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Research Article

Impact of Natural Oil Blend Formulation (NOBF) on Calves during Transportation

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Abstract

Background and Objective: Transportation causes a high level of stress in cattle. Stress is in terms of blood parameters, growth rate, prone to get diseased and mortality. The present trial aimed to reduce the stress level and death in calves occurred during transportation. **Materials and Methods:** Natural Oil Blend Formulation (NOBF) was designed and developed by combining the natural oils having immunomodulating and stress-releasing properties. The NOBF applied to calves before and during transportation. The NOBF feed was prepared by mixing 2 kg of NOBF in the regular meal. A total of ten weeks trial was performed on 24 Brahman breeds of the calves. The growth performance was recorded in the form of ADG and FCR. Blood parameters were tested before, during and at the end of the experiment to determine the stress level and health conditions. Data analysis performed using SPSS software. **Results:** The application of NOBF had enhanced productivity and improved the calves' blood parameters during the transportation and rearing period. The diet of NOBF significantly affected final body weight on day 122. However, it did not affect daily gain ($p > 0.05$). The calves in NOBF treated group obtained 7.78% (12 kg) ($p < 0.05$) higher body weight gain as control during the rearing period. The increase in growth rate had a direct impact on economics, i.e., IDR 540,000 per herd compared to control. **Conclusion:** The obtained results showed that the application of Natural Oil Blend Formulation (NOBF) supplement had worked significantly reducing the stress in transported calves, which resulted in better blood parameters, better productivity and ultimately higher income.

Key words: Natural oil blend formulation (NOBF), transportation, anti-stress, Brahman cross calf, productivity, blood parameters improvement, profitability

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Transportation of cattle is an essential part of the industry cycle. Livestock transportation is developed first by ship, then by rail, road and finally by air¹. During this process, a high level of stress and fear may occur in the farm animal, including physical and physiological stimuli that cause sickness in calves, bruises, disease and economic losses. The US Department of Agriculture estimates that the cattle industry loses 1 billion USD each year from shipping-associated disease². During road transport, the truck environment and truck associated parameters can have characteristics with an underlying predisposition to cause tissue damage, discomfort and added stress. The truck design, stocking density, driver, road quality, ventilation and the ambient temperature of the hauling container are essential factors to consider during an assessment of transport conditions².

Historical accounts relate to high mortality during the early days of transport and concerns for cattle welfare, similar to those today. Behavior, pathology and physiology were used to identify stress in response to transportation¹. The physiological changes that occur during shipping begin with dehydration, lack of feed intake, tissue damage and fume inhalation². The ambient temperature in the transportation environment is a potential cause of dehydration. When the weather is outside the animal's normal critical limits, energy expenditure increases to maintain body temperature homeostasis. While energy is highly required and the access to food and water during the transportation is limited, an unavoidable loss of body weight may occur and increased defecation and urination. At the cellular level, tissue damage results in the release of the membrane phospholipid bilayer and incite an inflammatory cascade at the site of injury through the conversion of lipid membrane into prostaglandins and other metabolites^{2,3}. Physiological measures indicate that stress and inflammatory responses can mediate immune system signaling through changes in the peripheral blood cellular profile. Transportation is accountable for a stress-induced neutrophilia². Empirical evidence shows that the neutrophil (lymphocyte) ratio as the primary adaptive immune functioning cells increased when cattle are handled and transported¹. Agonistic behavior also seems to be decreased by crowding and motion of the truck. Loading, loss of balance and falling are distressful to cattle. For example, the mean heart rates of animals transported on smooth roads are lower than those of cattle transported on the rough country or suburban roads with frequent intersections. Age at transport might also play a role. Young animals (less than four weeks old) do not tolerate

transport as quickly as older cattle and young cattle do not show a typical physiological stress response, as seen in older cows¹.

Essential oils are volatile compounds obtained from aromatic plants; represent a small fraction of the plant's composition. Essential oils have biological properties like antioxidants and anti-inflammatory. The main action targets presented in this review for the therapy of chronic inflammations were the reduction in reactive oxygen and nitrogen species and the decrease in NF- κ B, reducing the expression of proinflammatory cytokines⁴⁻⁶.

Various remedial strategies attempted to decrease cattle response to transportation stress. These include preconditioning, administration of vitamins, vaccines, feeding high-energy diets and electrolyte therapy, but this has not had a significant effect. This study aimed to examine the impact of NOBF, a combination of three essential oils, i.e., *Eucalyptus globulus*, *Pinus sylvestris* and *Lavandula latifolia* to the productivity and blood parameters improvement in calves during its transportation period and the rearing period.

MATERIALS AND METHODS

Study area: The experiment was conducted at PT. Widodo Makmur Cianjur Farm, West Java, Indonesia, under the Faculty of Animal Science, University of Gadjah Mada, Yogyakarta Indonesia. The ten weeks trial was conducted from November, 2018 to January, 2019.

Experimental design and animals: The total study period was 10 weeks. It was distributed into the first two weeks as calf's adaptation and the next eight weeks for the growth period observation. A total of 24 Brahman Cross calves assigned to 2 groups treatment, each treatment consists of 2 replicates with twelve calves each. The procedures as follows: Control with no dietary natural oil blend formulation and treatment with dietary NOBF (2 kg ton⁻¹).

Water and feed were available *ad libitum* daily. Feed intake was recorded daily throughout the experiment period and the average daily meal was calculated. During the growth period, the diet consisted of forage (king grass) and concentration.

Calves were weighed individually on before treatment, week 4 and week 8. Body weight gains are calculated as weight gain (final body weight-initial body weight). Average Daily Gain (ADG) calculated for the growth period and the Feed Conversion Ratio (FCR) were calculated by dividing total feed intake per group by the entire body weight gain per the same animal for the growth period.

Blood samples were collected from the caudal vein at the end of the trial (3 hrs after the morning feeding) by a heparinized syringe. Samples were centrifuged at 3,500 g for 15 min at 4°C and collected plasma was immediately transported to the laboratory and frozen at -20°C until analyzed. Plasma concentrations of glucose, urea-N, total protein, albumin, globulin were determined using an autoanalyzer (Alcyon 300i Abbott, USA).

Statistical analysis: Data analyzed using SPSS software, version 16 (IBM, 2011). Data variables were determined using the T-test comparisons made at a 5% level of significance.

RESULTS

Effect of natural oil blend formulation (NOBF) on calves during the growth period: There is an increase in final body weight in both the control and NOBF group of cattle after 10 weeks of observation. However, the NOBF group performed a higher growth rate. The dry matter intake of NOBF (4.40 kg/day) was higher than control (4.42 kg/day). The average daily gain weight of the NOBF group was higher (0.82) than the control (0.68). The Gain: Feed ratio of the NOBF group was better (0.192) than control (Table 1).

Calves transportation and recovery period: The effect of NOBF on calves' body weight (kg) during transportation and recovery period are shown in Table 2. The initial body weight of control was 70.08 kg whereas of NOBF group was 73.58 kg. After transportation, the weight reduced in control of 65.52 and 69.90 kg in the NOBF group. The data shown in Table 2 states that the weight recovery in the NOBF group was higher (83.25 kg) than control (79.62 kg). It is significantly different to each other. The loss in weight in control was higher than NOBF after transportation.

The dietary of NOBF had body weight heavier ($p < 0.05$) than the control. The NOBF increased body weight until 5.6% (4 kg) compared to control. Body weight after transportation was lower than before the transportation and after the recovery period. No interaction was observed between period and treatment ($p > 0.05$) (Fig. 1). Dietary of NOBF did

not affect body weight loss during transportation and gain of the recovery period ($p > 0.05$). However, the treatment reduced 1.39 kg body weight loss compared to control.

The NOBF effect on the biochemical parameters before transportation shown in Table 3 and after transportation is discussed in Table 4. This study has shown that the supplementation of NOBF had a significant effect on uric acid and albumin content ($p < 0.05$). However, no difference was observed in calcium, phosphorous and total protein between NOBF salt supplementation and control. The level of uric acid and albumin increased during NOBF supplementation before transportation. Hematology evaluation indicated no effect of dietary NOBF. Among biochemical parameters, uric acid was significantly lower in the NOBF group (Table 3). The total protein was slightly higher, whereas albumin content remains the same (Table 4). It shows the biochemical parameters not influenced by NOBF application ($p > 0.05$).

The effect of dietary NOBF on hematological parameters has been discussed in Table 5 (before transportation) and table 6 (after transportation). The blood parameters, like, hemoglobin, hematocrit, leukocyte, erythrocyte, mean corpuscular volume, mean cell hemoglobin, neutrophil and lymphocytes were analyzed before and after transportation. The hemoglobin count in both control and NOBF reduced after transportation. The hematocrit count reduced significantly in both control and NOBF. The leucocyte level increased in both control and NOBF but not significantly. Erythrocyte level increased in control after transportation but reduced in the NOBF group. The MCV count significantly increased after transportation specially in NOBF. The MCH value increased in both control and NOBF significantly. The

Table 1: Effect of NOBF on productivity in Brahman cross calves for 10 weeks

Items	Treatments		SEM	p-value
	Control	NOBF		
Initial BW (kg)	87.55	88.35	1.69	0.932
Final BW (kg)	128.50	137.30	3.71	0.360
DMI (kg day)	4.42	4.40	0.05	0.146
ADG (kg day)	0.68	0.82	0.03	0.942
G:F	0.156	0.192	0.01	0.168

BW: Body weight, DMI: Dry matter intake, ADG: Average daily gain, G:F: Gain: Feed

Table 2: Effect of NOBF on calf's body weight (kg) during transportation and recovery period

Treatments	Periods			
	Before transportation	After transportation	Recovery	Average
Control (kg)	70.08 ± 10.07	65.52 ± 10.31	79.62 ± 11.23	71.74 ± 11.85 ^x
NOBF (kg)	73.58 ± 9.07	69.90 ± 9.40	83.25 ± 10.47	75.59 ± 10.98 ^y
Average	71.83 ± 9.54 ^b	67.71 ± 9.90 ^a	81.44 ± 10.77 ^c	

^{a-c}Mean value with different of superscript within a row differ significantly ($p < 0.05$), x, y: Deal with other of superscript within a column differ significantly ($p < 0.05$)

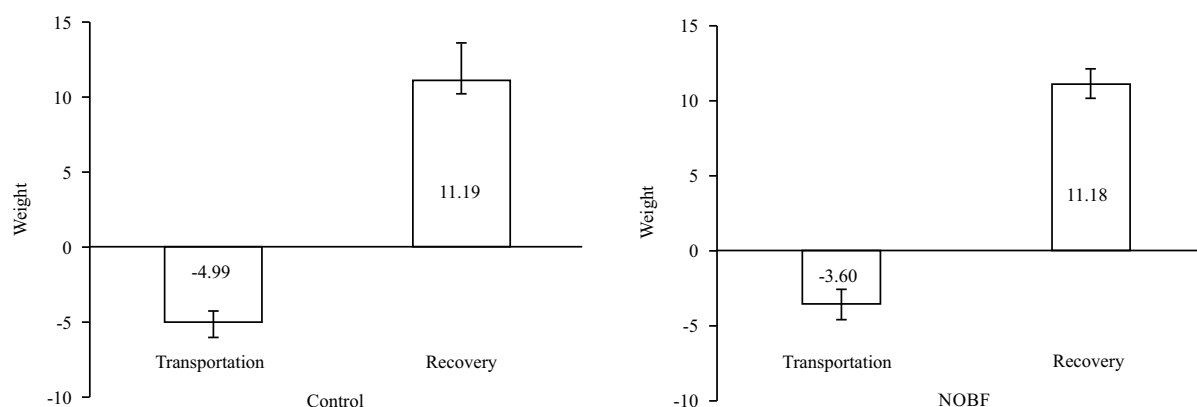


Fig. 1: Effect of NOBF supplementation on body weight during transportation and recovery period

Table 3: Effect of dietary NOBF on the biochemical parameter in calves before transportation

Treatments	Calcium	Phosphorus	Uric acid	Total protein	Albumin
Control	12.499	8.340	1.343	7.294	2.929
NOBF	12.083	8.632	1.912	6.953	3.176
Significant	ns	ns	***	ns	*
p-value	0.427	0.394	0.002	0.256	0.039

Table 4: Effect of dietary NOBF on the biochemical parameter in calves after transportation

Treatments	Calcium	Phosphorus	Uric acid	Total protein	Albumin
Control	11.850	9.179	2.002	7.924	3.455
NOBF	11.691	8.949	1.212	8.296	3.803
Significant	ns	ns	***	ns	ns
p-value	0.751	0.588	0.002	0.254	0.155

Ns: Non significant, ***Differ significantly

Table 5: Effect of dietary NOBF on hematology parameter on calves before transportation

Treatments	Hemoglobin	Hematocrit	Leukocyte	Erythrocyte	MCV	MCH	MCHC	Neutrophil	Lymphocyte
Control	12.68	38.88	11.90	111.15	34.24	11.38	33.28	3.64	8.04
NOBF	12.64	39.64	16.10	110.64	34.86	11.40	33.44	2.87	9.70
Significant	ns	ns	*	ns	ns	ns	ns	ns	ns
p-value	0.967	0.789	0.017	0.577	0.613	0.948	0.689	0.099	0.192

MCV: Mean corpuscular volume, MCH: Mean cell hemoglobin, ns: Not significant, ***Differ significantly

Table 6: Effect of dietary NOBF on haematology parameter on calves after transportation

Treatments	Hemoglobin	Hematocrit	Leukocyte	Erythrocyte	MCV	MCH	MCHC	Neutrophil	Lymphocyte
Control	11.85	4.41	20.88	120.25	37.88	15.82	41.22	8.75	11.86
NOBF	12.00	8.25	26.00	89.67	88.20	22.73	25.80	12.00	12.00
Significant	ns	*	ns	ns	***	*	***	ns	ns
p-value	0.664	0.027	0.312	0.189	0.004	0.022	0.00	0.193	0.928

MCV: Mean corpuscular volume, MCH: Mean cell haemoglobin, MCHC: Mean cell hemoglobin concentration, ns: Not significant, ***Differ significantly

MCHC value increased significantly in both control and treatment. The neutrophil value increased in both the groups whereas lymphocyte value reduced after transportation.

Effect of NOBF on cattle productivity during the feedlot period: The effect of NOBF on cattle productivity and economic analysis during the feedlot period is shown in

Table 7. Dietary of NOBF have a significant effect in final body weight ($p < 0.05$) at day 70. The daily weight gain of NOBF group was higher (1.36 kg) than control (1.26 kg), however, they were not significantly ($p > 0.05$) different. Dietary of NOBF increased body weight 634 kg compared to control 625 kg (12 kg) during the feedlot period. Economic analysis of different benefits from cattle, that dietary NOBF IDR 540,000 per herd compared to control (Table 7).

Table 7: Effect of NOBF essential oils on cattle productivity and economic analysis during the feedlot period

Treatments	Initial body weight	Final body weight	Daily gain per herd (kg)	Total gain per herd (kg) in 70 day	Economic analysis of gain (IDR) per herd
Control (n = 1395)	471	625	1.26	154	IDR 6,930,000
NOBF (n = 1386)	468	634	1.36	166	IDR 7,470,000
Different	3	9	0.10	12	IDR 540,000

1 kg body weight: IDR 45,000, NOBF: 2 kg ton⁻¹, Fattening period: 70 day

DISCUSSION

The inclusion of NOBF resulted in higher weight gain. The natural oil blend acted as an immunomodulator and maintained the body metabolism at the optimum level by reducing the stress level, which resulted in a better growth rate⁹⁻¹³. Dietary of NOBF before the transportation process can compressed body weight loss during transportation; economic losses can be suppressed. Transportation involves livestock stress, including rough handling, mixing with new animals of different ages, lack of feed and drinking water, transport facilities design and poor road conditions, load density, inadequate ventilation, temperature and extreme humidity and wind speed⁷. Therefore, it is challenging to determine which components are most responsible for transport stress⁸.

The intake of NOBF during transportation helped calves optimum metabolism and less energy wastage in body management¹⁴⁻¹⁷. Transportation stress on livestock can occur due to various factors, such as transport duration, cattle density, distance, the weather during transportation, poor road conditions and other factors. The shipment stressed livestock might become hyperactive behavior. More extended transportation of animals will be more experienced in shocks and higher fatigue levels. It is demonstrated by the physiological changes indicated by the abnormal hematological changes. The better biochemical parameters and hematological parameters showed the positive effect of NOBF on cattle metabolism, which resulted in higher weight gain. The lower uric acid showed a better oxidation process. Transportation involves livestock stress, including rough handling, mixing with new animals of different ages, lack of feed and drinking water, transport facilities design and poor road conditions, load density, inadequate ventilation, temperature and extreme humidity and wind speed⁷. Therefore, it is challenging to determine which components are most responsible for transport stress⁸.

Several research and publications support the crucial role of natural oil in reducing stress and increasing the production in cattle⁹⁻¹³. Natural oil acts as an immunomodulator and with anti-pathogenic properties, which ultimately helps in the productivity enhancement¹⁴⁻¹⁷. The improvement in

biochemical and hematological parameters and faster recovery in NOBF groups of calves made a better growth rate.

The addition of NOBF would optimize the digestion process by increasing the rumen's fermentation activity, especially with reducing methanogenesis and energy waste, increasing the production of microbial protein and removing ammonia concentration. Essential oils help to boost rumen microflora proliferation, increases the amount of propionate. It reduces acetate and methane production without changing the total amount of VFA¹⁸⁻²².

The overall better performance of calves fed on NOBF resulted in higher profit. The addition of NOBF (2 kg ton⁻¹ feed) increased the health status and tended to reduce the bodyweight loss during transportation. The NOBF improved the economic value after the 70 days feedlot program.

Even though, the uses of NOBF showed post transportation recovery better than control but at a slower rate. That could be the limitation of using natural products. In terms of recommendations, it would be useful to apply the NOBF formulations since the beginning in calves farming to keep the biochemical and hematological parameters at the optimum level. It would help lower or no stress during transportation and so faster the rate of recovery.

CONCLUSION

The obtained results showed that the application of Natural Oil Blend Formulation (NOBF) mixed in the feed had worked significantly. NOBF helped in reducing the stress in the transported calf, which resulted in better blood parameters, better productivity and ultimately higher income.

SIGNIFICANCE STATEMENT

This study discovered the development of a natural oil blend formulation that can be beneficial for reducing the stress level in the calf. This study will help researchers to uncover the critical areas of weight loss and productivity in cattle farming that many researchers were not able to explore. Thus a new theory on natural oil uses and applications may open an era of drug-free and stress-free farming systems.

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