

Immunoglobulin G response and performance in Holstein calves supplemented with garlic powder and probiotics

T.W. Kekana^{1#}, V.F. Nherera-Chokuda², J.J. Baloyi³ & C.M. Muya⁴

¹Western Cape Department of Agriculture, SAET, P.O. Box 313, Oudtshoorn, 6625, South Africa

²NERPO, 160 Garsfontein, Pretoria. 0081, South Africa

³School of Agriculture, University of Venda, P/Bag X5050, Thohoyandou, 0950, South Africa

⁴Agriculture Research Council-Animal Production, P/Bag X 02, Irene, 0062, South Africa

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Abstract

The study evaluated the effects of garlic, probiotics, and in combination on levels of immunoglobulin G (IgG) and growth performance in new-born Holstein calves. Thirty-two Holstein calves were randomly allocated to treatments at four days old and were maintained on them until they were 42 days old. The treatments consisted of control (C), garlic powder at 5 g/calf/day (GA), probiotics at 4 g/calf/day (PB), and the combination of garlic and probiotics (GP). Bodyweight, body length and heart girth measurements were taken to determine growth and blood was drawn to determine glucose and IgG. Faecal score and body temperature were recorded daily. Calves in GA and GP had higher IgG levels than calves in C and PB (28.0 g/L and 27.5 g/L versus 23.5 g/L and 25.5 g/L, respectively). Calves in GP and PB groups had lower faecal scores than C and GA (2.1 and 2.1 versus 2.3 and 2.2, respectively). Supplementation of GA, PB, and in combination did not affect feed intake and growth performance negatively, but improved serum IgG levels. Higher serum IgG in GP may indicate an improved intake and utilisation of nutrients that are responsible for immunity modulation and regulation. Probiotics and their combinations with garlic have the potential to reduce the incidence of diarrhoea when fed to young calves.

Keywords: dairy neonates, direct-fed microbes, natural herb

#Corresponding author: ThapeloK@elsenburg.com

Introduction

The cost of rearing replacement heifers is high, representing a quarter of dairy farm expenses (Drackley *et al.*, 2004). Reducing exposure to pathogens, improving immunity, and applying chemotherapeutic agents are focus areas in calf and heifer management (Earley & Fallon, 1999). Calves have little or no immunity at birth, and colostrum is a crucial source of immunoglobulins for initiating innate defence systems (Lawrence & Pierce, 1983; Piccone *et al.*, 2009). However, early establishment of innate immunity in confined dairy calf management systems is often associated with failure of availability of colostrum and a low rate of intestinal absorption of IgG (Godden, 2008; Piccone *et al.*, 2009). Antibiotics are vital in pathogen control. However, concerns about antibiotic residues in animal-sourced feeds and pathogen resistance are increasing, and alternative eco-friendly strategies are needed (Calsamiglia *et al.*, 2000).

Probiotics alone are safe and effective feed additives that affect the performance of calves favourably and are highly recommended as an alternative to antibiotics (Zhang *et al.*, 2015; Zhong *et al.*, 2019). However, in semi-intensive feeding systems, the use of supplemental micro-nutrients, antibiotics, and even probiotics is limited.

Natural herbs enhance performance and health in dairy cattle and could serve as alternatives to both antibiotics and probiotics (Kekana *et al.*, 2019; Seifzadeh *et al.*, 2019). Supplementation with garlic (*Allium sativum*) enhances detoxification and restores physical strength and resilience (Amagase *et al.*, 2001; Calsamiglia *et al.*, 2000). The anti-parasitic, insecticidal, anti-oxidant, anti-inflammatory, bactericidal and hypoglycaemic characteristics of garlic result from allicin, an active secondary metabolite (Ghosh *et al.*, 2010). Allicin is formed catalytically when garlic cloves are crushed and the enzyme alliinase of the bundle

sheath cells mixes with its substrate, alliin, which is released from mesophyll cells (Lawson, 1996). Many commercial garlic preparations are available, including garlic powder. Garlic powder is a preparation of sliced, dried, and pulverized garlic cloves, which forms allicin when added to water or milk (Lawson, 1996).

Church (1976) and Busquet *et al.* (2005) postulated that direct-fed microbes and secondary metabolites of plants have similar effects on rumen development. Novak *et al.* (2012) reported that when combined with other supplements, direct-fed microbials could synergistically affect the immunity of calves. Mixtures of herbs, including garlic powder, combined with probiotics have improved blood glucose, beta hydroxyl butyrate and total serum proteins in dairy calves (Seifzadeh *et al.*, 2019). Therefore, this study aimed to investigate IgG response and growth of pre-weaned Holstein calves supplemented with probiotics, garlic powder, and in combination.

Materials and Methods

The experiment was conducted at the Agricultural Research Council–Animal Production Institute (ARC-API) Irene, South Africa (S: 28°13'0" and E: 25°55'0", altitude 1523 m). It was approved by the ARC-API Ethics Committee (APIEC 12/008).

Thirty-two healthy new-born Holstein calves (36.2 ± 4.5 kg) were separated from their dam, after parturition and cleaning, and taken to the calf unit, where they received colostrum (2 L at 09h00 and 2 L at 14h00) for three days. All colostrum was tested with a colostrometer™ (Biogenics, Napa, Calif, USA), which indicated that it was of high quality. On day 4, the calves were stratified by birth weight and randomly allocated to one of four treatments (two males and six females per treatment). The treatments consisted of i) no additives (C); ii) 5 g/calf/d/garlic powder (GA), iii) 4 g/calf/d/probiotic (PB), and iv) 5 g/calf/d/garlic powder + 4 g/calf/d/probiotics (GP). One half of each treatment dosage was fed at each of two daily feedings of milk (9h00 and 14h00) from day 4 until day 35. From days 4 to 7 the calves were fed 2 L of whole milk (3.23% CP, 3.47% fat, and 4.69% lactose) at 08h00 and 14h00. From days 8 to 35, the calves were fed 3 L of milk at 8h00 and another 3 L at 14h00. From days 36 to 42 the calves were fed 4 L of milk at 08h00, only.

The probiotic used contained *Lactobacillus bavaricus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus coryniformis*, *Lactobacillus curvatus*, *Lactobacillus sake*, *Streptococcus* species and *Leuconostoc* species with a total viable count of 1.3 x 10⁷ cfu/g. It was fed according to the manufacturer's guidelines (4 g/calf/d). Garlic powder was purchased from Deli Spice (Cape Town, South Africa). According to the company, the cloves of *A. sativum* were dried under shade and made into coarse powder (0.4 mm), which contained 3.9 g/100 g moisture, 19.5 g/100 g protein, 0.53 g/100 g fat, ash of 3.4 g/100 g, 1.1 mg/100 g zinc, 12.5 mg/100 g selenium, 40.8 mg/100 g vitamin C, and 7.6 mg/100 g vitamin E. The main active ingredient of this powder was allicin (an organo-sulfur compound) at 1.5 mg/100 g dry matter (DM). A recommended garlic dosage level for dairy calves was not found, and thus the garlic dose in the current study was extrapolated from the recommended dose for human consumption, which is 6.5 g for a person weighing 65 kg (O'Gara *et al.*, 2000).

Commercial calf starter consisted of 91.2% DM, 17.5% crude protein (CP), 18.75% neutral detergent fibre (NDF), 8.69% acid detergent fibre (ADF), 0.65% calcium (C), 0.44% (P), 1.17% potassium (K), 0.22% magnesium (Mg), 250.16 ppm iron (Fe), 46.08 ppm manganese (Mn), and 10.28 ppm copper (Cu). (Meadow Feeds, Randfontein, South Africa, registration no. V 12012) and fresh water were available ad libitum from days 4 to 42. The calves were housed individually in 15 m² pens throughout the trial. Every pen had a plaited rubber matt and grass hay on the concrete floor as bedding. Daily intake of the starter, milk and water were measured throughout the experiment to determine feed conversion ratio (FCR). Bodyweight (BW), heart girth (HG), and body length (BL) were measured weekly. Heart girth was defined as the circumference of the thoracic cavity immediately behind the fore limbs and BL was the distance from point of the shoulder to the point of tuber ischia (Ugur, 2005). The faecal scoring system used a 4-point scale (Larson *et al.*, 1977). Faeces were scored and body temperature was measured daily. On days 2, 12, 22, 32, and 42, blood samples were collected from the jugular vein using vacutainer tubes that contained lithium heparin. The blood samples were centrifuged at 2500 rpm until the serum was separated from the cellular constituents, after which the serum was stored at -20 °C pending analysis. The levels of IgG in serum were analysed as described by Morrill and Howard (2012). Glucose was determined using the Accu-Chek Easy (ACE) method (Rumsey *et al.*, 1999).

Intake, measures of growth, serum IgG and glucose, body temperature and faecal score was analysed as repeated measures using the PROC MIXED model (SAS Institute Inc., Cary, North Carolina, USA). The daily observations were pooled by week before being analysed. The statistical model included treatment as a fixed effect, calf within treatment as a random effect, and time and its interaction with treatment as additional fixed effects. Time was modelled as a first-order autoregressive effect. Dry matter intake of starter (g/kg of bodyweight), average daily gain (ADG), initial and final BW, and days and frequency of diarrhoea were

subjected to ANOVA using PROC GLM (SAS Institute Inc., Cary, North Carolina, USA). Significance was declared at $P < 0.05$ and tendencies were considered to exist at $0.05 < P < 0.10$.

Results and Discussion

Dry matter intake of starter ranged from 0.22 kg/d for control calves to 0.27 kg/d for calves fed GP, but did not differ among treatment groups. However, calves fed GP tended to consume more ($P < 0.10$) starter (kg/d and kg/kg of BW) and water compared with those supplemented with C and GA (Table 1). Additives did not affect energy intake. However, there were effects ($P < 0.001$) of week and interaction between treatment and week for DMI of starter (Table 1). When DMI of starter was evaluated weekly, differences occurred during the last week of the trial, in which supplemented calves consumed more starter (kg/d) than control calves (Figure 1). Average water intake and FCR were not affected by treatment. However, calves did consume more water and starter as they grew ($P = 0.01$). Consumption of feed, milk, and water, and growth performance are presented in Table 1.

Table 1 Starter, milk and water intake, and growth performance of Holstein calves as affected by garlic and probiotics supplementation

Parameters	Treatment				SEM	P-value		
	C	GA	PB	GP		T	Week	T x Week
<i>Feed intake</i>								
Starter DMI (kg/day)	0.22	0.23	0.25	0.27	0.02	0.17	<0.001	0.01
Starter DMI (g/kg of BW)	4.8	4.9	5.2	5.6	0.42	0.09	-	-
Milk intake (l/day)	5.00	5.23	5.43	5.23	0.58	0.76	0.54	0.85
Water intake (l/day)	2.4	2.45	2.24	2.48	0.18	0.74	0.01	0.12
ME intake (MJ/day)	95.0	95.2	95.6	95.9	0.32	0.19	<0.001	0.02
FCR	0.52	0.48	0.32	0.27	0.11	0.22	0.006	0.51
<i>Growth performance</i>								
Initial BW (kg)	35.6	37.1	37.4	36.8	1.82	0.09	-	-
Final BW(kg)	56.0	56.6	59.0	60.3	1.33	0.34	-	-
ADG (kg)	0.48	0.46	0.57	0.53	0.05	0.41	-	-
Body length (cm)	72.7	72.6	72.9	72.3	0.92	0.67	0.04	0.54
Heart girth(cm)	84.3	84.0	83.9	84.1	0.57	0.72	0.04	0.61

C: control, GA: garlic, PB: probiotics, GP: garlic + probiotics, SEM: standard error of means. DMI: dry matter intake, ME: metabolizable energy, FCR: feed conversion ratio, ADG: average daily intake, BW: bodyweight

Average and weekly growth parameters (Table 1 and Figure 1) were not affected by the treatments ($P > 0.05$). However, there were notable effects of week ($P = 0.04$) on BL and HG. On average, serum IgG increased ($P < 0.05$) with supplemented groups, in which the GA and GP groups were the higher than PB.

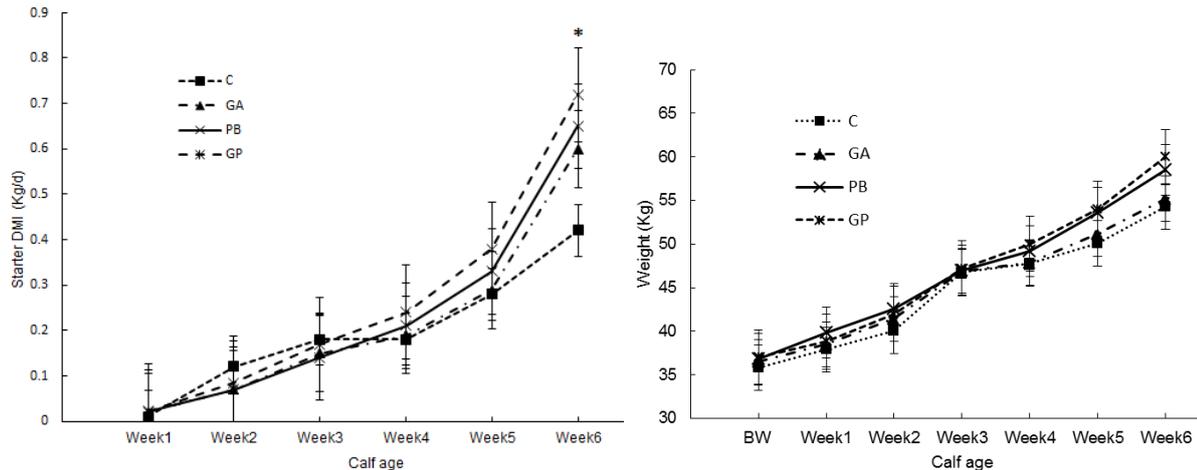


Figure 1 Starter dry matter intake and average weight of the calves fed control, garlic, probiotics and in combination over 42 days

GA: garlic; PB: probiotics; G+P: garlic plus probiotics

Starter DMI for C at week 5 was lower than the rest of the treatments ($P < 0.05$)

When evaluated weekly, the IgG levels increased with the age of the calves (Table 2). Group GA did not differ from GP, but both were higher ($P < 0.05$) than C and PB. Calves fed GA treatment tended ($P = 0.09$) to increase blood glucose with significant week effect. Faecal score in Group P did not differ from GP, but both were higher than C, whereas there was no difference between C and GA. Diarrhoea frequency tended to be higher in C ($P = 0.07$).

Table 2 Serum IgG, glucose levels, body temperature and diarrhoeal incidence of Holstein calves as affected by garlic and probiotics supplementation

Parameters	Treatment				SEM	<i>P</i> -value		
	C	GA	PB	GP		T	Week	T x Week
Average serum IgG (g/l)	23.5 ^c	28.0 ^a	25.5 ^b	27.5 ^a	0.48	0.02	<0.001	<0.001
Day 02	12.3	12.4	13.8	11.5	1.08	0.36	-	-
Day 12	16.9 ^b	20.2 ^a	18.7 ^a	18.9 ^a	1.08	0.02	-	-
Day 22	22.3 ^c	27.3 ^a	24.3 ^{bc}	26.7 ^{ab}	1.08	0.01	-	-
Day 32	28.7 ^b	35.3 ^a	31.2 ^b	36.3 ^a	1.08	0.03	-	-
Day 42	36.1 ^c	44.8 ^a	39.5 ^b	43.6 ^a	1.08	0.03	-	-
Average serum glucose (mmol/L)	5.2	6.2	5.6	5.1	0.41	0.09	0.01	0.21
Average body temperature (°C)	38.7	38.5	38.4	38.5	0.08	0.39	0.62	0.51
Average diarrhoea days	7	4	3	4	1.11	0.41	-	-
Diarrhoea frequency	2	1.1	1.1	1.0	0.30	0.07	-	-
Days fed electrolytes	5	3	2	3	0.83	0.72	-	-
Faecal score	2.3 ^a	2.2 ^{ab}	2.1 ^b	2.1 ^b	0.03	0.04	0.52	0.63

^{a,b,c} means with the same superscripts across the row do not differ at ($P < 0.05$). C: control, GA: 5 g: garlic, PB: 4 g: probiotics, GP: 5 g: garlic + 4 g: PB, IgG: immunoglobulin G; SEM: standard error of means

The GA, PB, and GP treatments did not affect feed, milk and water intakes in the current study. Nonetheless, the effects of garlic on DMI have been variable, with some authors reporting no effect on DMI (Khalelisizadeh *et al.*, 2011; Wanapat *et al.*, 2008) and others reporting improved DMI (Ahmed *et al.*, 2009; Hassan *et al.*, 2013). Similarly for PB group, Masucci *et al.* (2011) reported no effects on DMI while Di

Francia *et al.* (2007) observed improved feed intake and general performance. The disparity responses with feed additives on DMI have been attributed to the quality, dosage and mode of preparation for GA (Lu *et al.*, 2010) and species of microorganisms and their number in the PB products (Uyeno *et al.*, 2015). The observed tendency to increase both DMI and water intake in GP-supplemented calves confirmed a positive correlation, as reported by Soltan (2009). Nocek *et al.* (2003) hypothesized that the effects of garlic were similar to those of probiotics in terms of the balanced gut microbial populations and the subsequent overall digestive stimulant action. Overall digestive stimulant action signifies the balance on microbial populations and their ability to enhance the activity of pancreatic lipase (Rao *et al.*, 2003).

In the present study, final bodyweight did not respond to GA, PB, and GP treatments. This could be owing to the unaffected DMI that was observed in treated calves. Moreover, all calves from the present study had lower daily gain and feed intake than the breed standard for Holstein calves (Akayezu *et al.*, 1994). The low feed intake and lower BW gain throughout the trial could be attributed to low birth weight of the calves used in this trial. The average BW (36.2 kg) of the calves in the present study was 5 - 7 kg lower than the breed standard of Holstein (42.76 kg) (Aksakal & Bayram, 2009). It is well known that lighter birth weight decreases capability for adaptation to solid feed, resulting in increased morbidity because of disease (Morrill *et al.*, 1995). Nonetheless, the effects of P in calves have been associated with increased feed efficiency and weight gain (Lesmeister & Heinrichs, 2005; Timmerman *et al.*, 2005).

Serum IgG levels increased with age in all calves in agreement with a previous report (Athanasidou & Kyriazakis, 2004), although the noticeable IgG surge was in calves that received garlic-supplemented diets (G and GP). These results indicate that garlic-fed calves were in a healthier state than control calves. The increased effect of garlic supplementation on serum IgG was because of antioxidants such as alliin. Alliin modulates and regulates the early activation steps in immune development (Arreola *et al.*, 2015) and enhances stimulation of B-cells and the secretion of interleukin (Washiya *et al.*, 2013). The commonly recognized characteristic of B cells is their ability to produce antibodies (LeBien & Tedder, 2008). B cells are required for optimal T cell activation to certain antigens including low dose foreign proteins, pathogen challenge, and auto-antigens. Furthermore, their presence facilitates the genesis of the immune system, and maintains its integrity (Bouaziz *et al.*, 2007). High serum IgG was observed in supplemented calves at each sampling, except on day 23 for PB. Probiotics are reported to act as adjuvants to the immune system and therefore stimulate IgG production (Naqid *et al.*, 2015). However, reports on the effects of P on immunity have been equivocal. Likewise, Athanasidou and Kyriazakis (2004) and Naqid *et al.* (2015) reported increased serum IgG levels, while Masucci *et al.* (2011) observed no effects in calves' immune-status with PB supplementation. From these studies, the authors believe the discrepancies were because of varying concentrations of microbes, which determine the intensiveness of stimulus for immune cells, which affected the levels of circulating IgG. The unaffected serum glucose levels that were observed were within the recommended ranges (5.1 to 6.2 mmol/L) (Al-Saiady, 2010; Meyer *et al.*, 2009).

Reduced faecal score with P supplementation concurs with previous reports (Görgülü, 2003) and is attributed to antimicrobial-like compounds that are believed to act against gut pathogens (Roodposhti & Dabiri, 2012). Surprisingly, garlic supplementation exhibited minimal effects on faecal score amid numerous reports about its anti-bacterial properties against *Escherichia coli* and *Salmonella species* (Ahmed *et al.*, 2009) and inhibitory effects on coliform (Nikolic *et al.*, 2004). From this, it is clear that the daily supplementation of garlic at 5 g that was used in the current study did not supply sufficient active alliin compound. In the study of Lu *et al.* (2011), the inhibition from garlic powder extracts was proportional to the concentration of the organosulfur compounds, suggesting that the antimicrobial effect was dependent on the number of sulfur atoms in the diallyl sulphides (alliin). This verified that organosulfur compounds (diallyl sulfides and thiosulfinates) contributed most to the antimicrobial effect of garlic, substantially more than phenolic compounds. The significant decrease of faecal score in calves fed GP could be explained by the synergic effects of garlic and probiotics. This combination could be beneficial to pre-weaned calves for diarrhoeal treatment, because lactic acid bacteria (*Lactobacillus species* as major bacteria of the probiotics) are microorganisms that are resistant to the inhibitory effects of garlic (Rees *et al.*, 1993). In an in vitro study, supplementation with garlic powder induced the growth of *Lactobacillus* bacterial species in the gut and thus modulated gut microbiota (Filocamo *et al.*, 2015).

Conclusion

Supplementation of garlic powder alone and in combination with probiotics improved serum IgG, showing potential to improve calf health. Additionally, higher serum IgG in GP revealed recommendable synergistic effects on the utilization of nutrients responsible for immunity modulation and regulation. Feeding a combination of garlic powder and probiotics increased immunity and reduced faecal score in dairy calves.

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Authors' Contributions

WTK, CMM and JJB contributed to the idea, design and study execution. FVN-C and CMM helped in starter feed, milk composition and statistical analysis. All co-authors participated in interpretation of the results of the study and construction of the manuscript.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest and confirm that the manuscript has been read and approved by all the listed authors.

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