RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 43 Issue 2: 225-229 (June 2024)

Effect of *Azadirachta indica* and *Phyllanthus niruri* Plant Meal on Performance and Health in Growing Calves

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ABSTRACT

Background: During perinatal period, the immune system of calves is developing, making them vulnerable to infectious diseases that affect their growth or increasing mortality. Phytobiotics have various physiological effects on animals that could improve their performance and health. In this study, the effect of a feed herbal mixture made from *Azadirachta indica* and *Phyllanthus niruri* were evaluated in dairy calves.

Methods: Forty Holstein calves were assigned into four groups (doses of 0, 2, 3 and 4 g d^{-1}) and productive and health parameters were recorded. Results were analyzed according to a completely randomized design in which linear and quadratic effects of daily feed additive dose were tested.

Result: Increasing the dose of herbal mixture linearly reduced final body weight, body weight gain and daily calve starter intake, although it improved feed conversion. Blood tests showed that hematocrit and hemoglobin increased quadratically. The number of segmented neutrophils increased linearly. Finally, the number of pneumonia cases decreased quadratically. These results suggest that supplementation with herbal additives could improve feed efficiency and improve the health status of calves.

Key words: Growth performance, Health, Herbal mixture, Phytobiotics.

INTRODUCTION

In newborn calves, the perinatal period is critical because they have a high risk to develop infectious diseases that affect weight gain or increase mortality (Nehra *et al*., 2018). These animals start their life with an immature immune system, during the first two months as contain a lower number of leukocytes in mucosal tissue (Fries *et al*., 2011) and limited phagocytic activity against infectious agents (Kampen *et al*., 2006). A variety of nutritional strategies can be used in calves during the first few months of life to ensure higher growth performance and better health and to reduce the incidence of infectious diseases (Devant and Marti, 2020). The use of feed supplements, such as phytobiotics, has been associated with improved immunity of early weaned calves and higher daily weight gain associated with digestibility efficiency (Korake *et al*., 2016). Similarly, in experiments with calve leukocytes treated with phytobiotic the results showed an increase in bactericidal activity and reduction of cases of diarrhea in newborn calves (Teixeira *et al*., 2015). The feed herbal mixture composed with the plants *Phyllanthus niruri* and *Azadirachta indica*, have shown antimicrobial, antioxidant and immunomodulatory properties and improved liver metabolism (Lee *et al*., 2016; Islas *et al*., 2020; Kaur *et al*., 2020). Considering all the above, in this research, supplementation of calves with a feed herbal mixture (*Phyllanthus niruri* and *Azadirachta indica*) in the pre-weaning period was evaluated in the context that it could improve performance and health status, by assisting in enteric and respiratory diseases.

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How to cite this article: Pulido-Huertas, S., Mendoza-Martínez, G.D., Espinosa-Ayala, E., Hernández-García, P.A., Martínez-Gómez, D., González-Sánchez, J.F., Martínez-Cortés, I., Velázquez-Cruz, L.A. and Díaz-Galván, C. (2024). Effect of *Azadirachta indica* and *Phyllanthus niruri* Plant Meal on Performance and Health in Growing Calves. Asian Journal of Dairy and Food Research. 43(2): 225-229. doi: 10.18805/ajdfr.DRF-314.

Submitted: 10-02-2023 **Accepted:** 14-04-2023 **Online:** 29-04-2023

MATERIALS AND METHODS.

This research was conducted at the Autonomous Metropolitan University, Xochimilco (UAM-X), within a duration of two years (2020 to 2021). The experimental phase was carried out on a commercial dairy farm in Mexico (25°39′14.4″N 103°27′27.8″W; Altitude 1139 m), the protocol was approved by the Academic Committee of the Doctoral Program in Agriculture and Livestock of UAM-X.

Selection and feeding of calves

Forty Holstein calves were used in this research, all animals ingested colostrum at birth. Passive antibody transfer was assessed by initial total serum protein with digital refractometer (McCracken *et al*., 2017). Calves were housed in individual pens, fed milk replacer (Grupo Nu-3 Alimentos Balanceados, México; 22% CP, 15% Fat) and starter

supplement (Iniciación Premium Destete Precoz; Nuplen, México; 21.5% CP).

Experimental design and/or treatments

Calves were randomly assigned to treatment groups (n= 10 for each group). For 65 days (25 to 90 days of age) calves were supplemented daily with different doses of an herbal mixture with *Phyllantus niruri* and *Azadirachta indica* (Livoliv, Nuproxa México S. de RL de C.V.). The doses were 0, 2, 3 and 4 g d^{-1} which were administered orally individually in gel suspension. During the experimental period, milk replacer and starter were offered daily and water ad libitum. Calves were housed outdoors in individual pens, in a dairy farm localized in northeast of Mexico, in this area animals were exposed to 11 hrs of natural light.

Growth performance and disease incidence

Calves were weighted at the beginning and at the end of the experiment to estimate average daily gain and feed conversion ratio (Dida *et al*., 2019). The intake of milk replacer and starter supplement was recorded daily. On day 65, hip and wither heights and thoracic girth were measured (Korake *et al*., 2016). The clinical cases pneumonia, diarrhea, or otitis were recorded, diagnosed and treated with aminoglycoside or beta-lactam antibiotics.

Collection and analysis of blood samples

In day 65, blood samples were extracted from the jugular vein (pre-prandial 06:00 h), using tubes with EDTA and sodium citrate. Blood was analyzed to perform a complete blood count cell (CBC). The CBC differential leukocyte count and blood count were performed on the QS Kontrolab® EasyVet hematology analyzer.

Statistical analysis

Shapiro-Wilk test was used to test the normal distribution of variables. Initial body weight was used as a covariate in daily gain and final body weight (Steel *et al*., 1997). The results were analyzed according to a completely randomized design testing. Linear and quadratic effect of treatments inclusion levels on response variables were evaluated with non-orthogonal polynomial contrasts. The model used was

$$Yij=\mu+\tau i+eij,$$

μ= Mean value.
τi= Treatment effect (fixed).
eij= Error term.

Non-orthogonal polynomial contrasts used: Linear effect -0.760639-0.084515+0.25354463+0.591608; Quadratic effect +0.4029115-0.644658-0.322329+0.5640761. The data were analyzed using R software (Mirman, 2014).

RESULTS AND DISCUSSION

Growth performance

The values obtained from the evaluation of calve growth performance are shown in Table 1. Supplementation with herbal mixture at a higher dose generated a decrease in final body weight (BW) without modifying the average daily weight gain in calves (linear effect, P=0.05 and P=0.07 respectively). However, a reduction in daily starter intake (linear effect, P=0.0001) and an increase in daily milk replacer intake (linear effect, P=0.0001) were observed, resulting in a significant change in feed conversion ratio efficiency (linear effect, P=0.0004). Hip and withers heights and thoracic diameter were not affected by the herbal-based feed mix. These data are consistent with research in other ruminants, Dida *et al.* (2019), reported no changes in body weight and daily weight gain in goats (BW 14.1±1.5) fed a mixture of *Cajanus cajan* and *Azadirachta indica*. In another study, the incorporation of *Azadirachta indica* fruits in the diet of 10-12 months old rams was evaluated, observing an increase in feed intake and weight gain (Jack *et al*., 2020), in both studies they did not observe changes in feed conversion ratio (Dida *et al*., 2019; Jack *et al*., 2020). The difference observed with that reported by Jack *et al*. (2020), could be explained because in this study adult rams were used. The gastrointestinal tract and microbiota of adult rams, are already developed, compared to the calves used in this study. Jami *et al*. (2013), analyzed microbiomes of adult and young cattle, found differences in the composition and

Table 1: Effect of the supplementation of an herbal feed mixture (*Azadirachta indica* and *Phyllanthus niruri*) on the performance of growing calves.

	0	2	3	4	SEM	P-value	
	g d^{-1}					L	Q
Initial body weight, kg	43.83	41.71	42.37	42.37	1.27	0.39	0.39
Final body weight, kg	91.83	81.57	82.62	83.50	2.86	0.05	0.11
Average daily gain, kg d^{-1}	0.73	0.61	0.62	0.63	0.04	0.07	0.18
Daily starter intake, kg d^{-1}	1.68	0.97	0.84	0.91	0.12	0.0001	0.01
Daily intake of milk replacer, L d^{-1}	2.49	2.85	2.80	2.91	0.04	0.0001	0.05
Feed conversion ratio	2.27	1.55	1.33	1.43	0.09	0.0004	0.04
Withers height, cm	92.16	90.71	90.75	90.87	1.26	0.41	0.57
Hip height, cm	96.83	96.14	97.12	97.00	1.07	0.82	0.65
Thorax diameter, cm	102.83	100.42	101.00	102.75	1.64	0.84	0.17

SEM: Standard error of the mean, L: Linear effect; Q: Quadratic effect.

structure of the bacterial community in the rumen. In adult animals there is a higher proportion of bacteria of the genus *Bacteroides*, *Clostridium*, *Ruminococcus*, *Butyrivibrio* and *Lactobacillus*.

In our study, the feed conversion rate was more efficient, as calves showed a lower intake of starter, with the same weight gains per kg of feed in all treatment groups. The decrease in starter intake in animals supplemented with the herbal mixture could be explained by a physiological modification involved in appetite control. Hussein *et al*. (2011) reported that supplementation of mice, fed a high-fat diet, with *Ilex extract* (50 and 100 mg kg^{-1}) reduced food intake, generating a lower final body weight compared to the control group. In this study, an increase in the levels of leptin and glucagon (GLP-1), hormones involved in appetite inhibition, was observed. Similarly, Amiri *et al*. (2019) reported that supplementation with extract of *Allium cepa* L (150 and 250 mg kg^{-1}) increased food intake and daily weight gain by inhibiting leptin levels and increasing ghrelin, a hormone that stimulates appetite. Considering this information, one explanation for the effects obtained with *Azadirachta indica* and *Phyllanthus niruri* could be the presence of similar components; however, further analysis is needed to explain the phytochemical effect.

Disease incidence

Passive antibody transfer obtained a total serum protein value of 6.6±0.6 g dl^{-1}. The values obtained for disease incidence are presented in Table 2. The number of cases of otitis and diarrhea did not show a linear or quadratic effect due to the influence of the herbal mixture; however, the cases of pneumonia showed a decrease with doses of 2-3 g d^{-1} (quadratic effect, P=0.02) (Table 2). In addition, calves treated with 4 g d^{-1} of herbal mixture showed a decrease in cases of diarrhea. These results could be explained by the broad antimicrobial activity reported for *Phyllanthus niruri* and *Azadirachta indica* (Lee *et al*., 2016; Kharwar *et al*., 2020). For both herbs, an effect against pathogens causing respiratory and gastrointestinal diseases has been reported (Lee *et al*., 2016; Kharwar *et al*., 2020). Considering that many infectious diseases produced by bacteria or viruses, such as pneumonia and diarrhea, occur during calve growth (Nehra *et al*., 2018). Supplementation with *Phyllanthus niruri* and *Azadirachta indica* could be useful to prevent this type of diseases and reduce the use of antibiotics. In most cases, the use of antimicrobial drugs generates side effects, such as bacterial resistance (Durairajan *et al*., 2021).

Evaluation of health status

The hematological parameters of the calves are presented in Table 3. The results showed that supplementation with the herbal mixture significantly increased hematocrit values (quadratic effect, P= 0.03), with a tendency to also increase hemoglobin levels (quadratic effect, P= 0.06), mean corpuscular volume (MCV; linear effect, P= 0.07) and mean corpuscular hemoglobin (MCH; linear effect, P= 0.1). Few studies have analyzed blood parameters in animals treated with extracts based on *Phyllanthus niruri* and *Azadirachta indica*, however Kaur *et al*. (2019) showed similar results. In contrast, Nagalakshmi *et al*. (1999), did not observe changes in the hemoglobin levels of broilers treated with *Azadirachta indica* extract; Gupta *et al*. (2001) observed similar results with administration of *Azadirachta* indica extracts orally in rats for 60 days. Other investigations with mice and rats treated with *Phyllanthus niruri* extracts did not show changes in hematological parameters (Montejo *et al*., 2015; Asare *et al*., 2012).

To evaluate the effect of supplementation with *Phyllanthus niruri* or *Azadirachta indica* on the immune response, the number of blood cells, including cells involved in this response, was evaluated. The results showed that the herbal feed mixture did not modify the number of erythrocytes and white blood cells, but interestingly, it decreased the number of band neutrophils (linear effect, P=0.0001). In addition, supplementation with the herbal mixture showed an increase in the number of segmented neutrophils (linear effect, P=0.09) and monocytes (quadratic effect, P=0.24). These results suggest that supplementation could enhance the immune response involved in the control of infectious diseases, as observed in the pneumonia cases in our experiment. A similar phenomenon was observed in broiler chickens and carp fed *Azadirachta indica* extract, evidently increasing the number of WBC (Kwawukume *et al*., 2013; Kaur *et al*., 2019). A previous study showed that an extract of *Phyllanthus niruri* increased the functional maturation of dendritic cells (Nworu *et al*., 2010). Furthermore, in consensus with the data observed in calves, previous studies showed that both *Phyllanthus niruri* and *Azadirachta indica* extracts do not modify the number of WBC in mice (Haque *et al*., 2006 and Montejo *et al*., 2015).

Table 2: Effect of the supplementation of an herbal feed mixture (*Azadirachta indica* and *Phyllanthus niruri*) on the clinical cases of the growing calves.

	0	2	3	4	SEM	P-value	
	g d^{-1}					L	Q
Otitis, number cases	7.17	3.86	8.37	2.87	3.20	0.52	0.81
Pneumonia, number cases	5.17	2.71	1.12	6.75	1.72	0.94	0.02
Diarrhea, number cases	0.02	0.70	0.87	0.37	0.46	0.39	0.22

SEM: Standard error of the mean, L: Linear effect; Q: Quadratic effect; Number of clinical cases per group in a 65-day period.

Table 3: Effect of the supplementation of an herbal feed mixture (*Azadirachta indica* and *Phyllanthus niruri*) on the blood biometry in growing calves.

	0	2	3	4	SEM	P-Value	
	g d^{-1}					L	Q
Hematocrit, %	34.16	38.07	37.12	35.56	1.10	0.31	0.03
Hemoglobin, g dL^{-1}	11.76	12.90	12.55	12.15	0.35	0.40	0.06
Erythrocytes, × 10^{12} L^{-1}	5.50	5.65	5.36	5.12	0.27	0.24	0.39
Mean corpuscular volume, fl	63.40	67.37	69.75	69.55	2.41	0.07	0.65
Mean corpuscular hemoglobin, pg	21.83	22.82	23.61	23.77	0.96	0.10	0.87
MCHC* g dl^{-1}	34.43	33.88	33.83	34.20	0.27	0.35	0.10
Platelets, × 10^{9} L^{-1}	380.83	397.00	465.88	435.88	43.09	0.20	0.86
Erythrocyte sedimentation rate ml/h	1.43	0.83	0.00	0.00	0.84	0.35	0.45
White blood cells (WBC), × 10^{9} L^{-1}	10.11	9.80	10.20	11.28	1.04	0.45	0.38
Lymphocytes, × 10^{9} L^{-1}	46.50	33.85	44.87	37.12	4.23	0.23	0.39
Monocytes, × 10^{9} L^{-1}	3.83	5.00	5.25	4.25	0.90	0.55	0.24
Segmented neutrophils, × 10^{9} L^{-1}	4.00	5.47	4.51	5.48	0.46	0.09	0.58
Band neutrophils, × 10^{9} L^{-1}	0.68	0.30	0.18	0.06	0.09	0.0001	0.53
Eosinophils, × 10^{9} L^{-1}	2.66	3.42	2.75	3.12	0.90	0.79	0.75
Basophils, × 10^{9} L^{-1}	0.16	0.00	0.12	0.00	0.11	0.35	0.78
Plasma proteins, g L^{-1}	9.25	8.91	8.45	9.02	0.26	0.23	0.16

SEM: Standard error of the mean, L: Linear effect; Q: Quadratic effect; * MCHC: Mean corpuscular hemoglobin concentration.

Finally, considering all results obtained in this research, is possible suggest that supplementation with *Phyllanthus niruri* or *Azadirachta indica* mixture represent an option to control infectious diseases in calves. The low proportion of band neutrophils with an increasing tendency of segmented neutrophils in the blood of growing calves, indicates that this phytobiotics could have components that favor the maturation of immune response cells.

CONCLUSION

The supplementation with *Azadirachta indica* and *Phyllanthus niruri* in growing calves, is useful to make more efficient the feed conversion (calves treated decreased their starter supplement intake, without altering their increasing in body weight per food consumed). Also, health status, in supplement animals showed an increase. In these animals the number of segmented neutrophils was increased. This could be related with the immune response against infectious agents in calves where the number of pneumonia cases showed a decrease. All data suggest that the herbal feed mixture had positive effects on the health aspects of the calves, but these effects were not seen in increased weight gains.

Conflict of interest

All authors declare that they have no conflict of interest.

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