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**Effect of soy co-products in supplements for grazing cattle on growth,
complete blood cell counts, and physiological response following a
lipopolysaccharide challenge**

An Honors Thesis submitted in partial fulfillment of the requirements for Honors
Studies in Biology

By

Bralee Lansdell

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Biology

Fulbright College of Arts and Sciences

The University of Arkansas

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Abstract

The objective of this study was to determine the effect of soy co-products (soybean meal and soy oil) in the diet on the growth and hemocytology of cattle during a 56-day growing phase and the physiological/behavioral response to an endotoxin challenge. Angus crossbred steers ($n = 36$; 289 ± 31 kg, initial body weight \pm SD) were stratified by body weight and sire; and assigned randomly to pastures ($n = 9$; 0.45 ha/mixed-grass pasture). Pastures were assigned randomly to 1 of 3 dietary treatments: 1) a control supplement containing no soy co-products, 2) a supplement containing soybean meal, or 3) a supplement containing soy oil. All supplements were isonitrogenous and isoenergetic. Cattle were fed supplements (2.45 kg of dry matter/day) for a period of 56 days during which weight and blood samples for complete blood count (CBC) were taken every 14 days. At the conclusion of the growing phase, cattle were assigned randomly to 1 of 2 challenge groups (conducted 6 days apart) for a lipopolysaccharide (LPS) challenge (i.v. infusion of $0.5 \mu\text{g}$ LPS/kg of body weight). A minimum of 18 hours before sampling, cattle were fitted with jugular vein catheters and placed into stanchions. Sickness behavior scores and rectal temperatures were collected every 30 minutes for a duration of 8 hours following LPS infusion. Body weights were analyzed using pen means and the MIXED procedure of SAS specific for repeated measures with treatment, day, and the treatment by day interaction as fixed effects and replicate as a random effect. The CBC were analyzed using the MIXED procedure of SAS specific for repeated measures with treatment, day, and the treatment by day interaction as fixed effects, replicate as a random effect, and pen specified as the subject. Rectal temperatures and behavior scores were analyzed using the MIXED procedure of SAS specific for repeated measures with treatment, time, and the treatment by time interaction as fixed effects and challenge group as a random effect with calf as the subject. Dietary inclusion of soy co-products

did not affect the body weights of steers for the 56-day growing phase (treatment and treatment by day, $P \geq 0.20$), nor were any hemocytology measurements affected (treatment and treatment by day, $P \geq 0.12$) during the growing phase. Following the endotoxin challenge there was no effect of treatment or treatment by time ($P \geq 0.57$) for rectal temperatures or sickness behavior scores. Therefore, results indicate that inclusion of soy co-products in cattle diets did not affect growth or complete blood counts during a 56-day growing phase, neither did diet affect body temperature or sickness behavior in response to an endotoxin challenge.

Introduction

The effect of the inclusion of dietary seed co-products has been studied in various animal species with many of these studies indicating that seeds have properties that alter the body's growth rate and physiological response. However, most of these studies have used dairy animals as subjects rather than beef cattle. The co-products of soybeans, such as soybean meal and soy oil, are an accessible resource in the United States, and could offer health benefits to beef cattle in stocker and feedlot scenarios when included into the diet. Cattle in these stress-inducing environments are at risk for harmful diseases, such as bovine respiratory disease (BRD), which costs the industry almost one billion dollars annually. The physiological response to stressors, including the inflammatory response, is a balancing act. Inflammation is a natural response to sickness, but when that response is prolonged or overreactive, the effect can be damaging to an animal's health. Soybeans contain omega-3 and omega-6 fatty acids, which are both regulators of the inflammatory response. Successful utilization of soybean co-products could show that effectively activating the immune system ahead of illness can mitigate the losses associated with disease. This study aimed to determine the effect of supplementation of soy co-products in the diet on growth, complete blood counts, and physiological response in calves. Calves were fed for

a 56-day growing period, during which body weights and complete blood counts were recorded, and then cattle were subjected to a lipopolysaccharide challenge to determine physiological response using rectal temperatures, and sickness behaviors scores.

Literature Review

Soybeans

Soybeans are a plentiful resource in the United States, with the United States leading soybean production, as well as being the second largest exporter (USDA ERS, 2022). However, soy co-products are rarely utilized in the beef cattle industry. This can be explained by the high cost of soy co-products in comparison to other common feed products. On average, soybean meal is 25 to 35% more expensive than cottonseed meal, which is a co-product sometimes used in beef cattle diets (Mullenix & Stewart, 2021). In recent years, the beef cattle industry has accounted for only a small portion of soybean meal use at 6.8% (Annual Soy Stats Results, 2021). Similarly, the market for soybean oil is slim in the stocker and feedlot industry as the majority of soy oil is used for human consumption at 68% (Stowe et al., 2022).

Soybeans are a plant-derived source of both protein and energy. The composition of a soybean seed can be broken down into 20% soy oil and 80% soybean meal (Stowe et al., 2022). Soybean meal is the main source of protein, in both the livestock and poultry industries across the world, due to its high amino acid quality (Stein et al., 2008). Soy oil is seen as a source of omega-6 fatty acids and is made up of approximately 84% unsaturated fatty acids (X. S. Oliveira et al., 2021).

Benefits of Seed Co-Product Supplementation

Co-products from seeds, including sunflowers, soybean, and flaxseed have been shown to improve health and decrease disease incidence in various livestock species. These co-products

contain both omega-3 and omega-6 fatty acids, which are known to alter the immune response. Omega-3 fatty acids have anti-inflammatory properties, whereas omega-6 fatty acids are precursors to pro-inflammatory mediators (Cholewski et al., 2018; Innes & Calder, 2018). When omega-3 and omega-6 fatty acids are both present in an organism, they compete for the same enzymes, working within an organism to regulate the immune response (Gutiérrez et al., 2019).

The effect of seed co-product supplementation on growth has been explored in previous studies. Dairy cattle fed fennel seed powder had greater weight gain and final body weights when compared to those that received no fennel seed powder (Nowroozinia et al, 2022). However, fenugreek seed supplementation in dairy goats had no significant impact on live weight gain (Akbag et al., 2022). Inclusion of black cumin seed in the diet of lambs increased average daily gain, as they were in the growing period, but had no effect on sheep growth (Sadarman et al., 2021). These varying results indicate that the effect of seed supplementation on growth have yet to reach a consensus in the available literature.

The inclusion of seed co-products in the diets of ruminants have been attributed to several health benefits. Dairy cows supplemented with whole flaxseed had a lower number of postpartum diseases, including mastitis, milk fever, and diarrhea than those fed a diet containing no seed oil (Petit et al., 2007). Similarly, dairy cattle fed extruded flaxseed had a decreased incidence of ketosis and mortality, as well as a tendency toward increased fertility performance (Moallem et al., 2020). In dairy goats, soybean oil supplementation improved milk production, metabolism, and fatty acid profile (Hamzoui et al., 2021). Though there are several studies showing the benefits of seed co-product supplementation in dairy cattle, there are no studies

currently that utilize soybean products in stocker or feedlot cattle systems to observe the effects on the immune response.

Indicators of Physiological Response

Lipopolysaccharide (LPS) is an endotoxin found in the outer membrane of gram-negative bacteria that, when released, or introduced into an organism, causes an inflammatory response. Free LPS is recognized by receptors on the surface of myeloid cells, which begins a signaling pathway that results in the release of mediators of the inflammatory response (Lyu et al., 2017). There are indicators of the change in physiological factors during an inflammatory response, some of which are rectal temperatures and sickness behavior scores.

In wether lambs, an LPS challenge was used to induce an acute immune challenge and rectal temperature was taken to investigate the physiological response. The lambs experienced an increase in rectal temperature which peaked at 4 h and was still greater than controls at the completion of the challenge. Rectal temperatures moderately predicted white blood cell, lymphocyte, and monocyte concentrations in these lambs (Cadaret et al., 2021). Rectal temperature was used as an indicator of physiological response to injection of an endotoxin in beef steer calves, as an increase in body temperature has long been associated with an immune response (Elsasser et al., 1996).

In Brahman bulls, sickness behavior scores and rectal temperatures were taken as indicators of the physiological response to an LPS challenge to determine if temperament affected the severity of the immune response (Burdick et al., 2010). Sickness behavior scores allow inference to the extent of physiological changes during illness because behavioral changes often occur simultaneously. In dairy cattle, behavior was monitored, along with rectal temperature, during an LPS challenge to determine if behavioral changes could be early

indicators of illness. Calves exhibited some behavioral changes during the challenge, such as reduced self-grooming, rumination and ingestion of feed and increased time spent lying and standing inactive during temperature increase (Borderas et al., 2008).

Materials and Methods

Growing Period

Due to the inclusion of calves in the study, the following procedures were approved by the University of Arkansas System Division of Agriculture's Animal Care and Use Committee (IACUC). This experiment took place at the University of Arkansas System's Milo J. Schult Agricultural Research and Extension Center in the Spring of 2022 and continued into the Fall of 2022. Angus crossbred steers ($n = 36$), weaned approximately 1 month earlier from a single herd, were stratified by initial body weight (289 ± 31 kg) and sire and assigned randomly to pastures ($n = 9$, 0.45 ha/mixed-grass pastures). Pastures were assigned randomly to 1 of 3 treatments ($n = 3$ pastures/treatment; resulting in 12 calves/treatment). Treatment A was considered the control and was a grain supplement that contained no soy product. Treatment B was a supplement containing soybean meal. Treatment C was a supplement containing soy oil. Cattle were offered supplements (2.45 kg/day) for a 56-day period. All diets were isonitrogenous and isocaloric (Table 1). Water was available for ad libitum intake and cattle were supplemented with hay when necessary. Cattle were observed daily for signs of morbidity. On days 0, 14, 28, 42, and 56, cattle were brought to the chute where body weight and blood samples were taken. Blood samples were collected via jugular venipuncture in EDTA-containing vacuum tubes.

Lipopolysaccharide Challenge

After the completion of the growing phase, cattle were assigned randomly to 1 of 2 challenge groups ($n = 18$) for a lipopolysaccharide (LPS) challenge. The 2 challenges took place 6 days apart from each other. Cattle were transported 8.7 km a minimum of 18 hours before catheterization. A minimum of 18 hours before each challenge, a jugular vein catheter was inserted on the right side of the neck and cattle were placed into stanchions for acclimation. Each catheter was secured overhead, and cattle were regularly monitored until the challenge began. During this time, cattle were offered hay and water for ad libitum intake. Two hours prior to sampling, cattle were offered 2.7 kilograms of the respective supplement of their treatment group and were provided water for ad libitum intake throughout the challenge. Lipopolysaccharide (LPS), which is found in the gram-negative bacterial cell wall, was administered through the jugular cannula to activate the inflammatory response in these cattle (i.v. infusion of $0.5 \mu\text{g}$ LPS/kg of body weight). Rectal temperatures and sickness behavior scores were taken every 30 minutes beginning 2 hours prior to LPS injection (i.v. infusion of $0.5 \mu\text{g}$ LPS/kg of body weight) and continued to be taken until 8 hours post-injection. To maintain consistency, the time that each sample collection was completed was recorded. Each sampler measured rectal temperatures of 6 calves. A single DVM recorded sickness behavior scores for all cattle. Sickness behavior scores ranged from 1 to 5, with $\frac{1}{2}$ scores used as necessary (Table 2). Ambient temperatures were recorded every 30 minutes for the duration of the challenge. At the completion of the LPS challenge, jugular catheters were removed. Cattle were monitored 30 days for signs of morbidity.

Data Analysis

Blood samples for complete blood counts were refrigerated no longer than 3 hours before being analyzed for red and white blood cell values using an automated analyzer (HemaVet HV950; Drew Scientific, Miami Lakes, FL).

All statistical analysis was conducted using the PROC MIXED procedure of SAS 9.4. Body weights were analyzed using pen means and the MIXED procedure of SAS specific for repeated measures with treatment, day, and the treatment by day interaction as fixed effects and replicate as a random effect. The CBC were analyzed using the MIXED procedure of SAS specific for repeated measures with treatment, day, and the treatment by day interaction as fixed effects, replicate as a random effect, and pen specified as the subject. Rectal temperatures and sickness behavior scores were analyzed using the MIXED procedure of SAS specific for repeated measures with treatment, time, and the treatment by time interaction as fixed effects and challenge group as a random effect with calf as the subject. Statistical significance was declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.1$.

Results

Growing period

Over the growing period, there was no significant effect of treatment on body weight ($P = 0.20$). There was a significant day effect on body weight at $P < 0.0001$ which was expected, as the cattle continually gained weight over the growing period (**Figure 1**). There was no treatment by day interaction effect on body weight at $P = 0.91$.

White blood cell (WBC) counts had no treatment or treatment by day interaction effects, at $P = 0.90$ and $P = 0.36$, respectively. There was a day effect for WBC counts, at $P < 0.0001$ with day 0 having higher WBC counts than day 14 (**Figure 2**). From day 14, WBC counts

continued to increase until day 56, which had lower WBC counts than day 42, but higher counts than day 14 (Figure 2). Treatment had no effect on neutrophil percentages at $P = 0.94$. There was a day effect on neutrophil percentages ($P = 0.0008$) with day 0 having the lowest neutrophil percentages, and day 14 having a lower percentage than day 42 (**Figure 3**). There was no treatment by day interaction effect on neutrophils ($P = 0.84$). Lymphocyte percentage was not affected by treatment at $P = 0.95$. There was also no treatment by day interaction effect on lymphocyte percentages at $P = 0.88$. There was a day effect on lymphocyte percentages at $P < 0.0001$. Lymphocytes were highest on day 0 of the growing period (**Figure 4**). Neutrophil to lymphocyte ratio was taken into consideration as it is an indicator of immune system regulation (**Figure 5**). There was no treatment effect on this ratio ($P = 0.99$). There was no treatment by day interaction effect on the neutrophil to lymphocyte ratio at $P = 0.79$. However, there was a day effect on neutrophil to lymphocyte ratio at $P < 0.00001$.

Monocyte percentages had no day or treatment by day interaction effects, at $P = 0.68$ and $P = 0.40$, respectively. There was a day effect on monocyte percentage at $P < 0.0000$, with day 0 having the lowest percentages (**Figure 6**). Eosinophil percentages did not have a treatment effect at $P = 0.76$. There was a day effect at $P < 0.0001$, with day 0 being less than day 14 and day 28, but greater than day 56 (**Figure 7**). Overall, the highest eosinophil percentages were recorded on day 14 (**Figure 7**). There was no effect of treatment by day interaction on eosinophils, at $P = 0.73$.

Treatment did not have an effect on red blood cell (RBC) count ($P = 0.73$). Day did have an effect on RBC, with day 0 and day 14 having higher RBC counts than day 28, day 42, and day 56 (**Figure 8**). There was no treatment by day interaction effect on RBC counts, at $P = 0.99$. Hemoglobin concentration was not affected by treatment ($P = 0.91$) or a treatment by day

interaction ($P = 0.75$). There was a day effect on hemoglobin concentration ($P = 0.0004$) with day 0 having higher concentrations than days 14, 28, and 42 (Figure 9). Day 14 had higher concentrations than day 42, but lower concentrations than day 28 and day 56 (Figure 9). Day 28 had lower concentrations than day 56 (**Figure 9**). Hematocrit percentages had no treatment or treatment by day interaction effects at $P = 0.68$ and $P = 0.93$, respectively. There was a day effect on hematocrit percentages ($P = 0.0087$). Percentages decreased until day 28, then continued to increase to day 56 (**Figure 10**). Platelet counts had no treatment effect at $P = 0.29$. There was no treatment by day interaction effect for platelet count at $P = 0.98$. There was a tendency for day effect on platelet count ($P = 0.0898$). Days 0, 14, 28, and 42 tended to have lower platelet counts than day 56 (**Figure 11**).

LPS Challenge

Rectal temperature taken during the lipopolysaccharide challenge were not affected by treatment, at $P = 0.60$. There was also no treatment by day effect on rectal temperatures ($P = 0.99$) There was a time effect on rectal temperatures, at $P < 0.0001$. Rectal temperatures followed an expected pattern, as they were lowest between -2 h and 0 h. After LPS injection at 0 h, rectal temperatures began to increase, peaking at 3.5 h (**Figure 12**). Rectal temperatures then declined until 6.5 h, where they were similar until the conclusion of the trial, at 8 h (**Figure 12**). However, rectal temperatures from 6.5 h to 8 h were still higher than those taken prior to injection (**Figure 12**). Sickness behavior scores (SBS) (**Figure 13**) and rectal temperatures (**Figure 12**) followed a similar pattern, which was to be expected as both are indicators of the physiological response. There was no effect of treatment on SBS at $P = 0.19$. There was also no treatment by day effect on SBS ($P = 0.99$). There was a time effect on SBS, as expected ($P < 0.0001$). SBS was lowest prior to injection, between -2 and 0 h (**Figure 13**). SBS increased from 0.5 h to 3 h and then

began to decline until 7 h, where SBS remained similar until the completion of the trial at 8 h (**Figure 13**).

Discussion

Throughout this study, no differences in body weights or physiological responses were recorded that could be attributed to the supplementation of soybean meal or soy oil. Cattle receiving soy oil supplementation had greater body weights for the duration of the challenge, however this difference was not significant (**Figure 1**). Supplementation with soybean oil, which is high in omega-6 fatty acids (51%), which promote the inflammatory response, and contains omega-3 fatty acids that regulate the inflammatory response, did not cause any dramatic changes in hemocytology results in comparison to the control. From this, it can be inferred that feeding cattle a soy co-product supplement over a growing period is not harmful, as it did not cause any issues in the hemocytology or prevent weight gain. Rectal temperatures and sickness behavior scores followed the same pattern as seen in the literature, both peaking near 4 hours post-LPS injection before declining, with rectal temperature never coming down to the baseline for the remainder of the challenge (**Figure 12 & Figure 13**). Neither rectal temperature or sickness behavior score were significantly affected by the supplementation of soy co-products. This leads to the conclusion that the inclusion of soy in the diet of beef cattle does not inhibit or prolong the inflammatory response when introduced to a stressor.

There is a possibility that supplementing with products high in omega-6 fatty acids, like soybeans, may activate the immune response, predisposing cattle and allowing for production of an immune response that can more readily protect them from diseases, such as BRD. Inflammation in moderation can enhance an animal's ability to respond more quickly to infections of bacterial and viral agents (Broom and Kogut, 2018). Animals with more pro-

inflammatory mediator expression are more readily protected from disease and probable pathogens. Finding the balance between omega-3 and omega-6 fatty acid supplementation through the use of seed co-products could vastly improve animal health, by creating an ideal immune response, in which the animal can readily fight off the disease without a prolonged response.

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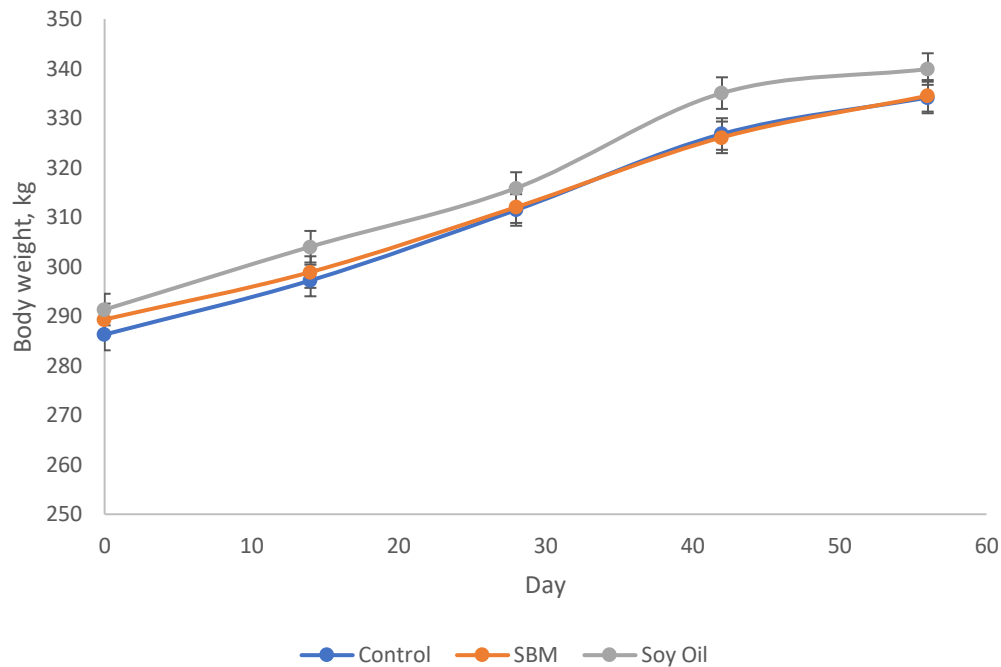
Tables and Figures

Table 1. Breakdown of supplements for each treatment group

Ingredient, % as fed	Supplements		
	Control	Soybean meal	Soy oil
Corn, cracked	53.5	60.9	53.4
Cottonseed meal	37.88	--	37.88
Soybean meal	--	31.05	--
Choice white grease	3.5	4.1	--
Soy oil	--	--	3.53
Molasses	1.0	1.0	1.0
Salt, white	0.69	0.69	0.688
Limestone	3.33	2.34	3.36
Vitamin A, D, E premix	0.056	0.056	0.056
Corn/Rumensin premix	0.267	0.267	0.267
Trace mineral premix	0.056	0.056	0.056

Table 2. Description of signs and behaviors that correspond to each sickness behavior score (SBS)

Score	Description
1	Normal, alert, ears erect: head level or high, eyes open, standing or lying, locomotor activity, responsive, performing maintenance behaviors
2	Calm but less alert, less activity, less responsive, standing or lying
3	Lying calm, head distended or tucked, less alert, signs of some mild respiratory problems (coughing, wheezing), slobbering/ hyper-salivation, nose dry
4	Clinical signs of sickness, respiratory problems, not responsive, head distended, lethargic
5	All/most respiratory problems, mucus/foam, head distended, not responsive, medical intervention required

**Figure 1.** The effects of soybean meal or soybean oil supplementation to growing cattle on body weight during a 56-day growing period

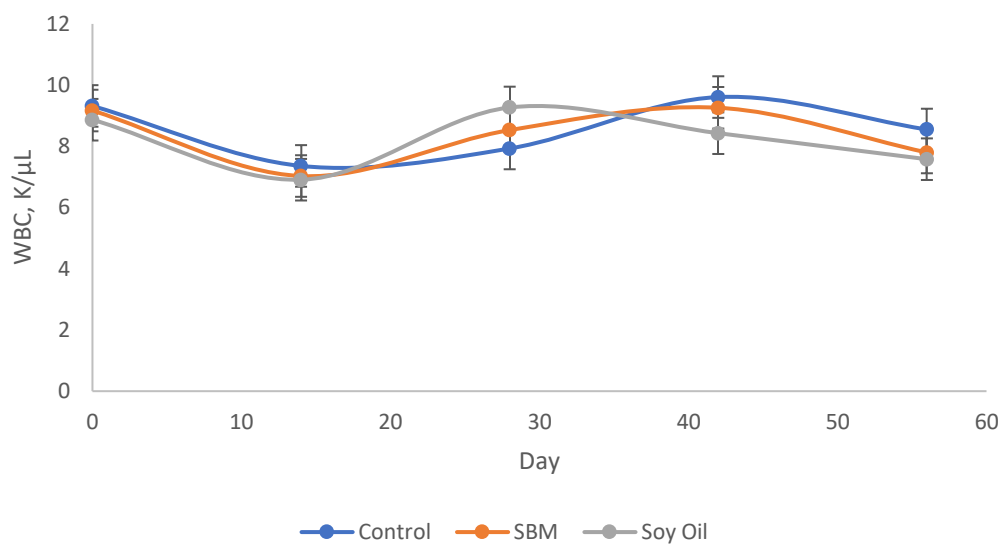


Figure 2 The effects of soybean meal or soybean oil supplementation to growing cattle on white blood cell count during a 56-day growing period

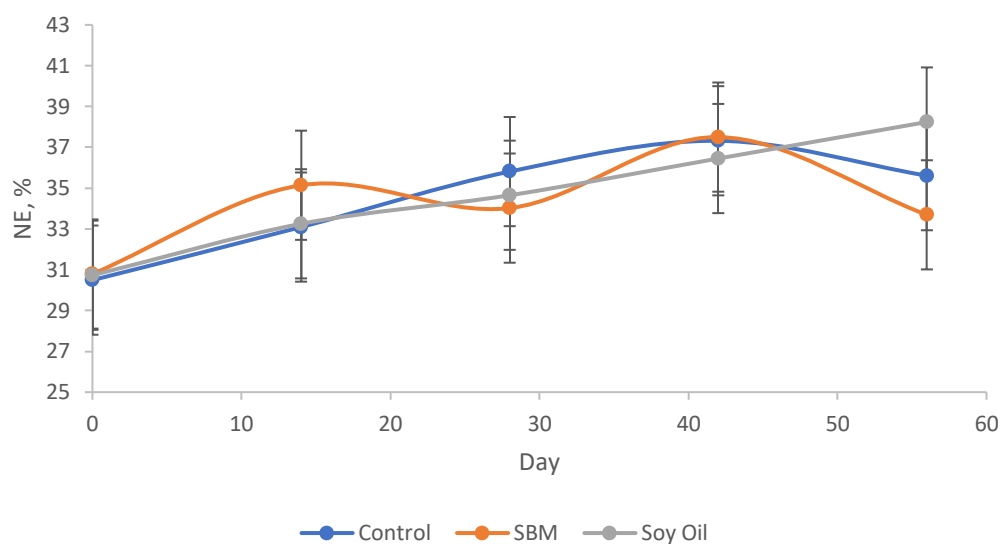


Figure 3. The effects of soybean meal or soybean oil supplementation to growing cattle on neutrophil percentage during a 56-day growing period

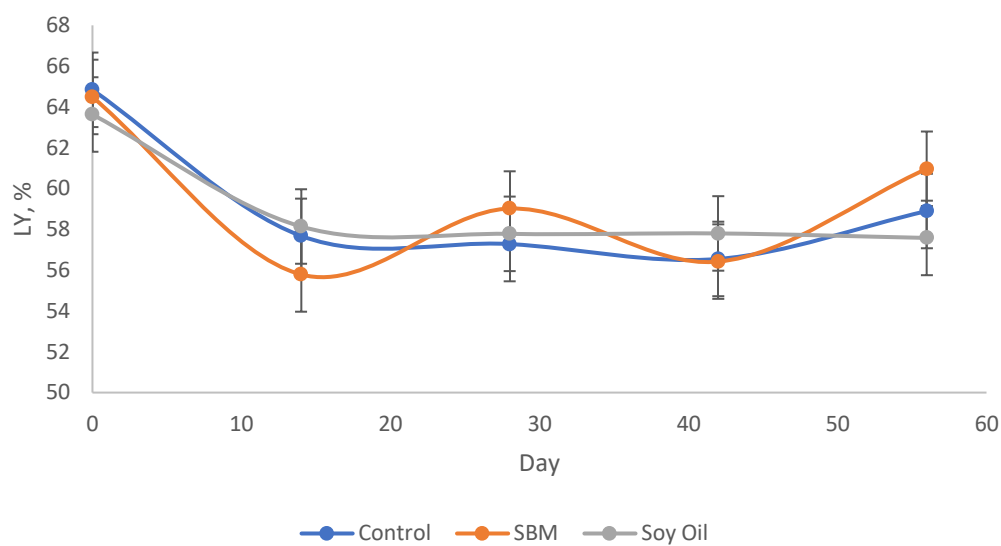


Figure 4. The effects of soybean meal or soybean oil supplementation to growing cattle on lymphocyte percentage during a 56-day growing period

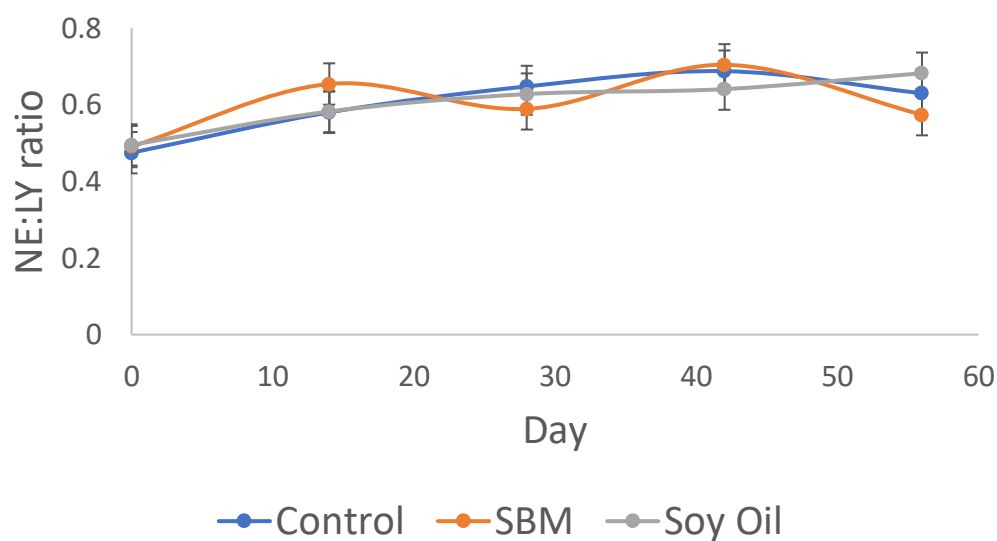


Figure 5. The effects of soybean meal or soybean oil supplementation to growing cattle on neutrophil to lymphocyte ratio during a 56-day growing period

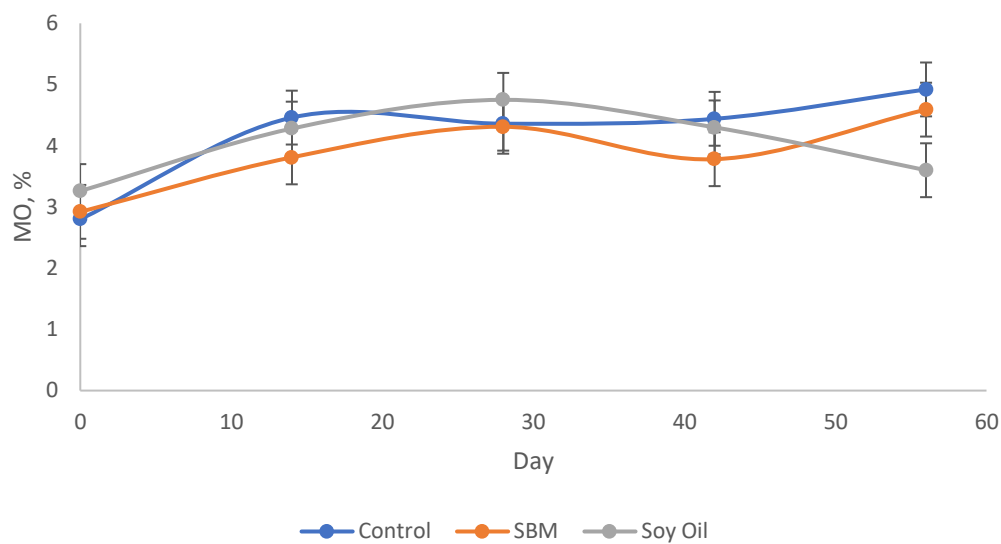


Figure 6. The effects of soybean meal or soybean oil supplementation to growing cattle on monocyte percentage during a 56-day growing period

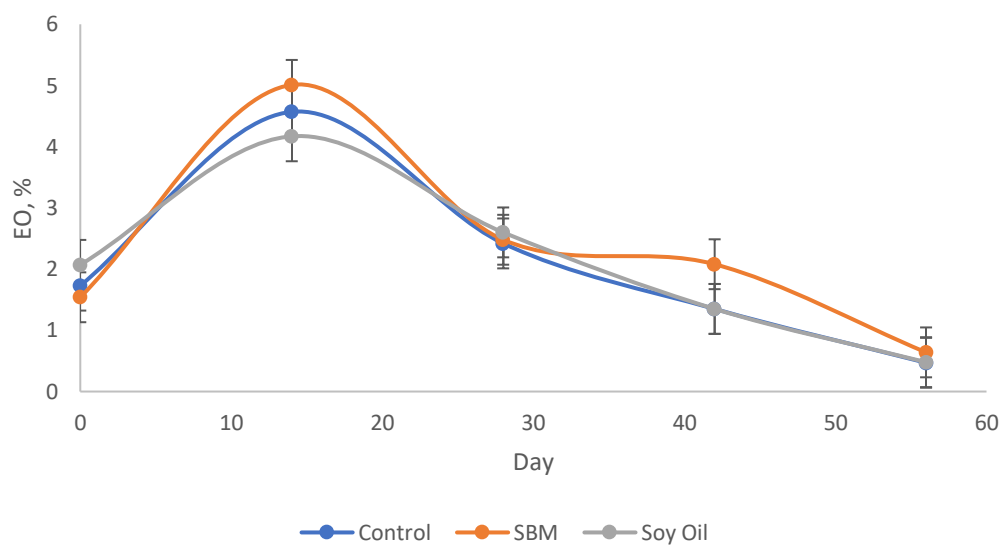


Figure 7. The effects of soybean meal or soybean oil supplementation to growing cattle on eosinophil percentage during a 56-day growing period

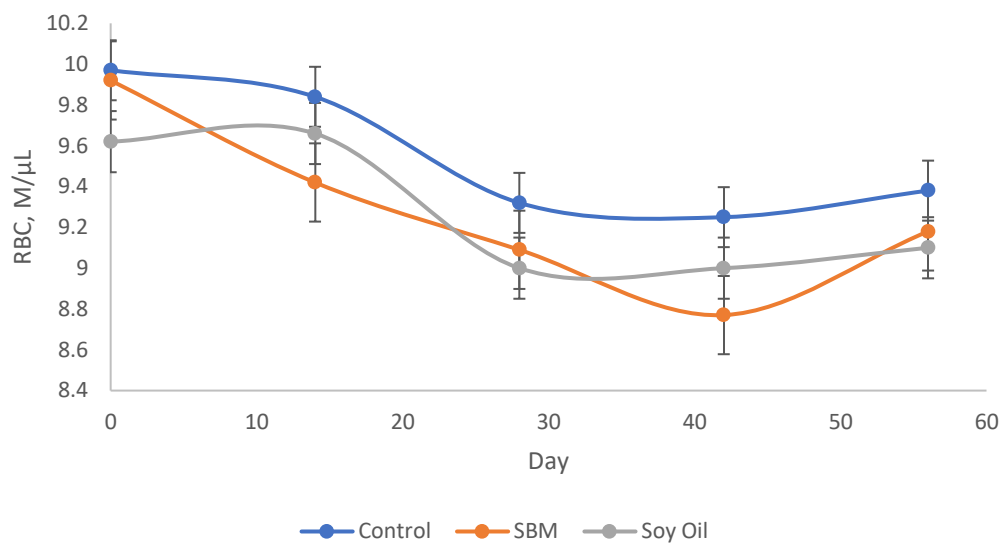


Figure 8. The effects of soybean meal or soybean oil supplementation to growing cattle on red blood cell count during a 56-day growing period

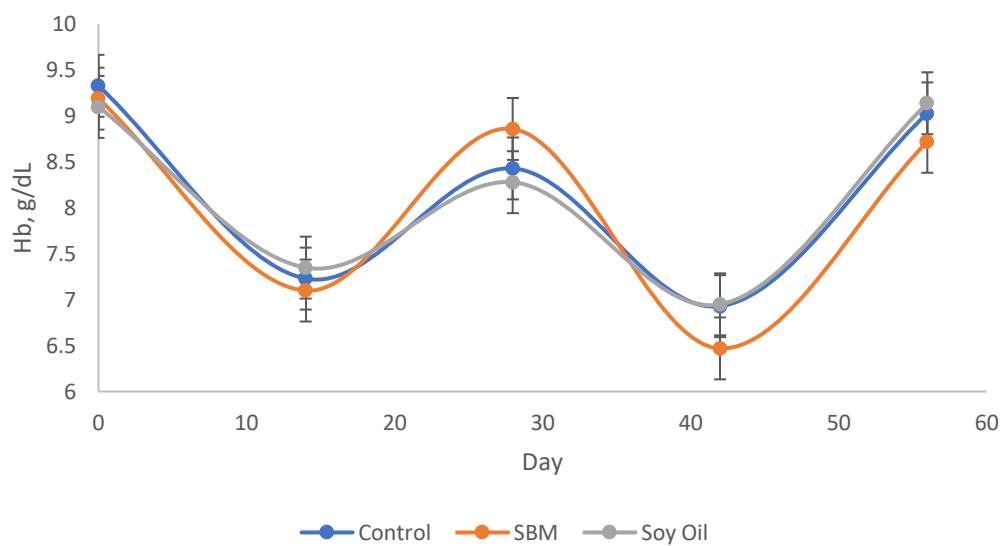


Figure 9. The effects of soybean meal or soybean oil supplementation to growing cattle on body hemoglobin concentration during a 56-day growing period

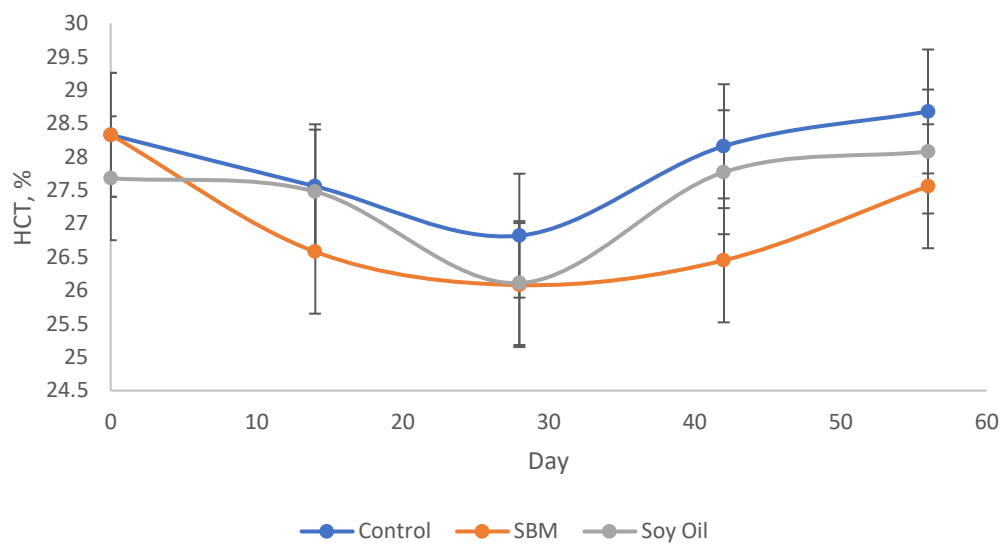


Figure 10. The effects of soybean meal or soybean oil supplementation to growing cattle on hematocrit percentage during a 56-day growing period

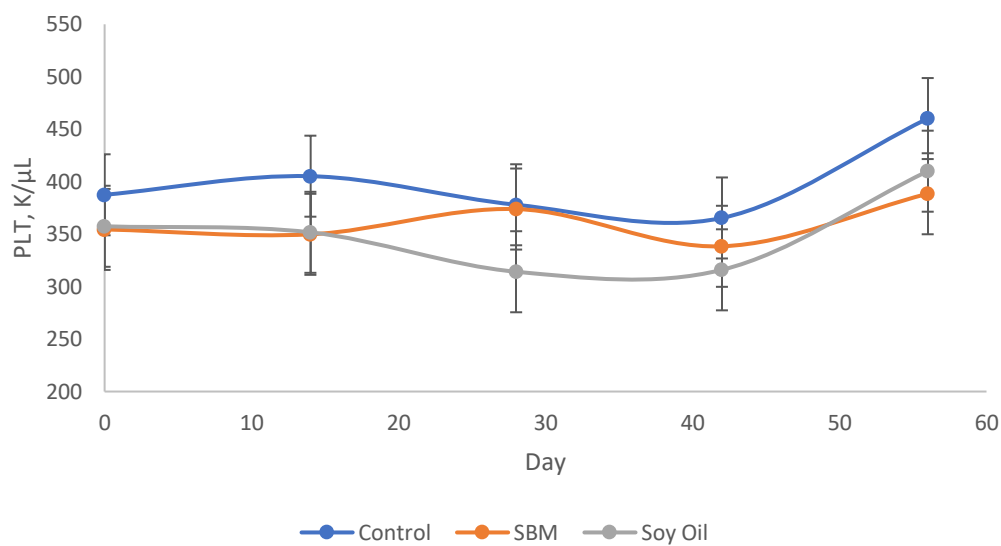


Figure 11. The effects of soybean meal or soybean oil supplementation to growing cattle on platelet count during a 56-day growing period

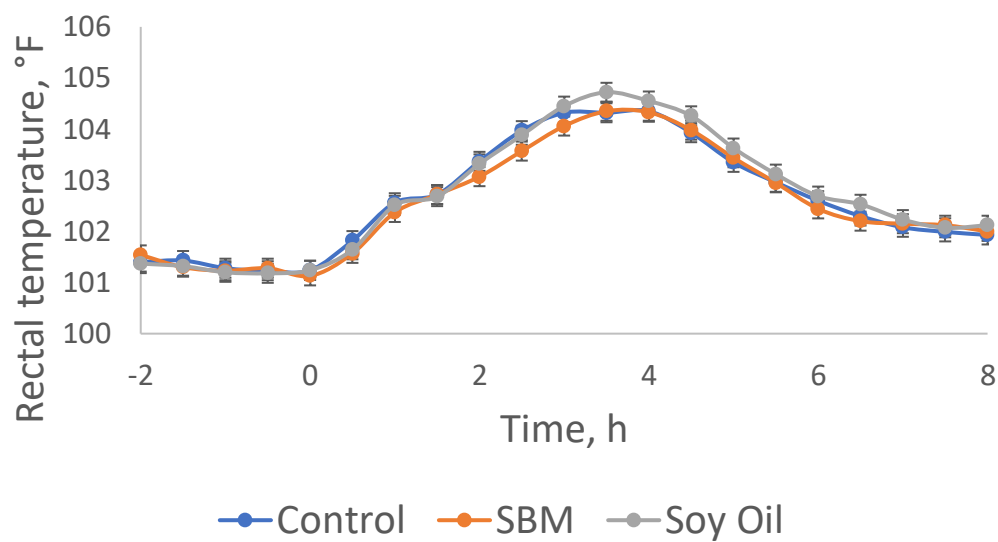


Figure 12. The effects of soybean meal or soybean oil supplementation to growing cattle on body rectal temperature following a lipopolysaccharide injection

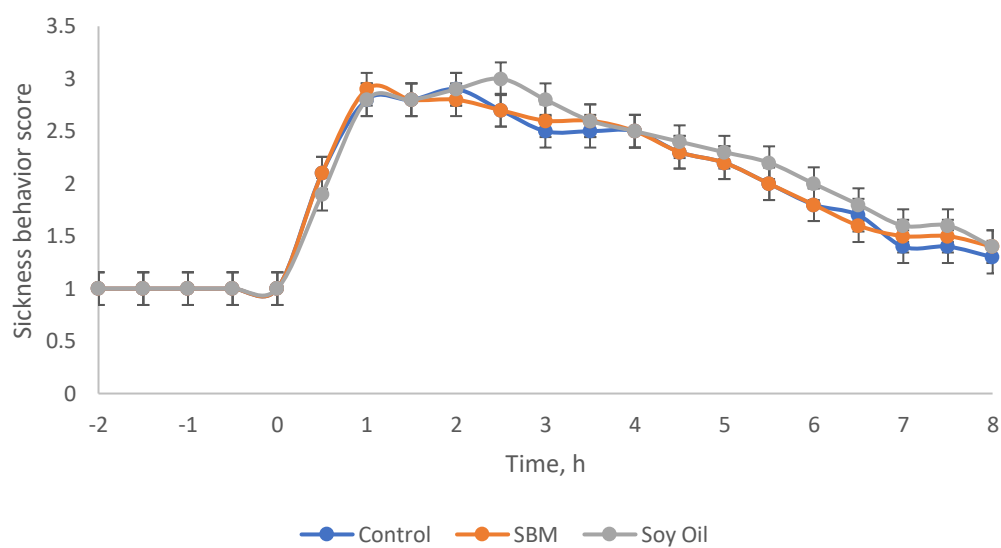


Figure 13. The effects of soybean meal or soybean oil supplementation to growing cattle on sickness behavior score following a lipopolysaccharide injection