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**Effect of feeding an immune modulator to multiparous Holstein cows during the dry period
and early lactation on health, milk and reproductive performance**

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Highlights

- The dry period immune status will carry over into the next lactation and alter performance and postpartum health.
- OmniGen-AF[®] modulate innate and adaptive immune markers in ruminants.
- An immunomodulatory feed additive resulted in significant improvements in daily milk yields.

Abstract

In this study we evaluated the effect of feeding an immune modulatory feed additive (OmniGen-AF[®]; OMN) during the dry period through early lactation on postpartum health, subsequent lactational and reproductive performance of multiparous Holstein cows. We hypothesized that feeding OMN beginning in the dry period and through 150 days in milk (DIM) would improve milk yield and reproductive performance as a result of improved immune response. To test our hypothesis, 1,392 multiparous pregnant Holstein cows of a commercial dairy herd were enrolled and randomly assigned at dry-off (47.6 ± 9.4 d before calving) to OMN (n = 706) or control (CON, n = 686) treatments. Both OMN and CON (placebo) supplements were fed once per day at a rate of 56 g/d per head from dry-off through 150 DIM. Cows were milked three times a day and daily milk yield was measured once per week, starting at the second week of lactation through 140 days in milk. Postpartum health disorders were monitored daily throughout the experiment. Cows were bred following a double OvSynch protocol after a 77-d voluntary waiting period (VWP). Final pregnancy status was assessed after supplementation ended at 230 DIM. Our results indicated that cows supplemented with OMN produced more milk than CON (40.8 kg/d vs.

40.1 kg/d; $P < 0.01$) during the first 140 d of lactation. Once they entered lactation, cows supplemented with OMN tended ($P < 0.10$) to have a reduced incidence of mastitis, retained placenta, displaced abomasum ($P < 0.11$) and reduced number of days spent in the hospital pen relative to CON. OmniGen feeding was also associated with a 10-d reduction in days open ($P < 0.05$) compared to CON. However, pregnancy rate at first, second and until ~230 DIM did not differ between treatments. In conclusion, supplementing an immunomodulatory feed additive to dry and early lactation dairy cows was associated with improved milk yield, a tendency to reduce transition cow disorders and reduced number of days open.

Abbreviations: OMN, Omnigen-AF; CON, Control; DIM, days in milk; VWP, Voluntary wait period; TMR, Total mixed ration; SCC, somatic cell count.

Key words: Milk, reproductive performance, health, OmniGen-AF[®].

1. Introduction

The dry period, a 6 to 8 week non-lactating period between two successive calvings, is essential for maximizing subsequent milk performance as it facilitates cell turnover and active replacement of damaged and senescent secretory mammary epithelial cells (Capuco et al., 1997). The dry period also coincides with the transition period which is commonly defined as the 3 weeks before and the 3 weeks after parturition (Drackley et al., 1999). The dry and transition periods are physiologically challenging and determinant for subsequent milk performance. Dairy cattle commonly experience immunosuppression during the transition period which makes them more susceptible to opportunistic pathogens and ultimately prone to diseases (Wu et al., 2017). Further, diminished immunocompetence during the transition period was not only reported to increase the number of new infections but may also result in a subclinical disease surfacing into clinical

(Kimura et al., 1999) in turn impairing subsequent milk yield and quality (Goff and Horst, 1997; Drackley, 1999). In addition, cows experiencing a postpartum disease or infection, such as mastitis or metritis, generally have poorer reproductive outcomes compared with healthy cohorts, despite the fact that the issue was resolved early in lactation before breeding (Santos et al., 2004; Sheldon et al., 2008).

Collectively, evidence suggest that dry and transition perio immune status will carry over into the next lactation and impact subsequent performance and postpartum health. However, information about the lingering impact of compromised immune status during the dry period on subsequent reproductive performance is scarce. Previous studies conducted by our group and others indicated that established markers of immune function, such as proinflammatory cytokines, are improved with feeding an immunomodulatory supplement, OmniGen-AF® (OMN; Phibro Animal Health Corporation, Teaneck, NJ), before and during the dry period (Brandão et al., 2016; Fabris et al., 2017b). Moreover, OMN supplementation at the recommended dose was shown to maintain the peripheral blood neutrophil function in transition cows (Wu et al. 2017). Feeding OMN when cows are exposed to high temperature-humidity index (THI) during the dry period was also reported to reduce the negative effects of heat stress on subsequent milk performance (Fabris et al., 2017a). With improvements in immune status from dry period to mid-lactation there appears to be a nexus between immunity, mammary gland function and health, which may include reproductive outcomes as well. However, previous studies with OMN supplementation have not evaluated the effect of a dry period and early lactation supplementation feeding strategy on reproduction.

The objective of this study was to determine if feeding OMN, an immunomodulatory feed additive, from dry-off through 150 DIM would improve reproductive performance and milk yield

of multiparous Holstein cows by enhancing immune status and promoting better health after parturition. Specifically, our hypothesis was that health, production and reproductive performance of multiparous Holstein cows could be enhanced by feeding OmniGen-AF beginning at dry-off and continuing through the second breeding cycle thereby encompassing a total of 210 days.

2. Materials and methods

2.1. Cows, experimental design, diets, and housing

The study was conducted between July 2018 and June 2019 at Alliance Dairies, a large commercial dairy in Florida (Trenton, FL). All treatments and procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

A total of 1,392 Holstein multiparous (mean \pm SD: 3.3 \pm 1.4 lactations) pregnant dairy cows were enrolled in the present study. Cows were on average (\pm SD) dried off at 47.6 \pm 9.4 d before calving and were randomly assigned to OMN (n = 706) or Control (CON: n = 686) treatments based on the last digit (i.e. odd or even) of their identification ear tag number. Treatments began at dry-off and continued until confirmation of pregnancy or after the second synchronized breeding (150 DIM). Cows were supplemented for approximately 210 days in total. A description of animals by treatment is presented in Table 1.

Throughout the entire experiment, cows on the OMN treatment were fed 56 g/d per head of OMN daily. OmniGen-AF[®] (Phibro Animal Health Corporation, Teaneck, NJ) is a patented branded product known to modulate innate and adaptive immune markers in ruminants and other livestock species that has been shown to support immune function (Hall et al., 2018) in replacement dairy heifers (Ryman et al., 2013), lactating dairy cows (Wang et al., 2009; Brandão et al., 2016), transition cows (Wu et al., 2017), and sheep (Wang et al., 2007). OmniGen-AF[®] is

proprietary a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, brewer's dehydrated yeast, mineral oil, calcium carbonate, rice hulls, niacin supplement, biotin, calcium d-pantothenate, vitamin B₁₂ supplement, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, and riboflavin-5-phosphate and folic acid. Cows that were not on the OMN treatment (CON) were fed 56 g/d per head of bentonite as a placebo control (AB20[®]; Phibro Animal Health Corporation, Teaneck, NJ) throughout the entire experiment.

During the dry-off (47.6 ± 9.4 d before calving) and close-up periods (24.6 ± 8.2 d before calving) cows received the treatments mixed with the total mixed ration (TMR) once a day. To ensure proper mixing with the TMR, supplements (OMN or CON) were added manually at the end of the TMR every morning following the manufacturers recommendation. Proper mixing was determined daily by visual observation. Cows of both treatments were housed in the same free stall maternity barn in neighboring pens of similar size, stocking density, environmental conditions (shade, fans, and soakers), and bedded with digester solids (Table 1).

After calving, supplements were top-dressed on the TMR for 14 d at a rate of 56 g/d per head while cows were locked up in head stanchions. Consumption of the supplements was ensured by visual assessment. During the fresh period, cows of both treatments were housed in the same sand bedded free-stall pen equipped with fans and soakers. After the fresh period (second week of lactation), supplements were mixed in the TMR and fed to the animals as they came back from the milking parlor. Throughout lactation, cows of both treatments were housed in the same free stall barn in neighboring sand-bedded pens of similar size, stocking density, and environmental conditions (shade, fans, and soakers) (Table 1).

Throughout the experiment, all diets were fed as TMR and formulated to meet or exceed Dairy NRC (2001) requirements for specific phases of the lactation cycle (Table 2). Total mixed

ration was offered *ab lib*, fed twice daily and pushed back several times per day. Free access to water was always provided.

2.2. Measurements and sampling

Cows were milked three times each day (OMN 0400, 1100 and 2000 h) (CON 0700, 1500 and 2300 h) and milk yield were monitored by DeLaval flow indicator F17 (DeLaval Inc.; Kansas City, Missouri) and manually recorded starting at the second week of lactation once every 7 d through 140 DIM during the second milking of the day. Further, milk samples (50ml) were collected during the second milking of the day every 14 days from the through 140 DIM, from a random subset of cows of OMN (n = 404) and CON (n = 384) treatments to assess milk composition, including protein, fat, lactose and somatic cell count (SCC). To do so, cows were randomly chosen as it was only possible to attach the milk samplers in the first 10 positions on each side of the milking parlor. The samples were collected from the cows placed in those positions during the second milking of the day. The samples were the shipped directly to the Dairy Herd Improvement Association (DHIA; Belleview, FL) laboratory for components analysis by Fourier transformed infrared spectrometry.

Postpartum disorders or disease incidence, including metritis, mastitis, retained placenta, laminitis, ketosis, pneumonia, and displaced abomasum, were monitored after calving through 150 DIM. Disease monitoring was done by the farm employees following the standard on-farm protocol of identification and treatment using direct farm reports from Dairy COMP 305 software (Valley Ag System; Tulare, California). Cows of both treatments treated with antibiotics were moved to a hospital free-stall pen and remained until they were cleared by to return to the lactation pens after the prescribed withdrawal period. While in the hospital pens, treatments

supplementation continued as it was added as top-dress on the TMR. Days a cow spent in the hospital pen were estimated by using the dairies antibiotic treatment protocol.

Cows were bred after a 77-day voluntary waiting period (VWP) using a double Ovsynch protocol (Souza et al., 2008) for first and second service breeding. Pregnancy diagnosis was performed using a portable ultrasound scanner at 30- and 60-days post-insemination. After dietary treatments ended, cows were still monitored for a reproductive performance until 231 ± 28.4 days of lactation (OMN = 232 ± 28.8 vs. CON = 231 ± 28.1 DIM). The number of inseminations and confirmed pregnancies were recorded weekly from the on-farm Dairy COMP 305 records and used to assess reproductive performance.

2.3. Statistical analyses

Data were analyzed using SAS (version 9.4, Statistical Analysis System Institute Inc., Cary, NC). Data for milk production and composition were analyzed using the MIXED procedure with week in milk as a repeated measure, cow (treatment) as the subject, treatment and the interaction between treatment and week as fixed effects and previous lactation yield and parity as covariates. Residuals were tested for normality and somatic cell count was transformed [$\log_{10}(x)$] to meet normality criteria. Health performance was compiled by treatment using PROC FREQ and analyzed with the GLIMMIX procedure. Survival analysis for time to pregnancy was analyzed by the PROC LIFETEST of SAS. The survival curves were then compared between treatments and the difference was statistically tested using the log-rank test. Differences were declared significant at $P < 0.05$ and a tendency at $0.05 \leq P \leq 0.10$.

3. Results

3.1. Milk Yield and Composition

Milk yield, composition, and quality recorded until 140 DIM are presented by treatment in Table 3. Figure 1 depicts milk yield throughout 140 DIM. Feeding OMN was associated with higher milk production as cows of the OMN treatment produced on average 0.7 kg/d more milk relative to CON cows (OMN = 40.8 vs. 40.1 ± 0.20 kg/d; $P < 0.01$). Milk fat (3.59 vs. $3.58 \pm 0.04\%$) and lactose (4.81 vs. $4.81 \pm 0.01\%$) percentages were similar between treatments (both $P > 0.05$), whereas milk protein percentage was higher ($P < 0.05$) in OMN ($2.95 \pm 0.01\%$) compared with CON ($2.91 \pm 0.01\%$) cows. Somatic cell counts differed between treatments as SCC was higher ($P < 0.05$) in OMN ($97 \pm 1.4 \times 10^3$ /ml) relative to CON ($75 \pm 1.4 \times 10^3$ ml; $P < 0.05$) cows.

3.2. Postpartum Health

Figure 2 depicts the frequency of postpartum disorders experienced by cows in both treatments during the entire study. Overall, there was no difference between treatments in the percent of cows presenting with at least one health disorder (no postpartum disorder; OMN = 37.1% vs. CON = 35.7%; $P = 0.58$). No significant difference between the groups in culling rate (OMN = 17.3% vs. CON = 17.5%; $P > 0.05$) and mortality (OMN = 3.7% vs. CON = 3.7%; $P > 0.05$) was detected during the experiment. However, specific indicators of health showed a trend for improvement in OMN cows with fewer diagnosed cases of mastitis (OMN = 36.8% vs. CON = 41.7%; $P = 0.06$); retained placenta (OMN = 5.4% vs. CON = 7.6%; $P = 0.09$) and displaced abomasum (OMN = 1.9% vs. CON = 3.2%; $P = 0.11$). Other postpartum disorders, such as metritis, ketosis, digestive disorders, pneumonia, and lameness did not differ between treatments. There was a significant difference in the number of days spent in the hospital pen between treatments, with OMN cows spending on average half a day less in the hospital than CON cows (OMN = 6.02 ± 0.09 d vs. CON = 6.56 ± 0.01 d; $P < 0.01$).

3.3. Reproductive Performance

Pregnancy rates at first, second and until ~230 DIM are presented by treatment in Table 3. At the first breeding, pregnancy rate did not differ between treatments (Table 3). After a 77-d VWP, there were 32.6% of OMN and 28.1% of CON cows were confirmed pregnant in the OMN and CON groups, respectively ($P = 0.31$; Table 2). After the second synchronized breeding cycle (\square 150 DIM), 52.3% and 47.7% ($P = 0.32$) of cows were confirmed pregnant in the OMN and CON groups, respectively. At 230 DIM, pregnancy rate was similar between treatments, as there were 76.1 and 73.3% of OMN and CON cows respectively confirmed pregnant. Although similar between treatments after second breeding ($P = 0.15$), OMN supplementation was associated with a reduced number of days open at 230 DIM with cows of the OMN confirmed pregnant 10 d earlier than CON (OMN = 139 vs. 149 ± 3.44 d; $P < 0.05$).

4. Discussion

The dry period and early lactation are known to be critical periods of immunosuppression, in which cows are highly susceptible to metabolic and health disorders that compromise performance outcomes (Santos et al., 2004). Supplementing cows with immunomodulatory additives is becoming a common management practice to improve immune competence during those critical windows of susceptibility in dairy cattle. Our objective was to determine if feeding OMN during the dry period and early lactation would improve health outcomes and subsequent productive and reproductive performance. Current knowledge and published literature about OMN supplementation to dairy cattle is limited, and no other research has evaluated the long-term effects of the supplementation on reproductive performance.

In the current study, feeding OMN from dry-off until 150 DIM was associated with an higher milk yield during the first 140 d of the lactation. Our results agree with Nickerson et al. (2019) who also reported improved milk yield when cows are supplemented OMN from dry-off until 60 DIM. Similarly, Brandão et al. (2016) observed an increase in milk yield when cows received OMN beginning during the dry period and extending through early lactation. In addition, Fabris et al. (2017) observed that feeding OMN to cows under heat stress contributed to rescue milk yield. In contrast, Leiva et al. (2017) offered OMN supplementation to lactating dairy cows but observed similar milk yield compared with CON cows, one possible explanation is that, cows in that experiment were already producing their maximum milk yield and the additional nutrients consumed from the supplementation were converted in body score condition, which was different between the groups. To elucidate the link between the immune function and milk performance, Kvidera et al. (2017) reported enhanced glucose utilization by the activated immune system under a local inflammatory response in the mammary gland, thereby limiting the amount glucose available for milk synthesis. Therefore, limiting the inflammatory response by providing an immune booster to the animal may be beneficial for milk production. Regarding milk components, in the current study, cows of both treatments had similar milk fat and lactose content whereas OMN supplementation was associated with higher protein percentage. The observed increase in protein composition was unexpected as Fabris et al. (2017), Leiva et al. (2017), Mammi et al. (2018), Hall et al. (2018) all reported no association between OMN feeding and milk components.

In addition to enhanced milk output, previous work suggests that feeding OMN in the dry period through 60 DIM is associated with improved postpartum health status (Mammi et al., 2018). In the present study, cows that were supplemented with OMN tended to have lower incidence of post-partum disorders, specifically mastitis, retained placenta and displaced abomasum. In

addition, cows fed OMN spent fewer days under antibiotic treatment in the hospital pen. It is suggested that the ability of the additive to enhance immune status may help explain the decreased incidence of post-partum health disorders observed in those studies. Further, previous reports in sheep and dairy cows indicate that OMN supplementation modulates innate immune response by increasing the concentration of neutrophil L-selectin, a surface adhesion protein, necessary for normal neutrophil function (Wang et al., 2007, 2009; Ryman et al., 2013). Improving the ability of the immune system to be more efficient in the clearance of pathogens could help to reduce development of clinical disease, which in turn would reduce the negative impact of a pathogen-induced stress thereby potentially allowing for improved productive and reproductive performance. Moreover, the improved health status associated with OMN feeding may also explain the improved milk performance of supplemented animals. Specifically, Akers and Nickerson (2011) reviewed the histological and ultrastructural changes that occur in the mammary gland during mastitis events. The authors highlighted the relationship between that loss in function and milk yield, is complementary to the changes in tissue architecture and alterations in cellular characteristics of the secretory mammary epithelial cells responsible for producing milk. In addition, Enger (2019) reviewed the importance of limiting the incidence of mastitis on the farm, due to the competition of nutrient utilization between immune and mammary systems.

Although cows fed OMN tended to have fewer cases of clinical mastitis, SCC were greater in those cows relative to CON cows. This contrasts with results reported by Chapman et al. (2016) that OMN supplementation to commercial dairy herds decreased milk SCC by up to 30%, and Hall et al. (2018) who reported that milk SCC was similar in OMN supplemented versus non-supplemented cows in a controlled setting. One possible explanation for the increased SCC in the OMN fed group is that the slight increase with OMN treatment was due to greater L-selectin

(CD62L) expression with OMN as previously reported (Wang et al., 2007, 2009), which may cause slightly increased migration of neutrophils from the circulation into the mammary gland relative to CON cows. Regardless of the underlying mechanism, the SCC values for both groups were less than 100,000 cell/mL, which were close to physiological minimums, indicative of a healthy udder (Dohoo and Meek, 1982).

Reproductive performance may be improved with a properly functioning immune system. Indeed, cows that experience several diseases postpartum are known to have impaired reproductive performance, even when the disease is resolved before breeding occurs (Santos et al., 2004; Sheldon et al., 2008). Huszenicza et al. (2005) indicated that mastitis can affect the resumption of ovarian activity in postpartum dairy cows and impair reproduction in cyclic cows, an effect that may be the consequence of premature luteolysis or a prolonged follicular phase. Because the innate immune system represents the first line of defense against a pathogen challenge and provides the adaptive system the time required to develop the appropriate antibody response, improvements in innate immune surveillance is expected to improve overall health, and potentially milk production and reproductive performance. In the present study we observed a reduced incidence of diseases from parturition through the end of the 150 DIM, higher milk yield and a reduction in the number of days open in cows receiving OMN.

Further, it is reported in the literature that metritis and other infections can impair the reproductive performance of dairy cows (Sheldon et al., 2006) as reflected by an increased number of days between first service and conception (Erb et al., 1981; Fourichon et al., 2000). Also, puerperal metritis impacts feed intake, long-term milk yield, and increases the chance of culling in multiparous cows (Writtrock et al., 2011). It is of interest that metritis incidence was not affected

during our study, despite a tendency for lower incidence of retained placenta within the OMN cows. The lack of difference in the reproductive disorders and outcomes (except for days open) observed between treatments may be reflective of the aggressive, effective reproductive management implemented at the farm. Studies have shown that reproductive performance can be compromised by certain diseases and disorders that occur around parturition. The occurrence of inflammatory disorders prior to breeding has been shown to reduce oocyte fertilization and conceptus development independently of estrous cyclicity and body condition score of cows (Ribeiro et al., 2016). Research suggests that reproductive efficiency is decreased by the presence of clinical mastitis in that a greater proportion of cows with mastitis remained non-pregnant over time and, the negative effects on reproduction were exacerbated when cows experienced both clinical mastitis and other diseases (Ahmadzadeh et al., 2010). Clinical or subclinical mastitis may influence reproductive response by alterations in endocrine profiles and follicular development, consequently mastitis could influence reproductive function via alterations in LH and FSH activity or function, thus affecting follicular development and/or oocyte maturation (Schrick et al., 2001). An indirect action of OMN may also positively impact reproductive outcomes. Specifically, feeding OMN is associated with greater progesterone concentrations at the time of embryo collection in super ovulated cows, which may be indicative of improved luteal function during this period of early embryonic development (Snider et al., 2019).

5. Conclusions

Feeding an immunomodulatory feed additive to multiparous Holstein cows beginning at dry-off and continuing through 150 DIM was associated with significant improvements in daily milk yields. In addition, incidence rates of mastitis and retained placenta tended to be less and days spent in the hospital pen was significantly reduced for cows supplemented with OMN. Days open

was significantly reduced in cows fed OMN at 230 DIM. In conclusion, feeding OmniGen-AF® to dairy cows in a commercial setting, from dry off to 150 DIM, was associated with improved health, milk yield and reproductive performance in the next lactation. Further investigation into the effects of hormonal and inflammatory marker responses in support of the immune system will be necessary to confirm the supplementation mechanism. Completion of such studies will also contribute to the identification of the most essential factors required for optimal immune cell function, which may contribute to improved immune response during the next lactation.

Author statement

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Conflict of interest

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References

- Ahmadzadeh, A., Mcguire, M.A., Dalton, J.C., 2010. Interaction between clinical mastitis, other diseases and reproductive performance in dairy cows. *WCDS Adv. Dairy Technol.* 22, 83–95.
- Akers, R.M., Nickerson, S.C., 2011. Mastitis and its impact on structure and function in the ruminant mammary gland. *J. Mammary Gland Biol. Neoplasia* 16, 275–289. <https://doi.org/10.1007/s10911-011-9231-3>.
- Brandão, A.P., Cooke, R.F., Corrá, F.N., Piccolo, M.B., Gennari, R., Leiva, T., Vasconcelos, J.L.M., 2016. Physiologic, health, and production responses of dairy cows supplemented with an immunomodulatory feed ingredient during the transition period. *J. Dairy Sci.* 99, 5562–5572. <https://dx.doi.org/10.3168/jds.2015-10621>.
- Capuco, A. V., Akers, R.M., Smith, J.J., 1997. Mammary growth in Holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80, 477–487. [https://doi.org/10.3168/jds.S0022-0302\(97\)75960-5](https://doi.org/10.3168/jds.S0022-0302(97)75960-5).
- Chapman, J.D., Bascom, S.S., Ely, L.O., Holub, G.A., Jarrett, J.P., Lanier, J.S., Kirk, D.J., Nuzback, D.E., Rowson, A.D., Wistuba, T.J., 2016. Health, milk yield and milk quality records evaluated in 787 dairy herds before and during OmniGen-AF® supplementation to dry and lactating cows. *J. Dairy Sci.* 99, 660. <https://doi.org/10.2527/jam2016-1365>.
- Dohoo, I.R., Meek, A.H., 1982. Somatic cell counts in bovine milk. *Can Vet J.* 23, 119–125.
- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82, 2259–2273. [https://doi.org/10.3168/jds.S0022-0302\(99\)75474-3](https://doi.org/10.3168/jds.S0022-0302(99)75474-3).

- Enger, B.D., 2019. Invited Review: Reevaluating how mastitis reduces milk yield: Discussion of competitive substrate utilization. *Appl. Anim. Sci.* 35, 408-415. <https://doi.org/10.15232/aas.2019-01876>.
- Erb, H. N., Martin, S.W., Ison, N., Swaminathan, S., 1981. Interrelationships between production and reproductive diseases in Holstein cows: Path analysis. *J. Dairy Sci.* 64, 282–289. [https://doi.org/10.3168/jds.S0022-0302\(81\)82565-9](https://doi.org/10.3168/jds.S0022-0302(81)82565-9).
- Fabris, T.F., Laporta, J., Corrá, F.N., Torres, Y.M., Kirk, D.J., McLean, D.J., Chapman, J.D., Dahl, G.E., 2017a. Effect of nutritional immunomodulation and heat stress during the dry period on subsequent performance of cows. *J. Dairy Sci.* 100, 6733–6742. <https://doi.org/10.3168/jds.2016-12313>.
- Fabris, T.F., Laporta, J., McLean, D.J., Kirk, D.J., Chapman, J.D., Corrá, F.N., Torres, Y.M., Dahl, G.E., 2017b. Strategies to ameliorate the negative impact of heat stress on immune status of cows during the dry period. *J. Dairy Sci.* 100, 70-71. Abstract M178.
- Fourichon, C., Seegers, H., Malher, X., 2000. Effect of disease on reproduction in the dairy cow: A meta-analysis. *Theriogenology.* 53, 1729–1759. [https://doi.org/10.1016/S0093-691X\(00\)00311-3](https://doi.org/10.1016/S0093-691X(00)00311-3).
- Goff, J.P., Horst, R.L., 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.* 80,1260–1268. [https://doi.org/10.3168/jds.S0022-0302\(97\)76055-7](https://doi.org/10.3168/jds.S0022-0302(97)76055-7).
- Hall ,L.W., Villar, F., Chapman, J.D., McLean, D.J., Long, N.M., Xiao, Y., Collier, J.L., Collier, R.J., 2018. An evaluation of an immunomodulatory feed ingredient in heat-stressed lactating

- Holstein cows: Effects on hormonal, physiological, and production responses. *J. Dairy Sci.* 101, 7095–7105. <https://doi.org/10.3168/jds.2017-14210>.
- Huszenicza, G., Janosi, S.z., Kulcsar, M., Korodi, P., Reiczigel, J., Katai, L., Peters, A.R., De Rensis, F., 2005. Effects of clinical mastitis on ovarian function in post-partum dairy cows. *Reprod. Dom. Anim.* 40, 199-104. <https://doi.org/10.1111/j.1439-0531.2005.00571.x>.
- Kimura, K., Goff, J.P., Kehrl Jr, M.E., 1999. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. *J. Dairy Sci.* 82, 2385–2392. [https://doi.org/10.3168/jds.S0022-0302\(99\)75489-5](https://doi.org/10.3168/jds.S0022-0302(99)75489-5).
- Kvidera, S.K., Horst, E.A., Abuajamieh, M., Mayorga, E.J., Fernandez, M.V., Baumgard, L.H., 2017. Glucose requirements of an activated immune system in lactating Holstein cows. *J. Dairy Sci.* 100, 2360–2374. <https://doi.org/10.3168/jds.2016-12001>.
- Leiva, T., Cooke, R.F., Brandão, A.P., Schubach, K.M., Batista, L.F.D., Miranda, M.F., Colombo, E.A., Rodrigues, R.O., Junior, J.R.G., Cerri, R.L.A., Vasconcelos, J.L.M., 2017. Supplementing an immunomodulatory feed ingredient to modulate thermoregulation, physiologic, and production responses in lactating dairy cows under heat stress conditions. *J. Dairy Sci.* 100, 4829–4838. <https://doi.org/10.3168/jds.2016-12258>.
- Mammi, L.M.E., Palmonari, A., Fustini, M., Cavallini, D., Canestrari, G., Chapman, J.D., McLean, D.J., Formigoni, A., 2018. Immunomodulant feed supplement to support dairy cows health and milk quality evaluated in Parmigiano Reggiano cheese production. *Anim. Feed Sci. Technol.* 242, 21–30. <https://doi.org/10.1016/j.anifeedsci.2018.05.011>.
- Nickerson, S.C., Kautz, F.M., Ely, L.O., Rowson, A.D., Hurley, D.J., Chapman, J.D., McLean,

- D.J., 2019. Effects of an immunomodulatory feed additive on intramammary infection prevalence and somatic cell counts in a dairy herd experiencing major health issues. *Res. Vet. Sci.* 124, 186–190. <https://doi.org/10.1016/j.rvsc.2019.03.013>.
- NRC, 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Riberio, E.S., Gomes, G., Greco, L.F., Cerri, R.L.A., Vieira-Neto, A., Monteiro, P.L.J., Lima, F.S., Bisinotto, R.S., Thatcher, W.W., Santos, J.E.P., 2016. Carryover effect of postpartum inflammatory disease on developmental biology and fertility in lactating dairy cows. *J. Dairy Sci.* 99, 2201–2220. <https://doi.org/10.3168/jds.2015-10337>.
- Ryman, V.E., Nickerson, S.C., Kautz, F.M., Hurley, D.J., Ely, L.O., Wang, Y.Q., Forsberg, N.E., 2013. Effect of dietary supplementation on the antimicrobial activity of blood leukocytes isolated from Holstein heifers. *Res. Vet. Sci.* 95, 969–974. <http://doi.org/10.1016/j.rvsc.2013.09.009>.
- Santos, J.E.P., Cerri, R.L.A., Ballou, M.A., Higginbotham, G.E., Kirk, J.H., 2004. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. *Anim. Repro. Sci.* 80, 31–45. [https://doi.org/10.1016/S0378-4320\(03\)00133-7](https://doi.org/10.1016/S0378-4320(03)00133-7).
- Schrack, F.N., Hockett, M.E., Saxton, A.M., Lewis, M.J., Dowlen, H.H., Oliver, S.P., 2001. Influence of subclinical mastitis during early lactation on reproductive parameters. *J. Dairy Sci.* 84, 1407–1412. [https://doi.org/10.3168/jds.S0022-0302\(01\)70172-5](https://doi.org/10.3168/jds.S0022-0302(01)70172-5).
- Sheldon, I. M., Lewis, G.S., LeBlanc, S., Gilbert, R.O., 2006. Defining postpartum uterine disease in cattle. *Theriogenology*. 65, 1516–1530. <https://doi.org/10.1016/j.theriogenology.2005.08.021>.

- Sheldon, I.M., Williams, E.J., Miller, A.N.A., Nash, D.M., S. Herath, S., 2008. Uterine diseases in cattle after parturition. *Vet. J.* 176, 115–121. <https://doi.org/10.1016/j.tvjl.2007.12.031>.
- Snider, A.P., Mclean, D., Menino Jr, A.R., 2019. Effects of feeding OmniGen-AF ® on superovulatory response in donor beef cows : I. Serum progesterone and cortisol, embryo recovery and quality. *Anim. Reprod. Sci.* 210, 106–174. <https://doi.org/10.1016/j.anireprosci.2019.106174>.
- Souza, A.H., Ayres, H., Ferreira, R.M., Wiltbank, M.C., 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology.* 70, 208–215. <https://doi.org/10.1016/j.theriogenology.2008.03.014>.
- Wang, Y.Q., Puntenney, S.B., Burton, J.L., Forsberg, N.E., 2007. Ability of a commercial feed additive to modulate expression of innate immunity in sheep immunosuppressed with dexamethasone. *Animal.* 1, 945–951. <https://doi.org/10.1017/S1751731107000365>.
- Wang, Y.Q., Puntenney, S.B., Burton, J.L., Forsberg, N.E., 2009. Use of gene profiling to evaluate the effects of a feed additive on immune function in periparturient dairy cattle. *J. Anim. Physiol. Anim. Nutr.* 93, 66–75. <https://doi.org/10.1111/j.1439-0396.2007.00780.x>.
- Wittrock, J.M, Proudfoot, K.L., Weary, D.M., von Keyserlingk, M.A.G., 2011. Metritis affects milk production and cull rate of Holstein multiparous and primiparous dairy cows differently. *J. Dairy Sci.* 94, 2408-2412. <https://doi.org/10.3168/jds.2010-3697>.
- Wu, Z.H., Yu, Y., Alugongo, G.M., Xiao, J.X., Li, J.H., Li, Y.X., Wang, Y.J., Li, S.L., Cao, Z.J., 2017. Effects of an immunomodulatory feed additive on phagocytic capacity of neutrophils and relative gene expression in circulating white blood cells of transition Holstein cows. *J. Dairy Sci.* 100, 7549-7555. <https://doi.org/10.3168/jds.2016-12528>.

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Table 1. Description of animals enrolled in the study by treatment. Data are presented as mean \pm SD.

	Treatments	
	OmniGen-AF ¹	Control ²
Number of animals, n	706	686
Parity at enrollment	3.3 \pm 1.4	3.3 \pm 1.4
Previous lactation yield, kg	11,955 \pm 2639	11,953 \pm 2691
Dry-off time, days before calving	47.6 \pm 10.2	47.6 \pm 8.4
Far-off pen stock density, animals/pen	261.1 \pm 39.2	258.2 \pm 44.2
Close-up pen, days before calving	24.8 \pm 8.8	24.5 \pm 7.4
Close-up pen stock density, animals/pen	155.3 \pm 58.1	140.2 \pm 67.2
Lactation pen stock density, animal/pen	489.1 \pm 23.5	506 \pm 24.6

¹Omnigen-AF cows were supplemented 56 g/d per head daily from dry-off until 150 DIM.

²Control cows were fed bentonite (placebo) at a rate of 56 g/d per head daily from dry-off until 150 DIM.

Table 2. Ingredient composition and nutrient content of far off, prepartum and postpartum diets fed to cows receiving 56g/d of OmniGen-AF (n = 706) or placebo (n = 686) from dry off through 150 DIM.

Item	Diet ¹		
	Dry-off	Prepartum	Postpartum
Ingredients, % of DM			
Corn silage	27.39	38.33	27.40
Bakery byproduct	-	-	5.20
Canola meal	-	-	7.44
Triticale silage	-	-	6.75
CSC brewers	-	-	7.69
Ground corn	-	-	3.85
Soybean hulls	-	-	1.27
Molasses blend	-	-	6.29
Nurisol ²	-	-	0.38
Gin trash	22.58	-	-
Sorghum silage	43.09	8.95	-
Soybean meal, solvent extract 48% CP	-	-	4.88
Mineral-vitamin premix, dry-off ³	6.97	-	-
Mineral-vitamin premix, prepartum ⁴	-	52.72	-
Mineral-vitamin premix, lactation ⁵	-	-	14.21
Nutrient content, DM basis			
NEL ⁶ , Mcal/kg	0.60	0.68	0.79
OM, %	93.24	89.48	90.23
CP, %	12.75	17.25	19.27
NDF, %	47.26	39.45	29.37
Forage NDF, %	31.74	21.79	15.05
ADF, %	33.71	24.57	18.47
NFC ⁷ , %	32.52	32.07	40.38
Ca, %	0.82	1.49	0.81
P, %	0.29	0.44	0.39
Mg, %	0.25	0.47	0.30
K, %	1.51	1.59	1.96
Vitamin A, IU/lb	931.8	2,160.3	1,442.2
Vitamin E, IU/lb	13.04	31.57	13.46
Salt, %	0.16	0.18	0.06
Fat, %	3.16	3.00	4.09
Ash, %	6.76	10.52	9.77

¹Far off diet was fed starting in the first day of the dry-off period. Prepartum diet was fed at least 30d before calving to calving and postpartum diet from calving to 150 DIM.

²Nurisol (Global Agri-trade Corporation; Rancho Dominguez, CA). 1.4% myristic acid, 40% palmitic acid, 3.3% stearic acid, 30.4% oleic acid, 8.0% linoleic acid, 8-10% calcium; 6.62 Mcal/kg net energy lactation.

³The dry off mineral and vitamin supplement contained (DM basis) 87.04% soybean meal-48%, 13.4% mineral; 50.67% protein, 14.04% soluble protein, 35.65% RDP(%DM), 0.86% lysine calc (%MP), 0.17% methionine calc (%MP), 0.36% histidine calc (%MP), 0.53% isoleucine calc (%MP), 1.02% arginine calc (%MP), 1.68 Mcal/kg NEI, 5.20% ADF, 6.76% NDF, 13.53% RDCHO (%DM), 12.14% sugar (%DM), 1.73% starch (%DM), 90% NDF, 1.03% fat, 0.83% RUFAL, 2.76% calcium, 0.62% phosphorus, 0.76% magnesium, 2.05% potassium, 0.95% sodium, 1.50% chlorine, 0.42% sulfur, 719.5 ppm zinc, 141.2 ppm copper, 433.7 ppm manganese, 13.5 ppm cobalt, 8.43 ppm iodine, 3.12 ppm selenium, 26,442 IU/kg vitamin A, 13,536 IU/kg vitamin D, 410.1 IU/kg vitamin E, -24.8 cation anion balance.

⁴The prepartum mineral and vitamin supplement contained (DM basis) 29.4% Bermuda grass silage, 4.38% bevachlor gen II, 11.2% canola meal, 9.9% CSC brewers, 13.5% ground corn, 10.1% oat straw, 12.1% soybean meal-48%, 9.4% mineral; 24% protein, 1.39 Mcal/kg NEI, 22.1% ADF, 35.7% NDF, 28.4% NFC, 2.56% calcium, 0.59% phosphorus, 0.76% magnesium, 1.64% potassium, 0.34% salt, 2.68% fat, 18.5% fiber, 15.1% ash, 9,030 IU/kg vitamin A, 132.1 IU/kg vitamin E.

⁵The lactation mineral and vitamin supplement contained (DM basis) 8.8% bakery byproduct, 28.8% canola meal, 10.1% ground corn, 1.0% nurisol (1.4% myristic acid, 40% palmitic acid, 3.3% stearic acid, 30.4% oleic acid, 8.0% linoleic acid, 8-10% calcium; 6.62 Mcal/kg net energy lactation), 6.3% soybean hulls, 7.7% soybean meal-48%; 30.4% protein, 1.59 Mcal/kg NEI, 19.4% ADF, 26.6% NDF, 27% NFC, 1.69% calcium, 0.55% phosphorus, 0.54% magnesium, 2.38% potassium, 0.15% salt, 4.41% fat, 12.4% fiber, 16.2% ash, 8,344 IU/kg vitamin A, 77.8 IU/kg vitamin E.

⁶Calculated using the NRC (2001) according to the chemical composition of the dietary ingredients and adjusted for the far-off, pre- and postpartum periods, respectively.

⁷Calculated as follows: $NFC = DM - (ash + CP + ether\ extract + NDF - NDF\ insoluble\ CP)$.

Table 3. Milk yield, milk composition, and conception rate through first, second, and third breeding of cows receiving OmniGen-AF (OMN; Phibro Animal Health Corporation, Teaneck, NJ) or Control (CON) throughout the dry period and early lactation. Data are presented as LSMEANS \pm SEM.

	Treatments			P-Values ¹		
	OMN	CON	SEM	Trt	Week	Trt \times week
Milk yield, kg/d	40.8	40.1	0.2	0.01	0.01	0.04
Fat, %	3.59	3.58	0.04	0.75	0.001	0.11
Protein, %	2.95	2.91	0.01	0.05	0.001	0.32
Lactose, %	4.81	4.81	0.01	0.57	0.82	0.44
SCC, 10^3 ml ²	97	75	16	0.003	0.41	0.76
Conception rate, %						
First breeding	32.5	28.1	0.12	0.31	—	—
Second breeding	52.3	47.7	0.17	0.32	—	—
After ~230 DIM	76.1	73.3	0.24	0.48	—	—

¹ Main effects of treatment (trt), week, and interaction between treatment and week are presented.

² Values were log transformed to meet normality assumption. Back-transformed values are presented in the table.

Figure 1. Milk yield of cows fed OmniGen-AF (OMN; Phibro Animal Health Corporation, Teaneck, NJ) or Control (CON) from dry off through 140 DIM. Overall means OMN = 40.8 ± 0.20 kg/d and CON = 40.1 ± 0.19 kg/d; $P < 0.01$.

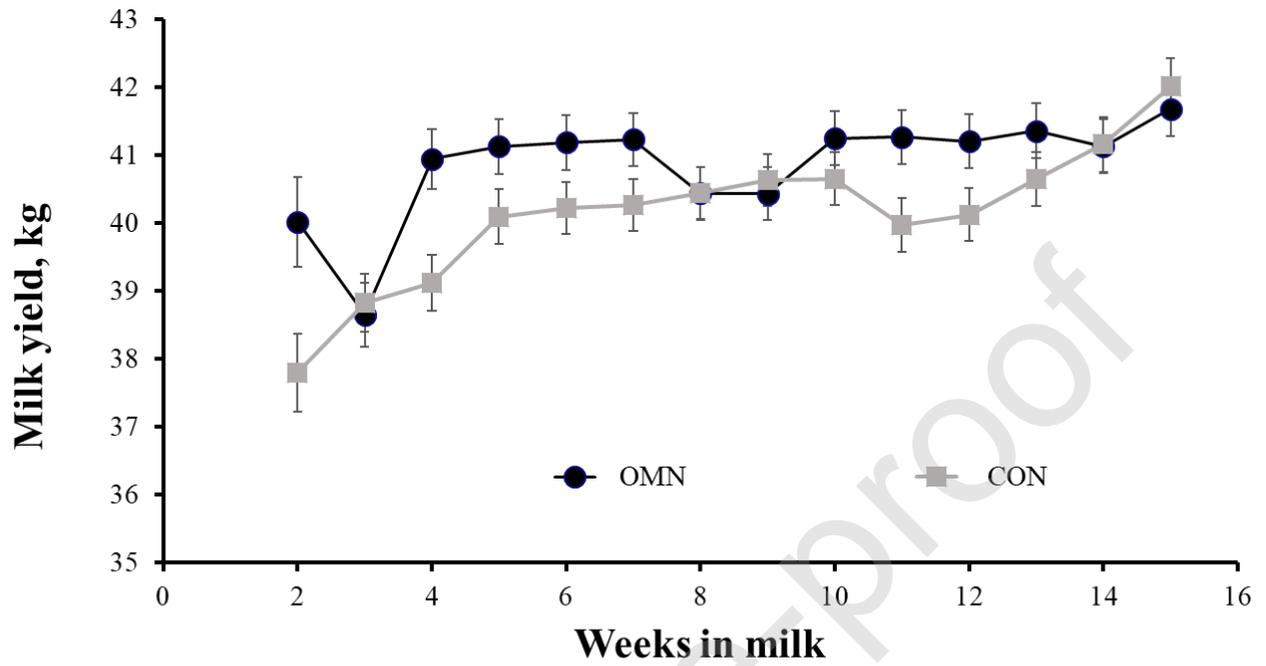


Figure 2. Frequency of postpartum disorders in Holstein dairy cows supplemented with OmniGen-AF (OMN; 56 g/d; n = 706) or placebo (CON, AB-20; 56 g/d; n = 686) from dry off through 150 days in milk.

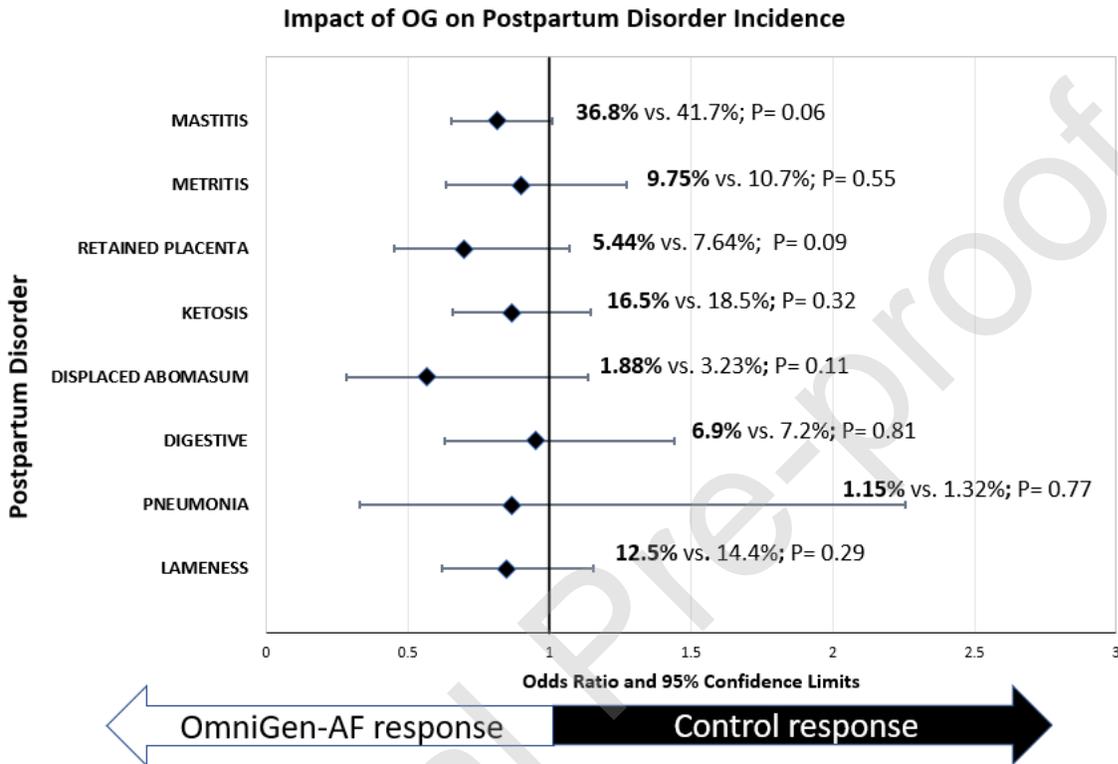


Figure 3. Reproductive performance of cows supplemented OmniGen-AF (OMN; 56 g/d; n = 706) or placebo (CON, AB-20; 56 g/d; n = 686) from dry off through 150 days in milk (DIM). Cows were bred using a Double OvSynch protocol with a voluntary waiting period of 77 days. After completing 150 DIM receiving OMN supplementation, cows were monitored for a general reproductive status until approximately 230 DIM (OMN = 232 ± 28.8 d vs. CON = 231 ± 28.1 d).

