Effects of antioxidants and prebiotics as vegetable pellet feed on production performance, hematological parameters and colostrum immunoglobulin content in transition dairy cows

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ABSTRACT
This research study addresses the hypothesis that supplementation of vegetable pellet feed (VPF) affects the quantity and quality of performance, blood parameters, and colostrum immunoglobulin concentration in dairy cows during the transition period. A total of 18 multiparous Holstein dairy cows were randomly assigned to one of 3 groups: 1- Control (basal diet; 0 g VPF/cow/day), 2- VPF10 (basal diet + 10 g VPF/cow/day), and 3- VPF20 (basal diet + 20 g VPF/cow/day). Cows were treated for 2 weeks before calving until 10 weeks after calving. Results showed that the VPF supplementation tends to increased milk yield ($P = .09$) and milk protein percentage linearly ($P < .05$) compared to the control group. Milk urea nitrogen (MUN) tended to be decreased linearly by VPF20 compared to control ($P < .05$) during week 2, while decreasing compared to control and VPF10 ($P < .01$) at week 8 of lactation. However, colostrum immunoglobulin concentration was not affected by the experimental diets. Blood lymphocyte and neutrophil concentration were lower and higher for the VPF10 group compared to control and VPF20 on week 2 of lactation, respectively ($P < .05$). Moreover, basophile concentration was lower for VPF supplementation than CTR group on week 2 of lactation (quadratic response; $P < .05$). It was concluded that feeding of VPF had a positive effect on production and several hematological items of early lactation dairy cows, especially at the first 2 weeks of lactation.

HIGHLIGHTS
- Vegetable pellet feed (VPF) supplementation positively increased milk protein percentage.
- Supplementation of VPF does not affect colostrum immunoglobulins.
- Feeding of VPF improved physiological responses of postpartum dairy cows through haematological parameters and white blood cells count during early lactation.

Introduction
Transition dairy cows are exposed to physiological and metabolic challenges from 3 weeks before calving to 3 weeks after calving (Bernabucci et al. 2005). Also, competition between cow and calf for energy, protein, mineral, and antioxidants intensified during this period (Grummer 1995). A decrease in dry matter intake and insulin-resistant, induced negative energy balance (NEB), which makes dairy cattle vulnerable to infectious and metabolic diseases (Nazifi et al. 2005). As a result, metabolic adjustment and energy partitioning towards the mammary gland induces mobilisation of stored adipose tissues that lead to increases in non-esterified fatty acid (NEFA) and lipid peroxidation (Pedernera et al. 2010; Lean et al. 2014).

Medicinal plants and their derivatives, known as phytobiotics, may have a useful impact on animal rumen fermentation, performance and health status depending on compounds (Kalantar et al. 2017). Other feed additives including essential oils, prebiotics as non-digestible compounds, as well as probiotics may have strong synergism with microbial flora (Alizadeh et al. 2010). Aung et al. (2019) showed that dry matter intake, milk yield, 3.5% fat corrected milk, milk fat percentage, body condition score, and blood parameters were not affected by $1.2 \times 10^{10}$ live yeast and 32 g/day mannan oligosaccharides. Westland et al. (2017) reported that colostrum production improved by supplementation of 1.33% (dry matter basis) mannan oligosaccharides than the control group (7.5 vs. 5.6 kg).
It was reported that Chinese medical herbs improved antioxidant indices, immunity system, IgG concentration, and interferon-gamma in Holstein dairy cows (Qiao et al. 2013). Gill (2000) demonstrated that the administration of herbs alone or in combination may have beneficial synergism on animal health and performance. To our knowledge, there is no report focusing on the combined effects of purified lignin, prebiotics, and herb essential oils as pellet forms for feeding dairy cows during the transition period. The present study hypothesised that supplementation containing purified lignin, mannan, and β-glucan polysaccharides in combination with essential oil as pellet form may positively affect the health status and therefore supports the production performance of Holstein dairy cows.

Materials and methods

All procedures used in this study were approved by the animal care advisory committee of the faculty of agriculture and food industries of Islamic Azad University, Science and Research Branch, Tehran, according to the guidelines of the Iranian Council of Animal Care (1995).

Animals, feeding and housing

The trial was conducted at a commercial dairy herd in Isfahan province, Iran. About 14 days before expected parturition time, 18 multiparous Holstein dairy cows were chosen based on parity (2.4 ± 0.5) and previous milk production (11,851 ± 551 kg 305 d mature equivalent milk yield) and randomly assigned to one of 3 dietary groups: 1- Control (basal diet; 0 g VPF/cow/day), 2- VPF10 (basal diet + 10 g VPF/cow/day), and 3- VPF20 (basal diet + 20 g VPF/cow/day). The treatments were statistically evaluated for previous milk production and no difference was observed between the groups for 305 d mature equivalent milk yield (Control: 11895 ± 554 kg, VPF10: 12148 ± 611 kg, and VPF20:11512 ± 489 kg; P = .72). Vegetable pellet feed consisting of mannan oligosaccharides (14.0%), β-glucan oligosaccharides (21.0%), purified lignin (50.0%) and Thyme, carvacrol, and Ocalypthol (8.0%), and moisture and water (7.0%), was provided from Animal Wellness Products (AWP) Co, Italy.

The cows were housed in free-stall barns with free access to water. Cows fed ad libitum twice daily (08:00 and 16:00) from 14 d before expected parturition date to 84 d postpartum. Total mixed diet (TMR) formulated to meet all requirements based on nutritionist recommendation and NRC (2001). Diets ingredients and composition are shown in Table 1. Vegetable pellet feed was fed orally through a liquid feeding tube (force-fed) by dissolving 10 and 20 g of VPF in 200 CC of water. The control group received 200 CC of water as a placebo.

Cows were milked 3 times daily and milk production of all cows was recorded during weeks 2, 4, and 8 after parturition. Milk samples were collected from 3 consecutive milkings and stored at 4°C in presence of a preservative (bronopol-B2). Milk protein, fat, somatic cell count (SCC), and urea nitrogen (MUN) were obtained using an automatic milk composition analyser (Milkoscan FT-120; Foss, Hillerød, Denmark). Samples of colostrum were taken individually at first milking.

Blood samples were taken from the coccygeal vein three hours after morning feeding during weeks 2, 4, and 8 postpartum using evacuated non-coagulation tubes. Blood serum was separated by centrifugation of 5 CC blood at 3000 × g for 15 min at 4°C and 2 aliquots of serum frozen at −20°C until analysis. Also, 5 CC of blood samples were drowned into tubes containing anti-coagulated with disodium-EDTA and then subjected to an automatic haematology analyser (Symex K 1000, TOA Ltd., Tokyo, Japan) to evaluate complete blood count (CBC). Differential blood count was performed based on Jain (1986), using the sectional method.

| Table 1. Diets ingredients and composition fed during transition period. |
|-------------------------------------------|-----------------|-----------------|
| Items (% of diet dry matter)   | Pre-partum | Post-partum |
|--------------------------------|-----------|------------|
| Alfalfa                        | 11.5      | 20.4       |
| Corn silage                    | 40.8      | 18.4       |
| Wheat straw                    | 3.51      | 0.50       |
| Beet pulp                      |           | 4.97       |
| Corn grain                     | 16.0      | 19.1       |
| Barley grain                   | 8.65      | 10.9       |
| Soybean meal                   | 2.4       | 11.2       |
| Soybean extruded               | 1.1       | 4.42       |
| Canola meal                    | 7.96      | 2.46       |
| Cottonseed                     | 2.21      | 2.49       |
| Wheat bran                     | 1.53      | 1.65       |
| Magnesium sulphate             | 0.78      | –          |
| Calcium chloride               | 0.54      | –          |
| Calcium carbonate              | 1.32      | 0.74       |
| Magnesium oxide                | 0.15      | 0.19       |
| Sodium bicarbonate             | –         | 0.93       |
| Salt                           | –         | 0.49       |
| Vitamin and mineral            | 1.46      | 1.12       |
| Chemical composition           |           |            |
| Crude protein                  | 12.9      | 16.9       |
| Ether extract                  | 3.3       | 3.7        |
| Neutral detergent fibre (NDF)  | 40.0      | 32.9       |
| Non fibrous carbohydrate (NFC) | 35.6      | 39.1       |
| Net energy for lactation (NEL) | 1.53      | 1.67       |
**Statistical analysis**

Data were analysed by the GLM procedure of SAS (2003; Institute Inc., Cary, NC, USA). The model has included the fixed effects of treatment, time, the interaction of treatment and time, and the random effect of cows nested within treatments.

The analytical model and its components were:

\[
Y_{ijk} = \mu + A_i + B_j + \delta(A)_{ik} + (A \times B)_{ij} + \beta(Xi - X) + \epsilon_{ijk}
\]

where, \(Y_{ijk}\): dependent variable, \(\mu\): mean of the population, \(A_i\): effect of treatment, \(B_j\): effect of time and \(j^{th}\) day of sampling as a repeated factor, \(\delta(A)_{ik}\): random effect of cow nested within treatment, \((A \times B)_{ij}\): 2-way interaction of treatment \(\times\) time of sampling, \(\beta(Xi - X)\): covariate variable, and \(\epsilon_{ijk}\): unexplained residual with normal distribution.

The significance level was declared at \(P < 0.05\), and a tendency towards significance was considered at \(0.05 < P \leq 0.1\) by Tukey test (SAS Institute, 2003).

**Results and discussion**

**Milk production and composition**

Milk yield and milk composition are presented in Table 2. A tendency to increase milk production \((P = 0.09)\) and an increase in milk protein percentage (linearly; \(P < 0.05\)) were observed in cows fed VPF compared with the CTR group during the experimental period. However, milk fat percentage was not affected by the experimental treatment. Faniyi et al. (2016) demonstrated that high affinity of plant extracts, especially essential oils, reduce the rate of protein degradation in the rumen due to inhibitory effects on specific rumen microorganisms (e.g. gram-positive bacteria) which may increase protein passage rate towards the small intestine and milk protein content. Moreover, feeding a blend of essential oils (capsaicin and pure forms of carvacrol, cinnamaldehyde, and eugenol) resulted in higher feed efficiency compared to the control group in mid-lactating dairy cows (Da Silva et al. 2020). Authors suggested that a change in ruminal fermentation pattern by essential oils (lower acetate to propionate ratio) may explain lower DMI and higher milk yield. In the case of oligosaccharides, increasing protein percentage was in line with Ząbek et al. (2013) and Zaleska et al. (2015) which reported \(\beta\)-glucan oligosaccharides enhanced milk, fat, and protein yield by 14, 30, and 11% in ewes, respectively. Limited data are available on purified lignin. However, in feedlot cattle, purified lignin was reported to increase feed efficiency by a slightly decrease in feed intake in the finishing period (Wang et al. 2016). Also, in calves addition of 12.5 to 50 gr of purified lignin did not change feed intake (Phillip et al. 2000). Since dry matter intake was not measured in the present study, a tendency to increase milk production may not be due to increased feed intake. Although our result showed that fat percentage was similar between experimental groups, previous studies revealed a positive effect of 10 g of yeast culture per day on milk fat content and fat corrected milk (Putnam et al. 1997). Furthermore, Wang et al. (2016) reported that FCM production was 2.5 kg higher in the first 30 days after calving in cows fed yeast compared to CTR.

Milk MUN concentration was affected by treatments. Cows fed VPF had linearly lower \((P < 0.01)\) MUN concentration than the CTR group, and VPF20 showed the least value compared with other treatments during week 2. Also, MUN concentration decreased \((P < 0.01)\) in VPF20 compared to CTR and VPF10 during

| Parameters                  | CTR     | VPF10 | VPF20 | Linear   | Quadratic |
|-----------------------------|---------|-------|-------|----------|-----------|
| Milk production (kg/d)      | 48.5 ± 1.62 | 51.1 ± 1.62 | 50.9 ± 1.62 | .09      | .16      |
| Milk fat (%)                | 2.78 ± 0.28 | 3.30 ± 0.28 | 3.43 ± 0.28 | .28      | .58      |
| Milk protein (%)            | 2.71 ± 0.06 | 2.99 ± 0.06 | 2.87 ± 0.06 | .04      | .21      |
| 2nd week of lactation \(\times 10^3/mL\) | 223 ± 29 | 274 ± 33 | 153 ± 30 | .46      | .09      |
| 4th week of lactation \(\times 10^3/mL\) | 15.2 ± 0.83 | 13.9 ± 0.89 | 11.9 ± 0.89 | .01      | .81      |
| 8th week of lactation \(\times 10^3/mL\) | 235 ± 44 | 272 ± 50 | 130 ± 45 | .60      | .13      |
| Somatic cell count \(\times 10^3/mL\) | 10.2 ± 0.83 | 9.30 ± 0.83 | 8.95 ± 0.83 | .30      | .79      |
| Milk urea nitrogen (mg/dl)  | 173.67 ± 44 | 158 ± 42 | 225.5 ± 45 | .54      | .83      |

*Experimental diets: CTR (basal diet), VPF10 (basal diet + 10 g vegetable pellet feed /cow/day), and VPF20 (basal diet + 20 g vegetable pellet feed /cow/day).*

**Table 2. Effects of feeding of vegetable pellet feed (VPF) on milk production and milk composition in Holstein dairy cows.**
week 8. The results of the present study showed that cows fed VPF10 and VPF20 tended to have a higher and lower SCC respectively and CTR presenting intermediate SCC during weeks 2 (quadratic effect; \( P = .09 \)). Decreasing in MUN concentration as VPF increased was in agreement with the finding of McIntosh et al. (2003) who reported that herbal components reduced the rate of deamination of amino acids in the rumen by reducing the growth of ammonia-producing microbes. Reducing ammonia production increases the efficiency of nitrogen utilisation in the rumen (Yang et al. 2010a). It is well demonstrated that the addition of essential oil of Thymol to casein (1 g/L) in the rumen resulted in the accumulation of amino acids by decreasing the concentration of ammonia nitrogen (NH3-N), which indicates the inhibition of amino acid deamination by rumen bacteria (Borchers 1965). Newbold et al. (2004) reported that a blend of essential oils reduced the amino acid deamination rate by 24% in sheep. Unexpectedly, we observed that cows fed VPF10 tended to have higher SCC during weeks 2 of lactation compared with CTR and VPF20, which was in contrast to Ząbek et al. (2013) who reported yeast β-glucan oligosaccharides could reduce releasing leucocytes in milk. However, lower SCC in cows fed VPF20, agreed with Shabtay et al. (2012) who reported that the hydrophilic and hydrophobic phenolic compounds of plant antioxidants can reverse the damaging effects of free radicals on mammary gland cells.

**Colostrum immunity parameters**

Mean concentrations of colostrum immunoglobulin (IgG, IgM, and IgA) produced by cows fed experimental diets are presented in Table 3. Concentrations of IgG, IgM, and IgA were not affected by VPF treatment. This was in agreement with Westland et al. (2017) who founded that pre-calving 2 g/cow/day MOS supplementation increased post-calving colostrum production with no positive effect on the total mass of immunoglobulin compared to the control group. Also administration of mannann oligosaccharide at 10 g/cow altered serum immunoglobulin levels, but not colostrum immunoglobulin after calving (Franklin et al. 2005). Franklin et al. (2005) reported cows fed similar concentrations of MOS in one group showed variation in colostral IgG, which may explain the absence of treatment effect of MOS in dairy cows. Furthermore, Ariza-Nieto et al. (2011) found that 250 mg/kg oregano essential oil (including oregano, thymol, and carvacrol) did not change the amount and concentration of serum and colostrum immunoglobulin in mother pigs. To our knowledge, there is no existing information on the effect of feeding purified lignin-containing supplement on colostrum immunoglobulins in dairy cows.

**Hematological parameters**

As shown in Tables 4 and 5, feeding of VPF did not affect erythrocyte levels, haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) on weeks 2, 4, and 8 of lactation. Gading et al. (2020) investigated the effect of the inclusion of pelleted-concentrate supplements containing essential oils and probiotics on the post-weaning diet of calves. They reported that the additive containing plant extracts and probiotics had no effect on any of the Hematological parameters except haemoglobin. The researchers attributed the increase in haemoglobin to antioxidants and other bioactive compounds in the additive. In the case of dairy cows, our results suggest that additive-containing plant extract did not affect the haematopoiesis process of the cows during the transition period.

White blood cell count (\( \times 10^3/\mu L \)) was not affected by the treatments during the experimental period. Of note, the CTR and VPF20 showed higher lymphocyte and lower neutrophil percentages compared to the VPF10 group during week 2 (quadratic effect; \( P < .05 \)). Basophil percentage is also decreased by feeding VPF (\( P < .05 \)). The trend for increasing basophils of VPF20 continued until the fourth week (linear effect; \( P = .07 \)). Haematologic parameters, such as white blood cells count are indicators used to assess health conditions and metabolic processes. Previously, the cows

| Immunoglobulin (mg/dL) | CTR | VPF10 | VPF20 | Linear | Quadratic |
|------------------------|-----|-------|-------|--------|-----------|
| IgG                    | 133.78 ± 0.78 | 132.77 ± 0.77 | 132.25 ± 0.78 | .18 | .79 |
| IgM                    | 6.90 ± 0.14   | 6.93 ± 0.15   | 6.86 ± 0.14   | .32 | .45 |
| IgA                    | 0.31 ± 0.15   | 0.35 ± 0.15   | 0.31 ± 0.15   | .91 | .38 |

*Experimental diets: CTR (basal diet), VPF10 (basal diet + 10 g vegetable pellet feed /cow/day), and VPF20 (basal diet + 20 g vegetable pellet feed /cow/day).*
receiving diets containing essential oil blends (clove, oregano, and juniper) showed no difference in WBCs count (Al-Suwaiegh et al. 2020). Also, a diet containing cinnamaldehyde did not affect white blood cell count in steers or beef heifers (Yang et al. 2010a, 2010b). Moreover, WBCs count or differential leukocyte count of dairy cows fed juniper or garlic oil were not affected (Yang et al. 2007). Due to the lack of effect of plant extracts on hematological parameters, it seems that changes in the count and ratio of leukocytes were due to other factors in VPF. In the case of prebiotic characteristics of VPF, it was suggested that MOS could stimulate an innate immune system leading to increasing the production of mannose-binding proteins which enhances phagocytosis (Franklin et al. 2005). Also, MOS would bind to macrophage receptor sites, which triggers a cascade reaction resulting in activating macrophages and release cytokines (Silva et al. 2009). In addition, the advantage of α- and β-D-glucans might be to bind pathogenic bacteria to prevent

**Table 4. Effects of feeding of vegetable pellet feed (VPF) on hematological parameters of Holstein dairy cows.**

| Parameters | CTR | VPF10 | VPF20 | Linear | Quadratic |
|------------|-----|-------|-------|--------|-----------|
| 2nd week of lactation |     |       |       |        |           |
| RBC (× 10⁶/μL) | 6.28 ± 0.19 | 6.31 ± 0.19 | 6.51 ± 0.19 | .41     | .72       |
| HGB (g/dL)    | 11.1 ± 0.25  | 11.2 ± 0.25  | 11.4 ± 0.25  | .41     | .72       |
| HCT (%)       | 31.7 ± 0.79  | 32 ± 0.79    | 32.4 ± 0.79  | .53     | .93       |
| MCV (fL)      | 50.6 ± 1.45  | 50.7 ± 1.45  | 50 ± 1.45    | .77     | .83       |
| MCH (pg)      | 17.8 ± 0.46  | 17.7 ± 0.46  | 17.6 ± 0.46  | .72     | .93       |
| MCHC (g/dL)   | 35.1 ± 0.24  | 34.9 ± 0.24  | 35 ± 0.24    | .81     | .60       |
| 4th week of lactation |     |       |       |        |           |
| RBC (× 10⁶/μL) | 5.43 ± 0.39  | 5.22 ± 0.32  | 5.13 ± 0.32  | .29     | .33       |
| HGB (g/dL)    | 9.6 ± 0.43   | 9.00 ± 0.35  | 9.00 ± 0.35  | .22     | .41       |
| HCT (%)       | 27.7 ± 1.18  | 25.9 ± 0.96  | 25.9 ± 0.96  | .25     | .47       |
| MCV (fL)      | 51 ± 2.04    | 49.8 ± 2.04  | 51.8 ± 2.04  | .47     | .36       |
| MCH (pg)      | 17.2 ± 0.75  | 17.3 ± 0.69  | 17.9 ± 0.69  | .12     | .89       |
| MCHC (g/dL)   | 32 ± 0.93    | 34.7 ± 0.93  | 34.7 ± 0.93  | .12     | .26       |
| 8th week of lactation |     |       |       |        |           |
| RBC (× 10⁶/μL) | 5.41 ± 0.19  | 5.46 ± 0.19  | 5.37 ± 0.21  | .30     | .47       |
| HGB (g/dL)    | 9.65 ± 0.25  | 9.5 ± 0.25   | 9.56 ± 0.25  | .25     | .67       |
| HCT (%)       | 27.8 ± 0.69  | 27.5 ± 0.69  | 27.3 ± 0.76  | .23     | .55       |
| MCV (fL)      | 51.2 ± 1.83  | 50.3 ± 1.83  | 52.3 ± 1.83  | .69     | .51       |
| MCH (pg)      | 17.9 ± 0.57  | 17.4 ± 0.57  | 18.1 ± 0.57  | .74     | .38       |
| MCHC (g/dL)   | 34.9 ± 0.21  | 34.6 ± 0.21  | 34.7 ± 0.21  | .46     | .32       |

*Experimental diets: CTR (basal diet), VPF10 (basal diet + 10 g vegetable pellet feed /cow/day), and VPF20 (basal diet + 20 g vegetable pellet feed /cow/day).

**Table 5. Effects of feeding of vegetable pellet feed (VPF) on white blood cell and differential leukocyte counting of Holstein dairy cows.**

| Parameters | CTR | VPF10 | VPF20 | Linear | Quadratic |
|------------|-----|-------|-------|--------|-----------|
| 2nd week of lactation |     |       |       |        |           |
| WBC (× 10⁶/μL) | 12.8 ± 2.04 | 9.79 ± 2.04 | 15.1 ± 2.28 | 0.68 | .37       |
| Neutrophil (%) | 34.2 ± 3.32 | 42.5 ± 3.03 | 34.1 ± 3.32 | 0.83 | .03       |
| Lymphocyte (%) | 57.5 ± 4.74 | 41.8 ± 4.74 | 59.5 ± 4.74 | 0.28 | .04       |
| Monocyte (%)   | 6.74 ± 2.40 | 11.7 ± 2.68 | 12 ± 2.68   | 0.12 | .19       |
| Eosinophil (%) | 1.56 ± 0.62 | 1.85 ± 0.53 | 1.90 ± 0.76 | 0.26 | .50       |
| Basophil (%)   | 0.88 ± 0.15 | 0.52 ± 0.15 | 0.52 ± 0.16 | 0.05 | .46       |
| 4th week of lactation |     |       |       |        |           |
| WBC (× 10⁶/μL) | 13.6 ± 3.68 | 11.4 ± 3.30 | 12.8 ± 3.68 | 0.93 | .54       |
| Neutrophil (%) | 31.1 ± 3.71 | 36.3 ± 4.15 | 31.5 ± 3.71 | 0.93 | .12       |
| Lymphocyte (%) | 57.4 ± 1.15 | 32.7 ± 1.15 | 55.7 ± 1.15 | 0.42 | .26       |
| Monocyte (%)   | 10.2 ± 6.53 | 23.8 ± 5.85 | 10.6 ± 6.53 | 0.17 | .93       |
| Eosinophil (%) | 0.62 ± 0.20 | 0.87 ± 0.26 | 0.20 ± 0.22 | 0.07 | .70       |
| Basophil (%)   | 0.42 ± 0.10 | 0.38 ± 0.10 | 0.72 ± 0.10 | 0.07 | .13       |
| 8th week of lactation |     |       |       |        |           |
| WBC (× 10⁶/μL) | 13.3 ± 4.02 | 14.5 ± 4.02 | 21.1 ± 4.50 | 0.71 | .27       |
| Neutrophil (%) | 24 ± 4.29   | 32.1 ± 4.30 | 21.5 ± 3.91 | 0.82 | .12       |
| Lymphocyte (%) | 74.6 ± 6.17 | 63.1 ± 6.75 | 74.6 ± 6.17 | 0.38 | .10       |
| Monocyte (%)   | 3.68 ± 0.88 | 5.40 ± 0.98 | 3.52 ± 0.88 | 0.23 | .52       |
| Eosinophil (%) | 0.53 ± 0.33 | 0.70 ± 0.34 | 0.67 ± 0.34 | 0.93 | .84       |
| Basophil (%)   | 0.72 ± 0.16 | 0.60 ± 0.16 | 0.85 ± 0.17 | 0.89 | .71       |

*Experimental diets: CTR (basal diet), VPF10 (basal diet + 10 g vegetable pellet feed /cow/day), and VPF20 (basal diet + 20 g vegetable pellet feed /cow/day).*
colonisation or interaction with immune cells directly discussed by Kogan and Kocher (2007). However, previous studies on various animal species have indicated that supplementation of MOS did not affect WBC and lymphocyte count in weanling pigs (Zhao et al. 2012) or monocyte, basophil, and eosinophil counts in broiler chickens (Attia et al. 2017). Based on our results, it seems likely that feeding VPF up to 20 grams per day could have positive effects on the aforementioned immune cells and may have positive implications when cows are immunosuppressed or undergoing a phase of stress. High levels of lymphocytes to neutrophils have been shown to indicate a reduction in inflammation in the body (Widowski et al. 1989). The results observed concerning VPF20 treatment are in contrast with previous findings, which reported increasing in neutrophil and decreasing in lymphocyte percentage by supplementation of autolysed yeast for lactating dairy cows under heat stress (Adili et al. 2020) and chickens (Ahiwe et al. 2019). To our knowledge, there is no research focussing on the evaluation of purified lignin on the immune system of lactating dairy cows. Also, more studies are needed to explore the link between a blend of plant extract, prebiotics, and purified lignin and immune cells distribution.

Conclusions

It was concluded that a mixture of antioxidants, and prebiotics, and purified lignin (VPF) may be useful for increasing milk yield, protein content, and decreasing MUN in Holstein dairy cows during the early lactation period. Although the addition of the substance led to changes in some hematological parameters, justifying its effects on livestock performance needs further investigation.

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Ethical approval

The protocols used in this experiment were approved by advisory committee of the faculty of agriculture and food industries of Islamic Azad University, Science and Research branch, Tehran, according to the guidelines of the Iranian Council of Animal Care (1995).

Disclosure statement

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [Ali Asghar Sadeghi], upon reasonable request.

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