2.77 | 30.3 ± 3,54 |
| Eosinophil (%) | 5.4 ± 0.26 | 5.5 ± 0.41 | 6.4 ± 0.39 |
| Monocyte (%) | 2.9 ± 0.09a | 4.0 ± 0.05c | 3,7 ± 0.22b |
| Basophil (%) | 1 ± 0.04 | 0.9 ± 0.09 | 1 ± 0.05 |
| Ratio N/L | 0.48 ± 9.58a | 0.59 ± 3,49b | 0.57 ± 1.24b |

Different letters in the same row showed significant differences ($p<0.05$)

Monocytes are the initial defense cells that fight against microbial infections after a foreign object enters the body (Santos et al., 2019). The results showed that the average monocyte was 2.9% - 4% (Table 3), which falls within the normal range of 0-4%. It demonstrates that the supplementation significantly affected the monocytes of weaned sheep ($P<0.05$). The highest monocyte level was observed in P1, which could be attributed to the sheep's immune system responding to the administration of whey protein supplement wafers. High monocytes indicate that the body is fighting the entry of foreign substances. The monocyte level in this study showed an increase but within a normal range, thus preventing the sheep from experiencing infection. A decreased level of monocytes can obstruct the bone marrow function, but increased monocytes can reduce body weight and cause impaired vision (Riadi and Akmal, 2021).

Basophils will react with allergy and antigens by releasing the chemical histamine, which can cause inflammation. The treatment showed that supplementary whey protein did not significantly affect the number of basophils in sheep ($P>0.05$). The average basophil in the study was 0.9 - 1% (Table 3), which falls within the normal range of basophil count, namely 0.3 – 3%. The percentage of basophils that increases in the blood makes blood circulation smooth and prevents blood clots that cause disease. The number of basophils that did not increase in the study showed that the livestock were in good health and were not injured. If animals are injured, basophil will release histamine that will trigger the capillaries to enlarge and increase its permeability. Basophil cells contain heparin, histamine, hyaluronic acid, chondroitin sulfate, serotonin, and several chemotactic factors (Setiawati and Sarjono, 2023).

The N/L ratio describes the stress condition and immune system of the sheep. Based on the statistical analysis results, whey protein wafers have significantly affected the N/L ratio in weaning sheep. The average N/L ratio in this study was 0.48 -0.59 (Table 3), which falls within the normal range as reported by Rahayu et al. (2017), namely under 1.6%. The contributing factors to N/L ration are environment, feed, transportation, and stress levels. An increase in N/L ratio in the blood can disrupt the immune system. In this study, wafer supplement increased due to changes in feed addition that remained within the normal range, causing the sheep to maintain their health.

**Blood Metabolites**

Blood metabolism is related to the absorption of nutrients in the blood, where macro-nutrient glucose acts as the main source of energy for the brain. The statistical analysis results showed that whey wafer supplement in this study significantly affected ($P<0.05$) the glucose level of weaning sheep. The average glucose value in the study ranged from 44.6 to 61.4 mg/dl, indicating that sheep fed on control treatment (P0) and 3% whey wafer (P2) had a glucose level below the normal (see Table 4).

Tabel 4. Metabolites percentage of released sheep blood

| Parameters | Treatments | | |
|---|---|---|---|
| | P0 (Control) | P1 (Whey protein 1.5 %) | P2 (Whey protein 3 %) |
| Glucose (mg/dl) | 52.2 ± 1.25[b] | 44.6 ± 1.95[a] | 61.4 ± 1.84[c] |
| Cholesterol (mg/dl) | 33.1 ± 0,7[a] | 44.1 ± 3.1[b] | 51.3 ±1.8[c] |
| Blood urea nitrogen (mg/dl) | 18.2 ± 1.74[b] | 16.3 ± 1.22 | 18.1 ± 1.35 |

Different letters in the same row showed significant differences ($p<0.05$)

According to Lendrawati et al. (2019), the normal range of glucose is 48 – 87 mg/dl. Glucose absorption in P2 treatment was due to the high lactose content in whey. Glucose levels in the blood are controlled by the interactions of breed, age, sex, health status, physiological response, and environmental factors (da Cruzl et al., 2017). A balanced glucose level in the blood is assisted by homeostatically regulating hormones insulin and glucagon (Suharti et al. 2017).

Blood cholesterol is a precursor in the biosynthesis of steroid hormones and bile acids. Based on the statistical analysis, whey wafer supplement significantly ($P<0.05$) affected the cholesterol level of weaning sheep. The average cholesterol value in the study ranged from 33.1 to 51.3% (Table 4). These results are aligned with the normal cholesterol level of weaning sheep, that is 31 – 68.5 mg/dl (Faisal et al., 2017) and lamb cholesterol of 36.80 mg/dl (Sarmin et al., 2021). The contributing factors to the quality of blood cholesterol are feed quality, feed consumption, unsaturated fatty acids intake, age, and sex. The quality of feed affects cholesterol biosynthesis because during metabolism, acetyl CoA is produced as a precursor in cholesterol biosynthesis (Rostini and Zakir, 2017).

Blood urea nitrogen is an indicator of protein status in livestock and identifies feeding problems. The statistical analysis showed that supplementing whey wafer did not significantly affect ($P<0.05$) sheep blood urea. The average blood urea ranged from 16-18% (Table 4), which falls under the normal range of healthy sheep (15-19 mg/dl) as reported by Widyawati et al., (2023). The blood urea nitrogen of sheep offered with 3% whey protein wafer treatment was similar to that of the control group, indicating that the treatment feed was safe for sheep. In contrast, sheep fed on 1.5% whey wafer had lower urea levels than the other treatments. Blood urea nitrogen increases with protein metabolism in the body. Increased blood urea nitrogen can be caused by dehydration or shock in livestock, which limits the release of urea (Widhyari et al., 2020).

**Effect of Feeding Treatments on Performance Sheep**

Body weight gain was analyzed to observe the effect of the treatment feed on sheep's performance. The effect of feeding treatment on sheep performance during the study is presented in Table 6. The results of the statistical analysis showed that supplementing whey protein wafers on sheep feed did not significantly affect ($P>0.05$) body weight gain after the sheep were weaned. The average daily gain (ADG) of sheep was 81.94 – 135.19 g/day (Table 5). The highest ADG was in the 3% whey wafer treatment compared to the control treatment. This was due to the high lactose content in whey as an energy source (Ryan and Walsh 2016). This is consistent with the higher glucose content in the blood compared to other treatments in table 5. Whey protein contains high branched-chain amino acids so that it can increase muscle development in sheep (Eseceli et al., 2021). Administration of whey at a percentage of 3% proved to increase Average daily gain higher than other treatments.

Table 6. The effect of whey protein wafer supplement on the average weight daily gain

| Parameters | Treatments | | |
|---|---|---|---|
| | P0 (Control) | P1 (Whey protein 1.5 %) | P2 (whey protein 3 %) |
| Average daily gain (g/head day) | 108.38 ± 4.05 | 81.94 ± 5.37 | 135.19 ± 15.76 |

## Conclusions

Supplementing 3% of whey protein wafer (P2) increased average daily gain of sheep to 135.19 g/head/day, higher than the control (P0). Hematology and blood metabolites of sheep receiving treatment feed were within the normal conditions, indicating effective treatment to maintain the health of weaning sheep and to increase average daily gain.

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