

Communication

# Effects of Replacing Corn with Hydroponically Sprouted Barley on the Growth Performance and Blood Metabolite Status of Holstein Dairy Heifers

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**Abstract:** This study aimed to determine the effects of replacing corn with hydroponically sprouted barley (HSB) in Holstein heifer feed on growth performance and blood metabolites. For this purpose, the barley was prepared with hydroponic sprouting under optimized conditions, and its chemical composition was analyzed on a dry matter (DM) basis. A total of 30 Holstein heifers that were 7 months old with an average body weight of 212.60 ± 55.00 kg were used in a completely randomized design comprising 10 repetitions and three groups over a period of 11 months. Dietary treatments included control (CON), basal diet (corn meal-based diets), and trail diets, in which 10% and 30% HSB gradually replaced corn meal. The growth performance and biochemical indices were analyzed every month. Results showed that the HSB diet increased chemical compositions compared with the control diets on a DM basis. In addition, diets with up to 30% HSB did not show any negative effects on the growth performance (body weight, height at withers, and height at rump) or selected blood metabolites of heifers compared with the CON diet. In conclusion, the use of HSB instead of corn meal did not lead to any adverse effects on growth performance or biochemical indices; therefore, HSB can be used as an alternative dietary source for raising Holstein heifers.

Keywords: hydroponic; barley; corn; body weight; blood metabolites; rectal temperature

# 1. Introduction

In animal agriculture production, feed is considered the most essential and expensive element. Thus, there is a need to investigate all possible biological feed resources for livestock production, including agricultural byproducts, forages, and weeds [1]. In recent years, hydroponic fodder production companies have been announced extensively to produce feed for ruminants all over the world, which has renewed the interest of livestock farmers and researchers as well [2,3]. Hydroponic fodder is produced from grains that are germinated and grown under optimized conditions in greenhouses within a short time period [4]. The utilization of sprouted fodder is not a new idea.



Since the 1600s, small sprouted grains have been used for fodder preparation [5]. Among the cereals, barley is the most important ancient cereal crop produced globally. The most important features of hydroponic fodder production are that 1 kg of barley grains can produce from 7 to 10 kg of green fodder within a week under well-controlled conditions [6]. Hydroponically produced green forage sprouts are highly palatable, have a height range between 15 and 20 cm, and are produced without the soil germination of cereal grains, such as barley and maize, using water and nutrient mineral solutions [7]. Freshly prepared hydroponically sprouted barley can be an important source of overall rations, which has been demonstrated to advance animal health, diminish heat stress, and increase productivity [8]. Germination activities in seeds involve many modifications, such as seed proteins being converted to essential amino acids, carbohydrates converted to sugars, and fats converted to essential fatty acids, and these increased activities lead to higher enzyme levels [3]. Hydroponically germinated barley is characterized by its level of crude protein, crude fiber, ash, minerals, and vitamins [9]. Furthermore, germination reduces phytic acid levels in seeds, which produces an insoluble complex, and minerals as well enzymes are produced during sprouting that can remove other anti-nutritional elements [10]. The digestive enzymes in sprouts act as biological catalysts in the digestion of carbohydrates, proteins, and fats. Furthermore, sprouting and germination processes neutralize the inhibitors that improve beneficial digestive enzymes [11]. During barley germination, the alkaloid hordenine is formed, which exhibits epinephrine-like activity, stimulating the heart, constricting vessels, and relaxing constricted bronchioles. Hordenine and other alkaloids are closely associated with palatability, intake, and weight gain in animals [12].

The use of methodology and engineering in sprouting makes it economically competitive with other feed choices. This technique may be particularly important for farmers who have little or no land for forage production [8]. Furthermore, this process typically requires a specific, controlled amount of water and nutrient mineral solution to avoid expensive commercial nutrition solutions that can minimize expected profits. An earlier study reported that hydroponic fodder germinated successfully with tap water without any supplementation of nutrients, but its nutritional content was affected. In addition, nitrogen is essential for the production of leafy and green plants and can be extracted from the atmosphere by the plant root or the stoma of the leaves and stems [13]. On the other hand, dairy heifers may represent excessive resource costs for feed, housing, and labor, yet return no money to the dairy farm until they calve or are sold, and feed cost may represent up to 60% of heifers' raising expenses and 12% of total dairy farm expenses. Few studies have been conducted to investigate the effect of the partial replacement of corn by HSB for dairy heifers. Thus, alternative feeds with a lower price must be produced. In this study, we aim to determine the effect of hydroponically sprouted barley (HSB) on Holstein dairy heifer growth performance and biochemical metabolites. This study was performed by comparing the value of feed with various concentrations of sprouted barley, with and without concentrates.

# 2. Materials and Methods

## 2.1. Production of Hydroponically Sprouted Barley Fodder

The sprouted barley was prepared by Gffa, Inc. (Seoul, Korea), following the method of Gebremedhin [6] using a hydroponic sprouting unit with a size of  $4.0 \times 6.0 \times 2.3$  m as length x width x height. A semi-automatic irrigation sprayer was used to irrigate the barley grains (*Keunalbori1ho*) with nutrient-added tap water. The greenhouse temperature was maintained at 16–23 °C, the humidity was 40–80%, and the water temperature was 16–23 °C. To promote leaf growth, fluorescent lighting pipes and irrigation equipment were arranged vertically on the walls. Barley grains were obtained from Nerundul Farming Association Corp, Gimje-si, Korea. To prevent fungal contamination, the purchased barley grain was first washed with fresh water and then soaked with 0.1% hypochlorite for 24 h. Afterward, the cleaned barley grains were transferred to a plastic bowl and covered with a wet cloth for another 24 h to induce germination. They were then spread on a tray at a rate of 300

g with a thickness of 1.5–2.0 cm. To irrigate the barley grains, on the first day, tap water (included nutrients) was provided at a flow rate of 0.47/min, and from the second day, the tap water was adjusted according to the water requirements. The total production of 6 days of hydroponically sprouted barley fodder was recorded. The samples were dried using an oven (Forced Convection Oven, Jeiotech, Daejeon, Korea (KOR)) at 63 °C for 72 h, ground using a cyclone mill (Foss Tecator Cyclotec 1093, Foss, Hillerød, Denmark) to pass through a 1-mm mesh screen sieve, and stored prior to the analysis of their chemical compositions.

### 2.2. Analysis of Chemical Compositions

The chemical compositions of the samples were analyzed based on the National Research Council guidelines [14]. The content of dry matter (DM), crude protein, crude fat, crude fiber, and crude ash, were determined as described in [15]. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) without the use of sodium sulfite or lignin were analyzed by the method described in [16].

#### 2.3. Animals, Management, Feeding, and Experimental Design

The study was conducted at the National Institute of Animal Science, Cheonan, Korea. Holstein heifers were selected for this study, and the experiment was carried out from June 2019 to April 2020. At the beginning of the study, the Holstein heifers (n = 30) were  $7 \pm 0.2$  months of age and had a body weight of  $210.98 \pm 2.10$  kg. The heifers were housed in one section of a free-stall barn, which had metal roofs over the free stalls and feeding area but was open between the roofs. Heifers were provided with supplemental cooling by fans. All heifers were maintained according to standard guidelines, and the experimental procedures of this research were approved by the Institutional Animal Care and Use Committee, protocol number (IACUC No: 2018-303) of the National Institute of Animal Science, Jeonju, Korea. The heifers were divided into three groups (with 10 animals in each group), and the treatment included (1) a control diet with corn meal as the major concentrate ingredients (CON), (2) 10% (DM basis) hydroponically sprouted barley replacing the corn meal, and (3) 30% (DM basis) hydroponically sprouted barley replacing the corn meal. All the heifers were fed a total mixed ration (TMR,  $NE_L$ 1.7 Mcal/kg, TDN 72.2%) twice daily at 09:00 and 17:00 h, water was provided ad libitum, and the TMR was formulated to meet the heifers' requirements [14] for energy, protein, minerals, and vitamins. The feed ingredients used in the treatment diets, and the nutrient compositions of the ration based on the realized TMR are presented in Table 1.

#### 2.4. Growth Performance and Biochemical Indices Analysis

For growth performance measurements, each heifer was weighed monthly, from 7 to 17 months of age. The measurements of height at the withers and rump, in addition to weight, were taken as the animal stood naturally and continued every month from age 7 to 17 months. The height of the animal was measured as the vertical distance from the top of the withers to the ground. A blood sample was collected via jugular venipuncture from each heifer once a month, from age 12 to 17 months, using sterile vacutainer tubes. The collected blood samples were centrifuged at  $1000 \times g$  for 15 min at 4 °C. The serum sample was collected and stored at -20 °C for further analysis. Serum samples were used to analyze the metabolic status (Wako Chemicals, Neuss, Germany) by using a blood auto-analyzer (Hitachi, 7180, Japan). The biochemical indices of total protein, albumin, urea nitrogen (UN), creatine kinase (CK), creatinine, calcium, phosphorous, magnesium, glucose, non-essential fatty acid (NEFA), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), total cholesterol, and triglycerides (TG) level were analyzed with a Clinical Analyzer (Hitachi 7180, Japan). The serum cortisol, progesterone, and estrogen concentrations were measured using a commercial ELISA kit (Wuhan Abebio Science, Wuhan, China) according to the manufacturer's instructions.

Item	CON	HSB (10%)	HSB (30%)			
Ingredient						
compositions (%DM)						
Corn	15.0	13.5	10.5			
Cotton seed	3.0	3.0	3.0			
Corn gluten feed	15.2	27.5	27.6			
Cotton seed hull pellet	19.2	11.1	5.0			
Molasses	3.0	3.0	3.0			
Soybean meal	14.8	10.6	7.6			
Brewer's spent grain	10.0	10	10			
Alfalfa hay	8.0	8.0	8.0			
Klein grass hay	3.0	3.0	3.0			
Timothy hay	3.0	3.0	3.0			
Oat hay	3.0	3.0	3.0			
Limestone	2.0	2.0	2.0			
Salt	0.3	0.3	0.3			
Vitamins & Minerals mix	0.5	0.5	0.5			
HSB		1.5	4.5			
Total	100	100	100			
Chemical compositions						
(%DM)						
DM (% as feed)	79.11 <sup>a</sup>	70.01 <sup>b</sup>	70.62 <sup>b</sup>			
Moisture	20.89 <sup>b</sup>	20.99 <sup>b</sup>	29.38 <sup>a</sup>			
Crude protein	19.14 <sup>ab</sup>	18.27 <sup>b</sup>	20.25 <sup>a</sup>			
Crude fat	5.23 <sup>ns</sup>	5.82 <sup>ns</sup>	5.33 <sup>ns</sup>			
Crude fiber	14.85 <sup>a</sup>	15.62 <sup>a</sup>	12.16 <sup>b</sup>			
Ash	8.34 <sup>a</sup>	7.65 <sup>a</sup>	6.11 <sup>b</sup>			
ADF	19.34 <sup>a</sup>	19.36 <sup>a</sup>	14.73 <sup>b</sup>			
NDF	37.53 <sup>a</sup>	39.56 <sup>a</sup>	32.39 <sup>b</sup>			
Energy (Mcal/kg)	3423.00 <sup>a</sup>	3404.33 <sup>a</sup>	3122.67 <sup>b</sup>			
Lignin	3.17 <sup>ns</sup>	3.05 <sup>ns</sup>	2.18 <sup>ns</sup>			
B-carotene (µg/100 g)	366.14 <sup>ns</sup>	397.26 <sup>ns</sup>	400.83 <sup>ns</sup>			
Feed cost (Korean won/kg DM)						
Corn	271		-			
HSB	-	5	583			

**Table 1.** Feed ingredients and chemical composition of the control (CON) and hydroponically sprouted barley (HSB) fed to growing Holstein heifers for 11 months.

Values are expressed as the mean  $\pm$  SEM of the 3 replicates in each group. <sup>a, b</sup> and <sup>ns</sup> denote comparisons made between columns (p < 0.05).

#### 2.5. Statistical Analysis

All data were subjected to statistical analysis in a randomized block design using the general least-squares (PROC GLM) procedures of the SAS program (SAS Institute Inc, Cary, NC, USA). The values were indicated as least-squares means and the standard error of the mean. The differences between the means of the treatment groups were determined using Duncan's multiple range test with a p < 0.05 representing statistical significance.

# 3. Results

#### 3.1. Nutritional Compositions of HSB

The chemical composition of the hydroponically sprouted barley and CON diet on a DM basis are shown in Table 1. The feed with corn replaced by 30% HSB had higher crude protein (20.25), and lower crude fiber (12.16), ash (6.11), ADF (14.73), and NDF (32.39) than the control (CON) diet on a DM basis. Together, corn replaced by 15% HSB showed similar chemical compositions to the CON diet. However,

no significant difference was noted for crude fat, lignin, and  $\beta$ - carotene content between the HSB (15% and 30% replacement) and CON diets on a DM basis.

#### 3.2. Effect of HSB on Growth Performance in Holstein Dairy Heifers

The effect of partially replacing corn with HSB in Holstein heifer feed on the heifers' live body weight is presented in Figure 1. The results of the present study showed that there was no significant difference noted in the live body weight of heifers fed with a diet having corn meal replaced with 10% and 30% HSB compared with the CON diet. Similarly, the values for height at withers and height at rump were not changed when the heifers were fed with 10% and 30% HSB compared with the CON diet (p < 0.05), as shown in Figure 2a and b, respectively. The activity volume was similar in heifers fed with 10% and 30% HSB and the CON diet (p < 0.05). However, a higher rectal temperature was observed in the heifers supplemented with the 30% HSB diet than those fed with the 10% HSB and CON diets, although the increased rectal temperature value was within the normal range (Table 2).

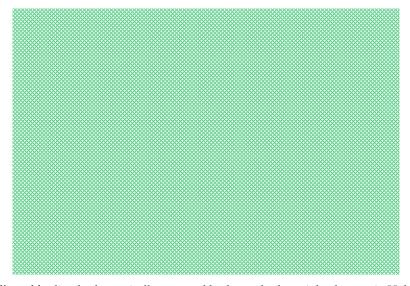
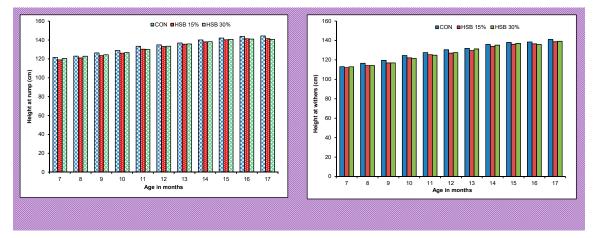


Figure 1. Effect of feeding hydroponically sprouted barley on body weight changes in Holstein heifers.



**Figure 2.** Effect of feeding hydroponically sprouted barley on height at withers (**a**) and height at rump (**b**) of Holstein heifers.

Months		CON	HSB (10%)	HSB (30%)
13	Activity volume (Unit)	$6247.40 \pm 376.80$ <sup>ns</sup>	$6265.50 \pm 394.40$ <sup>ns</sup>	$6155.27 \pm 347.45^{a}$
	RT (°C)	$38.55 \pm 0.03$ ns	$38.61 \pm 0.05$ ns	$38.84 \pm 0.04$ <sup>ns</sup>
14	Activity volume (Unit)	$6101.91 \pm 136.01^{\mathrm{b}}$	$6747.82 \pm 196.86^{a}$	$6232.05 \pm 150.11^{a}$
	RT (°C)	$38.57 \pm 0.01$ <sup>ns</sup>	$38.55 \pm 0.01$ <sup>ns</sup>	$38.81 \pm 0.01$ <sup>ns</sup>
15	Activity volume (Unit)	6232.51 ± 191.65 <sup>ns</sup>	$6346.50 \pm 220.05$ <sup>ns</sup>	5959.98 ± 182.86 <sup>ns</sup>
	RT (°C)	$38.33 \pm 0.03$ <sup>ns</sup>	$38.50 \pm 0.03$ <sup>ns</sup>	$38.67 \pm 0.02$ <sup>ns</sup>
16	Activity volume (Unit)	5992.65 ± 158.90 <sup>ns</sup>	$6374.03 \pm 160.91$ <sup>ns</sup>	5851.01 ± 151.09 <sup>a</sup>
	RT (°C)	$38.52 \pm 0.03^{\circ}$	$38.65\pm0.04^{\rm b}$	$38.85 \pm 0.03^{a}$
17	Activity volume (Unit)	5467.90 ± 169.91 <sup>ns</sup>	$6276.82 \pm 234.85$ <sup>ns</sup>	5427.25 ± 155.12 <sup>ns</sup>
	RT (°C)	$38.47 \pm 0.03$ <sup>ns</sup>	$38.60 \pm 0.02$ ns	$38.76 \pm 0.02$ ns

**Table 2.** Effects of feeding hydroponically sprouted barley on behavior activity volume and rectal temperature in Holstein heifers (n = 10).

Values are expressed as the mean  $\pm$  SEM of 10 dairy heifers in each group. <sup>a, b, c</sup> and <sup>ns</sup> denote comparisons made between columns (p < 0.05).

### 3.3. Effect of HSB on Blood Metabolites in Holstein Dairy Heifers

The data concerning the blood metabolites in Holstein heifers fed the CON and HSB diets are presented in Supplementary Tables S1–S5. During the study period, there were no significant changes observed in the selected blood metabolites in Holstein heifers fed a diet supplemented with 10% and 30% HSB as compared with those fed the CON diet (p < 0.05). However, blood glucose levels were slightly increased during the seventeenth month in heifers fed the HSB and CON diets (p < 0.05).

## 4. Discussion

The cost involved in raising heifers is an important issue for dairy farmers. Additional animals typically account for 15–20% of milk production costs. The replacement of heifers ranks as the second or third largest component of production costs after feed and possibly labor on animal agriculture production. The costs can vary from farm to farm, depending on individual management strategies. The aim of raising heifers should be to minimize costs while still maximizing the returns on the costs incurred. Therefore, the objective of a dairy replacement heifer management program depends on the successful rearing of heifers to minimize costs without compromising future lactation performance. Corn is a primary energy source that is mainly used as an ingredient in preparing feed for ruminants in Korea. However, corns are generally imported from other countries, and the cost of feed is high. Therefore, farmers and researchers have placed more attention on searching for an alternative to corn meal. Dietary energy, DM intake, body weight, and weight gain are closely associated with growth performance in ruminants [17]. Therefore, in this study, we evaluated the effects of HSB on growth performance and biochemical metabolites in Holstein heifers in Korea. The results of the present study revealed that there were no significant changes in the live body weight of heifers fed with a 10% and 30% HSB diet when compared with the CON diet. Moreover, the average daily weight gain was determined from body weight measured monthly, and no significant difference was noted between the 10% and 30% HSB diet-supplemented heifers and the CON-supplemented heifers. Therefore, the study findings confirmed that HSB could be a substitute for corn meal in feed for ruminants in Korea. The regulation of feed intake and energy balance are influenced by many factors, including age, initial live weight, nutrition, breed features, and management program [18], and this feed intake and energy balance is predicted for metabolic, digestive tract, and external factors [19]. Limited research has been published to evaluate the feed value of sprouting barley. Early study results have shown that a 10-day sprouted barley diet in pigs induced lower weight gain as compared with ground maize, but in beef cattle, sprouted barley green fodder showed a 200 g higher daily gain as compared with a maize control diet [20].

Biochemical indices are key markers of general health and vitality and may indicate the action of certain tissue, organ, and nutrient status. The increased concentration of blood glucose represented a high level of absorption of glucose due to the energy-yielding substrate and glucose consumption [21,22]. Our study finding also showed an increased concentration of glucose at the seventeenth month for heifers fed with 30% HSB. The increased blood glucose concentration was observed after the replacement of corn with HSB. Under normal physiological circumstances, blood glucose plays a key function in energy storage in tissues, organs, and the central nervous system, and it provides a suitable physiological guide for overall energy metabolism. The replacement of corn with HSB did not alter the total protein, albumin, BUN, CK, or creatinine concentration in Holstein heifers as compared with the CON-fed heifers. BUN is an end product of protein and amino acid catabolism in the body; it is negatively associated with nitrogen deposition and protein/amino acid utilization. When amino acids are imbalanced in the body, or when there is poor utilization of protein in the body, the BUN concentration increases [23]. Blood metabolites such as total cholesterol, TG, oxalo-acetate transaminase, and pyruvate transaminase, as well as total protein and LDH concentrations can be

used as predictors of fat and protein metabolism and of liver function. In this experiment, the above parameters were not significantly altered when heifers were fed the 10% and 30% HSB diet as compared with the CON diet, indicating that the replacement of corn with HSB in the diet of growing Holstein heifers did not adversely affect their carbohydrate, lipid, and protein metabolism.

Despite increasing livestock production, feed management practice is considered an important factor in animal agriculture. Cortisol is an important indicator that is used to determine the stress condition of an animal. Increased levels of cortisol in the blood indicate that an animal may be under stress, which leads to a decrease in reproduction. Therefore, cortisol levels are measured to evaluate whether an animal is experiencing stress conditions. In this study, we determined, every month for the entire study period, the blood cortisol level of heifers fed the 10% and 30% HSB diets and the CON diet. Our results showed that the 10% and 30% HSB diet did not negatively affect the cortisol levels when compared with the CON diet. Feed additives can indirectly alter the cortisol level in blood serum, but some studies only show that feeding may change the cortisol level in blood serum. Progesterone and estrogen are considered reproductive markers. Butler and Smith [24] demonstrated a strong relationship between negative energy balance and conception rate. In this study, we evaluated the level of reproductive hormones in blood, and these hormone levels were not significantly different between heifers fed the 10% and 30% HSB diets and the CON diet, which reflected the reproductive performance of the heifers. From these study findings, we confirmed an improved energy balance when heifers were fed the 10% and 30% HSB diet. These hormones increase cholesterol, lipoprotein, HDL, and sodium excretion. Further, these hormones are closely associated with the measurement of DM intake, average daily gain, and feed efficiency in heifers.

# 5. Conclusions

The findings of the present pilot study demonstrated that the partial replacement of corn with HSB had no adverse effects on live body weight, height at withers, or height at rump in Holstein heifers. Similarly, the substitution of corn with HSB had no negative effect on blood metabolites in Holstein heifers, and the feed cost of HSB was lower than corn. Thus, it is recommended to partially replace corn with HSB with the concentrate mixture to obtain optimal DM intake as well as high animal performance. However, further study is required in future concerning whether a higher level of substitution of corn meal with HSB has any adverse effect on Holstein dairy cattle growth performance and milk production.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/21/7442/s1.

**Author Contributions:** T.-I.K., D.-H.L., K.-S.K., and V.M. conceived and designed the experiments; T.-I.K., funding acquisition; T.-I.K., D.-H.L., V.M., H.-J.L., S.-M.P., Y.-J.K., H.-C.C., J.-H.P., and S.-C.K. performed most of the experiments; V.M. wrote the manuscript, and K.S.K. supervised and provided many helpful suggestions regarding the study and manuscript writing. All authors have read and agreed to the published version of the manuscript.

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